Diego Santiago-Alarcon
Alfonso Marzal *Editors*

Avian Malaria and Related Parasites in the Tropics

Ecology, Evolution and Systematics

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Editors
Diego Santiago-Alarcon
Red de Biología y Conservación de Vertebrados
Instituto de Ecología, A.C. - CONACYT
Xalapa, Veracruz, Mexico

Alfonso Marzal
Department of Anatomy, Cellular Biology and Zoology
University of Extremadura
Badajoz, Badajoz, Spain

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To my lovely wife Ana Marina who has taught me empathy and that living is enjoying the small everyday gifts that nature gives us for free. To my parents, Francisco Javier and María del Carmen, who taught me the value of education, hard work, and dedication. To Tuza, Oso, Chispa, and Goddi.

Diego Santiago-Alarcon

To my dear wife Rocío, for being my unconditional partner. You are and always will be my support and happiness. You are my little gift every day. To our children Rosario and Alfonso, for filling us with love and joyfulness. To my parents, Alfonso and María del Carmen, for teaching me the value of education and hard work.

Alfonso Marzal

Foreword

The tropics and temperate climates differ considerably in the mean and the variance in temperature, precipitation, and wind. While certain areas vary considerably in climate over the years, others show clear patterns of consistency with effects on life history of the numerous organisms that inhabit these areas. In particular, richness and abundance of parasites are predominantly high in the tropics, especially the haemosporida (Price 1980; Combes 2001; Valkiūnas 2005). In fact, ecological theory predicts a strong positive relationship between the abundance and diversity of parasites and their hosts (Morand and Krasnov 2010). The crucial difference between the temperate zones and the tropics is the dissimilarity in prevalence and parasitemia or intensity of infection (Morand and Krasnov 2010). Already Darwin's contemporary Alfred R. Wallace emphasized the greatly exaggerated difference in interspecific interactions between hosts and parasites in tropical regions and the fitness consequences for the hosts (reviews in Coley and Aide 1991; Schemske et al. 2009). Does biodiversity differences between temperate and tropical regions result in a higher tropical diversity due to a richer set of ecological interactions, or are they simply due to random sampling at different levels? Are haemosporidians different from all other parasites, or do they just constitute part of the combined coevolutionary interactions between hosts and parasites like any other kind of host–parasite interaction, with parasites in the tropics having more severe fitness consequences for their hosts?

Haemosporidians are an important cause of parasite virulence and are also the ancestors of malaria parasites including human malaria. Hence, they are at the origin of one of the most significant and debilitating human diseases. These effects also apply to the consequences of climate change on host–parasite interactions with different impacts on haemosporidians and other parasites (Møller et al. 2013). Because vector-borne parasite interactions involve hosts, parasites, and the important vectors that transmit them, a full understanding of all parties requires in-depth studies of each of these. Encounter rate probability and an array of anti-parasite defenses such as physical removal of parasites (e.g., preening intensity), chemical defenses (e.g., uropygial secretions), immune function, and micro-habitat choice by vectors all contribute to differences in host–parasite interactions. Furthermore,

different interspecific interactions (e.g., brood parasitism, predation, and competition) appear to be particularly susceptible to haemosporidian infections. Brood parasites are rarely infected with blood parasites be it common cuckoos *Cuculus canorus*, great spotted cuckoos *Clamator glandarius*, cowbirds, or any other species. This surprising finding either suggests that brood parasites are particularly resistant to haemosporidians and they rarely encounter vectors or their peculiar lack of or reduction in parental care reduces the prevalence and intensity of infection by haemosporidians. In contrast, predators suffer frequently from blood parasite infections, especially by the debilitating parasite genus *Plasmodium*. The fitness costs of haemosporidian infections have so far only been studied in a handful of cases. For example, blood parasites have been shown to affect the laying date (Marzal et al. 2005), reproductive success (Merino et al. 2000) and survival of their hosts (Martínez-de la Puente et al. 2010). So far, the number of experimental studies of haemosporidians that has been performed in the tropics is scarce (see Chap. 17 of this volume). Studies of temperate zone birds indicate that viability, predation, and future fitness prospects are all linked to blood parasitism.

Tropical species are commonly less abundant than their temperate counterparts, but with clear latitudinal trends in prevalence and parasitemia. Such latitudinal patterns of biodiversity show evidence of latitudinal maxima in both hosts and parasites (Morand and Krasnov 2010), but also in diseases caused by these parasites (Guernier et al. 2004), though there are exceptions to this general pattern (e.g., genera *Leucocytozoon*; Fecchio et al. 2019). Differences in life history of hosts and parasites are typically linked to differences in latitude and abundance. Indeed, Møller et al. (2009) showed that virulence in the tropics is much higher than in the temperate zone even when controlling for differences in host size, general ecology, and differences in similarity among taxa due to common phylogenetic descent. A number of different potential mechanisms have been suggested to account for such effects. If there are more parasite taxa in the tropics, this implies a higher diversity of infection being correlated with a higher diversity of potential food. In other words, multiple parasites select for more intense competition for limiting resources. Is competition more common and more intense in the tropics than elsewhere? We can make the prediction that experimental manipulation of the level of parasitism at different latitudes should result in higher virulence in the tropics.

Birds commonly migrate between tropics and temperate climates with hundreds of millions of migrants moving twice annually between the northern and the southern hemisphere. Several studies have shown that migration affects host–parasite interactions (see Chap. 16 of this volume). For example, barn swallows in the tropics have body condition depending on environmental conditions, but also on host–parasite interactions. Molt in the tropics affects timing of migration and subsequent timing of reproduction as shown by barn swallows (Møller et al. 1995).

Invasions may contribute to the history of host–parasite interactions, particularly for the evolution of novel interactions, immune adaptations, and native biodiversity loses (see Chap. 15). These interactions may be of recent origin partly due to invasions of parasites, vectors, and hosts as in Hawaii, Galapagos, and other tropical regions that may all contribute to the dynamics of haemosporidians in the tropics.

For instance, house sparrows (*Passer domesticus*) constitute a model system for the study of invasion biology, with the widespread occurrence of haemosporidians being determined by enemy release resulting in blood parasites doing better in areas where open niches are abundant. Moreover, blood parasites may do better in areas where novel weapons are particularly suitable for invasion of alien environments, where diversity and prevalence are low (Marzal et al. 2018). The first cases of interactions between haemosporidians and tropical birds derive from reports of the 1900s, but the link between hosts life history and parasite infection risk dates back to the mid-1950s (see Chap. 1).

The interaction between haemosporidians and their hosts constitutes an important model system for the study of host–parasite–vector interactions. There is an extensive development of research, in particular on malaria in humans, but also on malaria in domesticated animals and wild animals more recently as demonstrated in the present volume. A search on Web of Science resulted in 326,231 hits with most being for blood parasite*. Surprisingly, there has been very little experimental research on this system. Why are there only a couple of experimental studies that have attempted to use medication as a tool for understanding the consequences of prevalence and parasitemia of blood parasite infections? Likewise, there have only been few studies attempting to link haemosporidians infections with the ecological and evolutionary consequences of such infections. High prevalence and parasitemia, particularly in tropical host species, suggests that parasitologists and ecologists should increase their research effort to study such effects in the tropical zones and compare them with the same type of studies in the temperate areas. Why such studies are virtually absent is difficult to understand. This book constitutes an excellent basis for the development of such research agenda.

Anders Pape Møller
Ministry of Education Key Laboratory for Biodiversity Science
and Ecological Engineering, College of Life Sciences,
Beijing Normal University, Beijing, China

Ecologie Systématique Evolution, Université Paris-Sud, CNRS,
AgroParisTech, Université Paris-Saclay,
Orsay, Cedex, France

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Contents

Chapter 1
Research on Avian Haemosporidian Parasites in the Tropics Before the Year 2000

Diego Santiago-Alarcon and Alfonso Marzal

Abstract In 1884, only 5 years after A. Laveran discovered agents of human malaria, Vassily Danilewsky reported the first description of the pathological effects of avian malaria on their bird hosts. Shortly after, Sir Ronald Ross carried out the first investigation on the life cycle of avian *Plasmodium* parasites, being the first to show that the malaria parasite is transmitted by the bite of infected mosquitoes. Since its discovery until now, bird malaria parasites have played a major role as model organisms in human malaria research. Experiments with avian malaria have significantly contributed to the description of the life cycle of *Plasmodium* parasites and for early antimalaria drug screening. Avian malaria and related haemosporidian parasites are globally distributed; host diversity and environmental factors in the tropical regions favor the prevalence and diversity of this group of parasites. Thus, a large number of studies on bird haemosporidians during the last century, particularly taxonomic descriptions, were conducted in a wide range of tropical birds, mainly in African countries. Although the number of publications on this topic was not big during the first half of the last century, it significantly increased from late 1970s, reaching a maximum on early 1990s. During these periods, internationally recognized researchers such as G.F. Bennett, M.A. Peirce, and R.A. Earlé, among others, published a number of studies reporting data of avian haemosporidian infections in many host taxa covering many tropical regions, as well as the descriptions of 134 novel species. During the last three decades, important contributions by G. Valkiūnas have organized and enriched the knowledge on the taxonomy of this group of protozoans. Here, we have conducted an extensive search for avian haemosporidians publications in the Web of Science Core Collection and a bibliometric analysis of the found documents during the period of 1909–2000. In this

D. Santiago-Alarcon (✉)
Red de Biología y Conservación de Vertebrados, Instituto de Ecología, Xalapa, Mexico
e-mail: diego.santiago@inecol.mx

A. Marzal
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain

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chapter, we present an extensive synthesis of research conducted on avian malaria and related haemosporidian parasites across the different tropical regions (America, Africa, Asia, Australia, and Oceania).

Keywords Avian malaria · *Haemoproteus* · *Leucocytozoon* · Parasite taxonomy · *Plasmodium* · Tropical parasitology

1.1 Introduction

Avian haemosporidians are vector-borne parasites belonging to the genera *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and *Fallisia* (Valkiūnas 2005; Santiago-Alarcon et al. 2012a; see Chaps. 2 and 5). This group of parasites is globally distributed with the exception of polar regions (Clark et al. 2014), and a large genetic diversity (>3500 lineages) has been discovered during the past decade (Bensch et al. 2009). Such genetic diversity has allowed the experimental study of different lineages' virulence belonging to the same or different *Plasmodium* morphospecies (e.g., Palinauskas et al. 2011, 2018; Ilgūnas et al. 2019a, b). Additionally, a new simple centrifugation method to obtain high-quality DNA from haemosporidian parasites (Palinauskas et al. 2013) has allowed more in-depth genomics and transcriptomic studies, leading to the recovery of the first avian haemosporidian genome (*Haemoproteus tartakovskyi*, Bensch et al. 2016) and some transcriptoms as well (Videvall et al. 2017; see also Chap. 4). However, the history of avian haemosporidian research starts more than 100 years earlier, when in 1880 Charles Louis Alphonse Laveran discovered gametocytes circulating in the peripheral blood of infected human patients. Some years later, in 1884 Vassily Danilewsky (Danilewsky 1884) working in Ukraine discovered the broad distribution of intracellular malaria-like parasites infecting birds. These organisms were classified later in the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. Importantly, he developed the first investigations showing the pathological effects of these blood parasites on avian hosts and demonstrated similarities to pathologies caused by malaria parasites in humans (Danilewsky 1889; Valkiūnas 1985) and the initial seasonality patterns of the infections on wild birds (Marzal 2012). Later, in 1897 William MacCallum discovered sexual processes (e.g., exflagellation) in haemosporidians using *Haemoproteus columbae* infecting pigeons as model organisms (Cox 2010). Also, during 1897, Sir Ronald Ross, a British officer in the Indian Medical Service, who knew of V. Danilewsky's studies on bird malaria, discovered the involvement of mosquitoes (Diptera: Culicidae) in the transmission of *Plasmodium* sp. (probably *Plasmodium relictum*) in birds (Ross 1898). In 1902, Ross was awarded the Nobel Prize in Physiology and Medicine for these contributions, which were essential in understanding malaria epidemiology and transmission. After the discovery of the involvement of mosquitoes of several genera, mainly *Culex* and *Aedes* in the transmission of avian *Plasmodium* (Huff 1927), bird malaria parasites became an experimental model for human malaria research in many

laboratories. But with the discovery of rodent haemosporidians by 1948 (Killick-Kendrick 1974), and later primate malaria (Cox 2010; Santiago-Alarcon et al. 2012a), the avian haemosporidians decreased in popularity as a model system for the study of human malaria. However, studies on avian malaria parasites still have played an essential role in advances of medicine, parasitology, ecology, and evolution, among others fields. For example, the test of synthetic compounds (e.g., plasmochin and atebrin) in birds allowed the discovery of effective antimalarial drugs in humans and methods to study exo- and erythrocytic parasite stages in vitro (Coatney et al. 1953; Ball and Chao 1961; Ball 1964; McGhee et al. 1977). Furthermore, during the last 15 years, avian haemosporidians have become a model system to study the ecology and evolution of this antagonistic interaction (e.g., Bensch et al. 2000; Ricklefs and Fallon 2002; Marzal et al. 2008; Knowles et al. 2011; Santiago-Alarcon et al. 2014; Renner et al. 2016; Pacheco et al. 2018), which is paving the road to develop novel pathogen control and prevention strategies aiding both medical and animal health (e.g., Asghar et al. 2015, 2016).

The malaria disease generated by parasites of the genus *Plasmodium* has been recognized in ancient cultures around the globe, from Chinese documents by 2700 B.C. to Egyptian papyri by 1500 B.C. But more solid evidence comes from ancient Greeks 800–400 B.C., who provided the natural history of the disease, with its characteristic fevers and enlarged parenchymal internal organs such as livers and spleens (Cox 2010). The history of avian haemosporidian parasite studies in the tropics started at the beginning of the twentieth century, for example, with the discovery of louse flies (Diptera: Hippoboscidae) as vectors of *H. columbae* in Brazil (Aragão 1908), and expeditions from the Liverpool School of Tropical Medicine to West Africa (e.g., Gambia, Congo), where haemosporidian infections in humans, domestic and wild animals were reported (Todd and Wolbach 1912). Nonetheless, the first work conducted in tropical areas was the one leading to the discovery of the involvement of mosquitoes transmitting avian malaria (probably *P. relictum*) in India (Ross 1898). Thus, there is already a long history of avian haemosporidian research in tropical regions, but most of it was focused on reporting infections in bird species by parasites of different haemosporidian genera (e.g., Todd and Wolbach 1912; Greiner et al. 1975; Bennett and Herman 1976; McClure et al. 1978; White et al. 1978), as well as on taxonomic descriptions of new parasites, where 62 novel species were described from Asia (mainly in India), 48 from Africa (mainly in South Africa), 24 from America (mainly in Brazil), and 8 from Oceania (Table 1.1; summaries of taxonomic and nomenclature work and keys for haemosporidian parasite species are available in Valkiūnas 2005; Valkiūnas and Iezhova 2018).

In this chapter, we searched for publications in the Web of Science Core Collection (www.webofknowledge.com) from the year 1909–2000, including all languages and document types. We used the following Boolean search keywords:

TS = ((Haemosporid* OR avian malaria OR Plasmodium OR Haemoproteus OR Leucocytozoon) AND (Tropic* OR Neotropic* OR Afrotropic* OR Ethiopia* OR IndoMalay* OR Oriental OR Austral* OR Oceania*)).

Table 1.1 Avian haemosporidian species initially described in tropical regions and their current taxonomic status

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Africa (n = 48)					
Haemoproteus africanus	Valid	Green-backed twinspot (*Mandingoa nitidula*)	Africa	Tanzania/Amani	Bennett and Peirce (1991)
Haemoproteus balearicae	Valid	Black crowned crane (*Balearica pavonina*)	Africa	United Kingdom/Surrey[b]	Peirce (1973)
Haemoproteus balmorali	Valid	Spotted flycatcher (*Muscicapa striata*)	Africa	Zambia/Balmoral	Peirce (1984b)
Haemoproteus borgesi	Valid	Fine-spotted woodpecker (*Campethera punctuligera*)	Africa	Guinea	Tendeiro (1947)
Haemoproteus bubalornis	Valid	White-billed buffalo weaver (*Bubalornis albirostris*)	Africa	Kenya/Ngulia	Bennett and Peirce (1991)
Haemoproteus bucerotis	Valid	Northern red-billed hornbill (*Tockus erythrorhynchus*)	Africa	Botswana/Gaborone	Bennett et al. (1995)
Haemoproteus burhini	Valid	Spotted thick-knee (*Burhinus capensis*)	Africa	South Africa/Satara, Kruger National Park	Bennett et al. (1995)
Haemoproteus chelidonis	*Incertae sedis*	Northern house-martin (*Delichon urbicum*)	Africa		Franchini (1922), White and Bennett (1978)
Haemoproteus crumenium	Valid	Marabou stork (*Leptoptilos crumenifer*)	Africa	Kenya/Nairobi	Peirce and Cooper (1977)
Haemoproteus cublae	Valid	Black-backed puffback (*Dryoscopus cubla*)	Africa	Zambia/Balmoral	Peirce (1984c)
Haemoproteus enucleator	Valid	African pygmy kingfisher (*Ispidina picta*)	Africa	Uganda/Entebbe	Bennett et al. (1972)
Haemoproteus hirundinis	Valid	Hirundinidae (*Pseudhirundo sp.*)	Africa	Algeria	White and Bennett (1978)
Haemoproteus indicator	Valid	Greater honeyguide (*Indicator indicator*)	Africa	Uganda/Zika Forest	Bennett et al. (1986a)

Haemoproteus janovyi	Valid	White-backed vulture (*Gyps africanus*)	Africa	South Africa/Sengwa wildlife research area, Zimbabwe	Greiner and Mundy (1979)
Haemoproteus killangoi	Valid	African yellow white-eye (*Zosterops senegalensis*)	Africa	Uganda/Zika Forest	Bennett and Peirce (1981)
Haemoproteus lairdi	Valid	Blue-breasted bee-eater (*Merops variegatus*)	Africa	Uganda/Entebbe	Bennett et al. (1978)
Haemoproteus mcleani	*Species inquirenda*	Southern fiscal (*Lanius collaris*)	Africa	South Africa/Pretoria	Bennett et al. (1995)
Haemoproteus montezi	Valid	Purple-crested turaco (*Gallirex porphyreolophus*)	Africa	Mozambique	Bennett and Peirce (1990)
Haemoproteus motacillae	Valid	Western Yellow Wagtail (*Motacilla flava*)	Africa	Uganda/Entebbe	Bennett and Peirce (1990)
Haemoproteus neseri	Valid	Chorister robin-chat (*Cossypha dichroa*)	Africa	South Africa/Louis Trichardt, Transvaal	Bennett and Earlé (1992)
Haemoproteus pelouroi	Valid	Hadada ibis (*Bostrychia hagedash*)	Africa	Guinea	Tendeiro (1947)
Haemoproteus porzanae	Valid	Baillon's crake (*Zapornia pusilla*)	Africa	Tunisia/Kairouan	Coatney (1936)
Haemoproteus pratasi	Valid	Helmeted guineafowl (*Numida meleagris*)	Africa	Guinea	Tendeiro (1947)
Haemoproteus psittaci	Valid	Grey parrot (*Psittacus erithacus*)	Africa	United Kingdom/Wickford, Essex[b]	Bennett and Peirce (1992)
Haemoproteus queleae	Valid	Red-headed quelea (*Quelea erythrops*)	Africa	Equatorial Guinea/San Thomé	Bennett and Peirce (1991)
Haemoproteus sequeirae	Valid	Splendid sunbird (*Cinnyris coccinigastrus*)	Africa	Guinea	Tendeiro (1947)

(continued)

Table 1.1 (continued)

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Haemoproteus stellaris	Valid	Grey-rumped swallow (*Pseudhirundo griseopyga*)	Africa	Uganda/Kasenyi	White and Bennett (1978)
Haemoproteus telfordi	Valid	Black-bellied bustard (*Lissotis melanogaster*)	Africa	Zaire/Kundelungu, Katanga	Bennett et al. (1975)
Haemoproteus timalus	Valid	Rufous chatterer (*Argya rubiginosa*)	Africa	Kenya/South Horr	Bennett et al. (1991c)
Haemoproteus undulatus	Valid	Red-faced mousebird (*Urocolius indicus*)	Africa	South Africa/Kruger National Park	Bennett and Earlé (1992)
Haemoproteus uraeginthus	Valid	Red-cheeked cordon-bleu (*Uraeginthus bengalus*)	Africa	Chad/N'Djamena	Bennett and Peirce (1991)
Plasmodium durae	Valid	Wild turkey (*Meleagris gallopavo*)	Africa	Kenya/Langata, Nairobi	Herman (1941); see also Laird (1978)
Plasmodium fallax	Valid	African wood owl (*Strix woodfordii*)	Africa	Congo	Schwetz (1930)
Plasmodium garnhami	Valid	Eurasian hoopoe (*Upupa epops*)	Africa	Egypt/Imbaba District, Giza	Guindy et al. (1965)
Plasmodium gundersi	Valid	African wood owl (*Strix woodfordii*)	Africa	Liberia/Harbel, Marshall Territory	Garnham (1966)
Plasmodium rouxi	Valid	Spanish sparrow (*Passer hispaniolensis*)	Africa	Algeria/Mitidja plains, Algiers	Sergent et al. (1928)
Leucocytozoon balmorali	Valid	Black-backed puffback (*Dryoscopus cubla*)	Africa	Zambia/Balmoral	Peirce (1984d)
Leucocytozoon caprimulgi	Valid	Square-tailed nightjar (*Caprimulgus fossii*)	Africa	Congo/Haute-Sangha	Bennett et al. (1992b)
Leucocytozoon centropi	Valid	White-browed coucal (*Centropus superciliosus*)	Africa	South Africa/Pietermaritzburg	Bennett et al. (1993a)
Leucocytozoon colius	Valid	Speckled mousebird (*Colius striatus*)	Africa	South Africa/Lydenburg, Transvaal	Bennett et al. (1993b)

Leucocytozoon dizini	Valid	Western grey plantain-eater (*Crinifer piscator*)	Africa	Guinea Bisáu	Tendeiro (1947)
Leucocytozoon eurystomi	Valid	Blue-throated roller (*Eurystomus gularis*)	Africa	Congo/Haute-Sangha	Bennett et al. (1993b)
Leucocytozoon neavei	Valid	Helmeted guineafowl (*Numida meleagris*)	Africa	Sudan/Khartoum	Sambon (1909)
Leucocytozoon schoutedeni	Valid	Red junglefowl (*Gallus gallus*)	Africa	Congo/Bukama, Lake Upemba, Katanga	Rodhain et al. (1913)
Leucocytozoon sousadiasi	Valid	Black-headed lapwing (*Vanellus tectus*)	Africa	Guinea Bisáu	Tendeiro (1947)
Leucocytozoon struthionis	Valid	African ostrich (*Struthio camelus*)	Africa	South Africa/Cape Province, Middelburg District	Walker (1912)
Leucocytozoon toddi	Valid	Lizard buzzard (*Kaupifalco monogrammicus*)	Africa	Congo	Sambon (1908)
Leucocytozoon vandenbrandeni	Valid	African darter (*Anhinga rufa*)	Africa	Congo/Leopoldville	Rodhain (1931)
Asia and Oceania ($n = 66$)					
Haemoproteus aegithinae	Valid	Common iora (*Aegithina tiphia*)	Asia	India/Nagoa	de Mello (1935b)
Haemoproteus anthi	Valid	Paddyfield pipit (*Anthus novaeseelandiae*)	Asia	India/Goa	de Mello (1935b)
Haemoproteus antigonis	Valid	Demoiselle crane (*Grus virgo*)	Asia	India/Junagad	de Mello (1935b)
Haemoproteus bennetti	Valid	Greater yellownape (*Chrysophlegma flavinucha*)	Asia	India/Darjeeling W.B.	Greiner et al. (1977)
Haemoproteus bilobata	Valid	Fire-tufted barbet (*Psilopogon pyrolophus*)	Asia	India/Mt. Brinchang, Malaya	Bennett and Nandi (1981)

(continued)

Table 1.1 (continued)

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Haemoproteus centropi	Valid	Greater coucal (*Centropus sinensis*)	Asia	India/Diu	de Mello (1935b)
Haemoproteus contortus	Valid	Eurasian whimbrel (*Numenius phaeopus*)	Southeast Asia	Philippine Islands/Batangas	Bennett (1979)
Haemoproteus coraciae	Valid	Indian roller (*Coracias benghalensis*)	Asia	India/Goa	Bishop and Bennett (1986)
Haemoproteus cornuata	Valid	Blue-throated barbet (*Psilopogon asiaticus*)	Asia	Bhutan/West Bhutan	Bennett and Nandi (1981)
Haemoproteus dicaeus	Valid	Crimson-breasted flowerpecker (*Prionochilus percussus*)	Southeast Asia	Malaysia/Subang	Bennett and Bishop (1990)
Haemoproteus dicruri	Valid	Black drongo (*Dicrurus macrocercus*)	Asia	India/Pragana	Peirce (1984e)
Haemoproteus elani	Valid	Black-winged kite (*Elanus caeruleus*)	Asia	India/Daman	de Mello (1935b)
Haemoproteus eurylaimus	Valid	Silver-breasted broadbill (*Serilophus lunatus*)	Southeast Asia	Thailand/Chieng-mai	Bennett et al. (1991d)
Haemoproteus eurystomae	Valid	Oriental dollarbird (*Eurystomus orientalis*)	Southeast Asia	Malaysia/Rantau Panjang	Bishop and Bennett (1986)
Haemoproteus fuscae	Valid	White-throated kingfisher (*Halcyon smyrnensis*)	Asia	India/Santo Estevam	de Mello and da Fonseca (1937)
Haemoproteus gallinulae	Valid	Common moorhen (*Gallinula chloropus*)	Asia	India/Carambolim Lake, Ilhas, Goa	de Mello (1935b)
Haemoproteus halcyonis	Valid	White-throated kingfisher (*Halcyon smyrnensis*)	Asia	India/Canacona	de Mello (1935b)
Haemoproteus handai	Valid	Plum-headed parakeet (*Psittacula cyanocephala*)	Asia	Pakistan/Lahore	Bennett and Peirce (1986)

Haemoproteus herodiadis	Valid	Intermediate egret (*Ardea intermedia*)	Asia	India/Lake Carambolim, Ilhas Goa	de Mello (1935a)
Haemoproteus iwa	Valid (Levin et al. 2011)	Great frigatebird (*Fregata minor*)	Oceania	USA/Hawaii, Laysan Island	Work and Rameyer (1996)
Haemoproteus lanii	Valid	Long-tailed shrike (*Lanius schach*)	Asia	India/Pondá	de Mello (1936)
Haemoproteus manwelli	Valid	Little green bee-eater (*Merops orientalis*)	Asia	India/Maharashtra	Bennett et al. (1978)
Haemoproteus megapodius	Valid	Dusky scrubfowl (*Megapodius freycinet*)	Asia	India/Campbell Bay,Great Nicobar Island	Nandi and Mandal (1980)
Haemoproteus meropis	Valid	Little green bee-eater (*Merops orientalis*)	Asia	India/Nagpur	Bennett (1978)
Haemoproteus monarchus	Valid	Island monarch (*Monarcha cinerascens*)	Oceania	New Guinea	Bennett et al. (1991c)
Haemoproteus nettionis	Valid	Chestnut teal (*Anas castanea*)	Oceania	Australia/ New South Wales	Coatney (1936)
Haemoproteus nucleophilus	Valid	Black berrypecker (*Melanocharis nigra*)	Oceania	Papua New Guinea/L. Kopiago, Southern highlands	Bennett and Bishop (1990)
Haemoproteus orientalis	Valid	Little green bee-eater (*Merops orientalis*)	Asia	India/Maharashtra	Bennett et al. (1978)
Haemoproteus orioli	Valid	Eurasian golden-oriole (*Oriolus oriolus*)	Asia	India/Nova Goa	Peirce (1984f)
Haemoproteus orizivorae	Valid	Java sparrow (*Lonchura oryzivora*)	Southeast Asia	Indonesia/West Java	Peirce (1984h)
Haemoproteus otocompsae	Valid	Red-whiskered bulbul (*Pycnonotus jocosus*)	Asia	India/Malim (Baedez)	Peirce (1984i), Rahal et al. (1987)

(continued)

Table 1.1 (continued)

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Haemoproteus pachycephalus	Valid	Golden whistler (*Pachycephala pectoralis*)	Southeast Asia	Philippine Islands/Mindanao	Bennett et al. (1991c)
Haemoproteus pastoris	Valid	Rosy starling (*Pastor roseus*)	Asia	India/Pragana	de Mello (1935b)
Haemoproteus philippinensis	Valid	Cinereous bulbul (*Hemixos flavala*)	Southeast Asia	Malaysia/Mount Brinchang, Pahang	Rahal et al. (1987)
Haemoproteus pittae	Valid	Blue-banded pitta (*Pitta arquata*)	Southeast Asia	Borneo	Bennett et al. (1991d)
Haemoproteus plataleae	Valid	Eurasian spoonbill (*Platalea leucorodia*)	Asia	India/Diu	de Mello (1935b)
Haemoproteus ptilotis	Valid	Yellow-faced honeyeater (*Caligavis chrysops*)	Oceania	Australia/Milson Island, New South Wales	Coatney (1936), Bennett et al. (1994)
Haemoproteus rileyi	Valid	Indian peafowl (*Pavo cristatus*)	Asia	India/Patna	Malkani (1936)
Haemoproteus rotator	Valid	Pin-tailed snipe (*Gallinago stenura*)	Southeast Asia	Philippine Islands/Palawan	Bennett (1979)
Haemoproteus sanguinis	Valid	Red-whiskered bulbul (*Pycnonotus jocosus*)	Asia	India/Calcutta, Bengal	Rahal et al. (1987)
Haemoproteus thereicerycis	Valid	Brown-headed barbet (*Psilopogon zeylanicus*)	Asia	India/Corlim, Nova Goa	de Mello (1935b), Bennett and Nandi (1981)
Haemoproteus trogonis	Valid	Scarlet-rumped trogon (*Harpactes duvaucelii*)	Southeast Asia	Malaysia/Subang	Bennett and Peirce (1990)
Haemoproteus upupae	Valid	Eurasian hoopoe (*Upupa epops*)	Asia	India/Daman	de Mello (1935b)
Haemoproteus wenyoni	Valid	Common tailordbird (*Orthotomus sutorius*)	Asia	India/Nova Goa	Peirce (1984g)
Haemoproteus xantholaemae	Valid	Coppersmith barbet (*Psilopogon haemacephalus*)	Asia	India/Telinkhery, Nagpur	Bennett and Nandi (1981)

Haemoproteus zosteropis	Valid	Oriental white-eye (*Zosterops palpebrosus*)	Asia	India/Calcutta	Bennett and Peirce (1981)
Plasmodium anasum	Valid	Northern Shoveler (*Spatula clypeata*)	Asia	China/Lin-pien Ping-tung Hsien, Taiwan	Manwell and Kuntz (1965)
Plasmodium coturnixi	Valid	Common quail (*Coturnix coturnix*)	Asia	Pakistán/Kohat	Bano and Abbasi (1983)
Plasmodium dissanaikei	Valid	Rose-ringed parakeet (*Psittacula krameri*)	Asia	Sri Lanka/Ja-ela	de Jong (1971)
Plasmodium formosanum	Valid	Taiwan partridge (*Arborophila crudigularis*)	Asia	China/Nan Tou Hsien, Taiwan	Manwell (1962)
Plasmodium gallinaceum	Valid	Red junglefowl (*Gallus gallus*)	Asia	Sri Lanka	Brumpt (1935)
Plasmodium gallinulae	*incertae sedis*	Common moorhen (*Gallinula chloropus*)	Asia	India/Lake Carambolim, Ilhas Goa	de Mello (1935b)
Plasmodium griffithsi	Valid	Wild turkey (*Meleagris gallopavo*)	Asia	Burma/Rangoon	Garnham (1966)
Plasmodium hegneri	Valid	Common teal (*Anas crecca*)	Asia	China/Lo-tung, I-lan Hsien, Taiwan	Manwell and Kuntz (1966)
Plasmodium herodiadis	*species inquirenda*	Intermediate egret (*Ardea intermedia*)	Asia	India/Lake Carambolim, Ilhas Goa	de Mello (1935a)
Plasmodium lophurae	Valid	Bornean fireback (*Lophura ignita*)	Asia	Borneo	Coggeshall (1938)
Leucocytozoon anellobiae	*Species inquirenda*	Little wattlebird (*Anthochaera chrysoptera*)	Oceania	Australia/Brisbane, Queensland	Bennett et al. (1994)
Leucocytozoon caulleryi	Valid	Red junglefowl (*Gallus gallus*)	Southeast Asia	Vietnam/Tonkin, Hanoi	Mathis and Léger (1909)

(continued)

Table 1.1 (continued)

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Leucocytozoon dubreuili	Valid	Redwing (*Turdus iliacus*)[c]	Southeast Asia	Vietnam/Tonkin, Hanoi	Bennett et al. (1993c)
Leucocytozoon leboeufi	Valid	Yellow bittern (*Ixobrychus sinensis*)	Southeast Asia	Vietnam/Tonkin, Hanoi	Mathis and Léger (1911)
Leucocytozoon maccluri	Valid	Dark-sided thrush (*Zoothera marginata*)[c]	Southeast Asia	Thailand/Chiengmai	Bennett et al. (1993c), Valkiūnas 2005
Leucocytozoon marchouxi	Valid	Red-collared dove (*Streptopelia tranquebarica*)	Southeast Asia	Vietnam/Tonkin, Hanoi	Bennett et al. (1992b)
Leucocytozoon nyctyornis	Valid	Blue-bearded bee-eater (*Nyctyornis athertoni*)	Asia	India/Rani, Kamrup District, Assam	Nandi (1986a)
Leucocytozoon simondi	Valid	Common teal (*Anas crecca*)	Southeast Asia	Vietnam/Tonkin, Hanoi	Mathis and Léger (1910)
Leucocytozoon squamatus	Valid	Scaly-bellied woodpecker (*Picus squamatus*)	Asia	India/Ptnitop, Uddampur, Jammu, Kashmir	Nandi (1986b)
Leucocytozoon tawaki	Valid	Fiordland penguin (*Eudyptes pachyrhynchus*)	Oceania	New Zeland/Kaikoura, Jackson's Head, South Island	Bennett et al. (1992b)
America (n = 24)					
Fallisia (*Plasmodioides*) *neotropicalis*[d]	Valid	Rock pigeon (*Columba livia*)	South America	Venezuela/El Saman, Villa Bruzual, Portuguesa	Gabaldon et al. (1985)
Haemoproteus apodus	Valid	Ashy-tailed swift (*Chaetura andrei*)	South America	Brazil/Sao Paulo, Guaratuba	Bennett et al. (1986a)
Haemoproteus bucconis	Valid	White-eared puffbird (*Nystalus chacuru*)	South America	Brazil/Sao Paulo, Itapetininga	Bennett et al. (1986a)
Haemoproteus circumnuclearis	Valid	Olive-striped flycatcher (*Mionectes olivaceus*)	South America	Colombia/Río Verde, Valle	Bennett et al. (1986b)
Haemoproteus cracidarum	Valid	Rufous-vented chachalaca (*Ortalis ruficauda*)	South America	Venezuela/San Juan de los Morros, Guarico	Bennett et al. (1982)

Haemoproteus formicarius	Valid	Plain antvireo (*Dysithamnus mentalis*)	South America	Brazil/Itapetininga	Bennett et al. (1987)
Haemoproteus furnarius	Valid	White-eyed foliage-gleaner (*Automolus leucophthalmus*)	South America	Brazil/Itapetininga	Bennett et al. (1987)
Haemoproteus ortalidum	Valid	Rufous-vented chachalaca (*Ortalis ruficauda*)	South America	Venezuela/Jácura, Falcón	Bennett et al. (1982)
Haemoproteus souzalopesi	Valid	Fuscous flycatcher (*Cnemotriccus fuscatus*)	South America	Brazil/ Sao Paulo, Guaratuba	Bennett et al. (1986b)
Haemoproteus trochili	Valid	White-tipped sicklebill (*Eutoxeres aquila*)	South America	Colombia/Río Zabaletas	White et al. (1979)
Haemoproteus vireonis	Valid	Red-eyed vireo (*Vireo olivaceus*)	South America	Brazil/Itapetininga	Bennett et al. (1987)
Haemoproteus witti	Valid	Red-billed streamertail (*Trochilus polytmus*)	South America	Jamaica/Greenhills	White et al. (1979)
Plasmodium bertii	Valid	Grey-necked wood rail (*Aramides cajaneus*)	South America	Venezuela/Guaquitas, Barinas	Gabaldon and Ulloa (1981)
Plasmodium columbae	Valid	Rock pigeon (*Columba livia*)	South America	Brazil/Sao Paulo	Carini (1912)
Plasmodium forresteri	Valid	Northern barred owl (*Strix varia*)	North America	USA/Trenton, Gilchrist County, Florida	Telford et al. (1997)
Plasmodium gabaldoni	Valid	Rock pigeon (*Columba livia*)	South America	Venezuela/Villa Brunzal, Portuguesa State	Garnham (1977)
Plasmodium hermani	Valid	Wild turkey (*Meleagris gallopavo*)	North America	USA/Palmdale, Glade's County, Florida	Telford and Forrester (1975)
Plasmodium huffi	Valid	Toco toucan (*Ramphastos toco*)	South America	Brazil	Muniz et al. (1951)

(continued)

Table 1.1 (continued)

Species	Taxonomic status[a]	Type host	Region	Country/locality	Reference
Plasmodium juxtanucleare	Valid	Red junglefowl (*Gallus gallus*)	South America	Brazil/Minas Gerais	Versiani and Gomes (1941)
Plasmodium lutzi	Valid	Grey-necked wood rail (*Aramides cajaneus*)	South America	Brazil/Sao Paulo	Lucena (1939)
Plasmodium paranucleophilum	Valid	Tanager (*Tachyphonus sp.*; Thraupidae)	South America	Northeast Brazil[e]	Manwell and Sessler (1971)
Plasmodium pinottii	Valid	Toco toucan (*Ramphastos toco*)	South America	Brazil	Muniz and Soares de (1954)
Plasmodium tejerai	Valid	Wild turkey (*Meleagris gallopavo*)	South America	Venezuela/Santa Inés, Miranda, Trujillo	Gabaldon and Ulloa (1977)
Leucocytozoon grusi	Valid	Sandhill crane (*Antigone canadensis*)	North America	USA/Payne's Prairie, Florida	Bennett et al. (1992b)

Synonyms are not listed in the table, only those species that are currently valid or species where not enough information exists yet to firmly establish their taxonomic identity

[a]Taxonomic status is given according to Valkiūnas (2005) review, in which it was specified and grounded. Many parasites described from tropical regions turned out to be synonyms of currently valid species names because the bird host family was used as the main taxonomic trait during their description, which is now well established that using bird families is not a valid taxonomic practice (Valkiūnas and Ashford 2002); using information about bird families is recommended only as an initial guide toward species identifications. Some of the synonyms might be validated in the future; however, additional research is needed to prove each case

[b]The type hosts were imported from Africa into Surrey and Essex; some were captive at a zoological garden and from a veterinary practice clinic in Essex; host species is endemic to West Africa or equatorial Africa (from Guinea to Tanzania)

[c]The work by Gabaldon et al. (1985) also included the description of a new subgenus, *Plasmodioides*

[d]The type host species was not provided in the original description, but it is suspected to be the one listed in the table based on the common name

[e]It is a likely location given that the parasite was described from an imported bird to the USA from South America

In total, we retrieved 671 articles, and after a thorough review, we eliminated papers that were not related to the field or that were outside of the tropics; at the end 176 research papers remained. Subsequently, we conducted a bibliometric analysis using the R package bibliometrix v. 2.0.0 (Aria and Cuccurullo 2017), with the objective of determining productivity across years, country, and author contributions. From the 176 research papers of the period 1912–2000, we found an average of 13.1 citations per manuscript. The beginning of last century was a slow start for the field of avian haemosporidian research in the tropics (an average of <1 article per year from 1912 to 1962); the field started in earnest approximately by the year 1976 with nine publications (the years with the most number of articles published were 1978 [11], 1984 [16], 1991 [14], 1992 [11], 1993 [13]) and had an annual percentage growth rate of 5.75 for the analyzed period. The top five journals where most tropical avian haemosporidian research was published during last century were *Canadian Journal of Zoology* (15), *Journal of Natural History* (12), *Journal of Parasitology* (11), *Journal of Wildlife Diseases* (8), and *South African Journal of Wildlife Research* (8). The most productive authors (i.e., >5 papers) were, in decreasing order, G.F. Bennett (45), M.A. Peirce (26), R.A. Earlé (16), N.C. Nandi (13), A. Gabaldon (12), G. Ulloa (12), M.A. Bishop (7), E.C. Greiner (6), F.W. Huchzermeyer (6), and A.K. Mandal (6) (Fig. 1.1). More recently, G. Valkiūnas has been the leading haemosporidian taxonomist; he and his colleagues have been the researchers in charge of organizing and enriching the taxonomy of avian haemosporidians during the past three decades around the world (e.g., Valkiūnas 2005; Valkiūnas and Iezhova 2018; Fig. 1.2). In terms of productivity by country, the most publications during the last century came from India with 60 publications, followed by South Africa and Brazil with 25 papers each, and then by Mexico, Venezuela, Argentina, Australia, and Malaysia with between 12 and 15 papers each (Fig. 1.3).

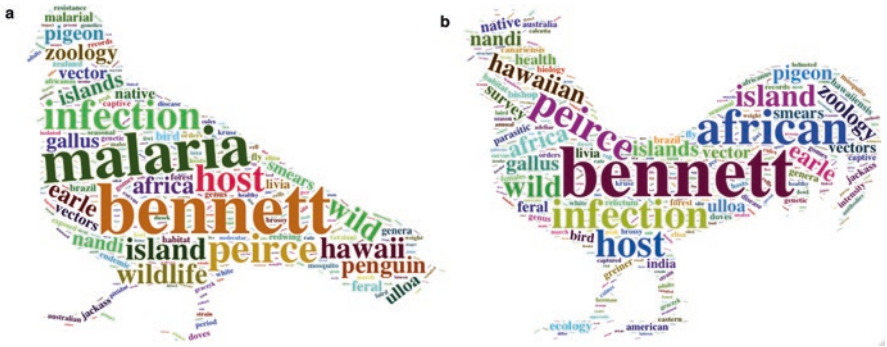

Fig. 1.1 Word cloud showing the most frequent words ($n = 250$) used in the titles, author lists, and abstracts from the 176 publications analyzed for tropical research on avian haemosporidian parasites. For the word clouds, we used the outlines of a rock pigeon (*Columba livia*) (**a**) and a red junglefowl (*Gallus gallus*) (**b**). We intend this as homage to these two widespread and charismatic species that have given so much in many different aspects (e.g., food, sport, service in war) to humans throughout the centuries. For the avian haemosporidian research, pigeons and chickens have provided material for the description of new species and have been model hosts for experimental infections, among other benefits

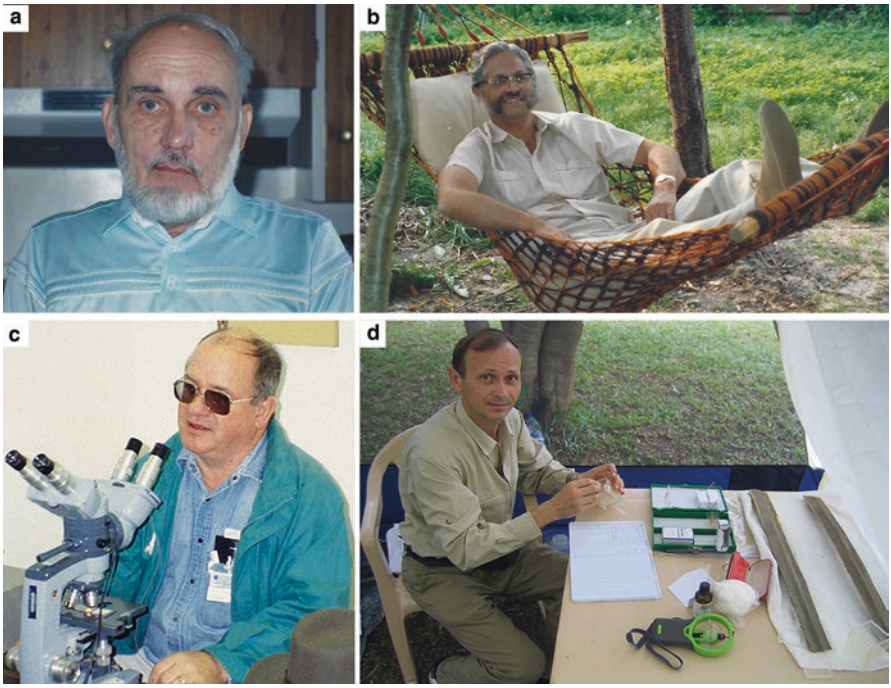

Fig. 1.2 Pictures of some important scientists for the field of tropical avian haemosporidian research during the last century (1900–2000): Gordon F. Bennett (**a**), Mike A. Peirce (**b**), Ellis C. Greiner (**c**), Gediminas Valkiūnas (**d**)

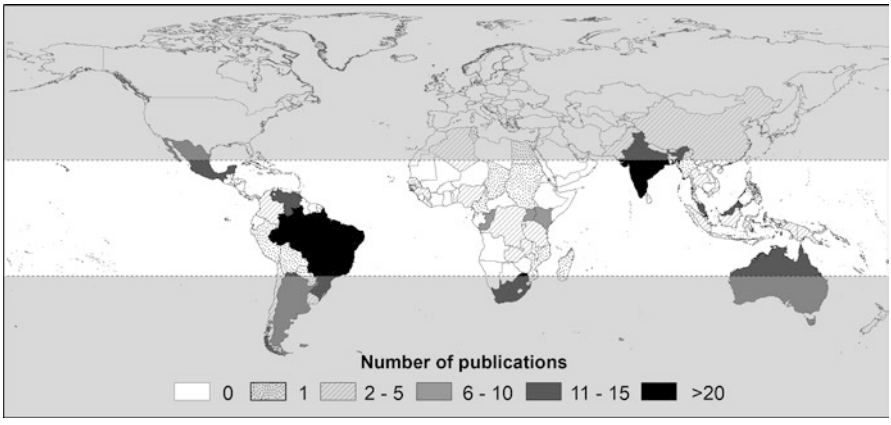

Fig. 1.3 Number of publications by country in tropical and some subtropical regions around the world. The light grey shaded region on the map delimits the tropical areas across the world

Below, we provide an exhaustive synthesis of the research conducted on avian haemosporidians in the different tropical regions (i.e., America, Africa, Asia, and Oceania; for practical purposes, we are combining Australia and Oceania into the same region). It is important to remember that all studies discussed here are based on microscopic examination of blood films. Thus, prevalence for different countries may not be precise because of researchers experience, and also when combined with molecular methods (polymerase chain reaction, PCR) researchers can detect low intensity infections missed by microscopic examination; however, PCR methods can also miss infections due to probes' high specificity, therefore making the use of both desirable (Valkiūnas et al. 2008a; see Chap. 2 for an introduction to haemosporidian life cycles and study methods).

1.2 Studies in the Tropics

1.2.1 Africa

A large expedition conducted at the beginning of the last century (1911) in Gambia by the Liverpool School of Tropical Medicine had the objective to study human trypanosomiasis, but researchers also collected samples from nonhuman mammals (n = 39), birds (n = 108), and reptiles (n = 28) (Todd and Wolbach 1912). *Leucocytozoon* was detected in vultures and bush fowl (francolins), and pigments producing Haemosporida parasites (i.e., *Haemoproteus*, *Plasmodium*) were detected in several bird species, but high parasitemia infections were recorded only in pigeons, bush fowl, and vultures (Todd and Wolbach 1912).

A parasite survey conducted in Senegal included a total of 809 birds from 43 species, where blood parasite prevalence was low (11.5%) compared to other parts of Africa (e.g., Ghana 32%, n = 135; Uganda 31%, n = 1998; Kenya 25%, n = 377; Tchad 23%, n = 389; Abyssinia 23%, n = 5046); most infections (80%) were of *Haemoproteus* parasites (Bennett et al. 1978; see Bennett et al. 1992a for sub-Saharan birds, where *Haemoproteus* parasites were also the most common [19%], followed by *Leucocytozoon* [8.3%] and *Plasmodium* species [3.5%]). There was similar avian haemosporidians prevalence across Africa considering an east-west comparison, with the exception of Tanzania and Zaire, where prevalence was higher (47% and 72.2%, respectively) compared to most other countries, particularly for the genera *Plasmodium* and *Leucocytozoon* (Bennett and Herman 1976; Bennett et al. 1978). Moreover, prevalence seasonality in Senegal was clearly defined, with highest infection rate from December to May (10–17%) and lowest from June to November (0–9%). This result was unexpected given that the rainy season is from July to September (higher prevalence was recorded during the rainy season in Uganda; Bennett et al. 1974, 1977) and those are favorable conditions for the development of insect vectors (Bennett et al. 1978). A study conducted in eastern Africa (Kenya, Tanzania, Zaire) surveyed 647 birds from 146 species, showing a 37.2% of

total hematozoan prevalence in either single or multiple infections; *Haemoproteus* parasites were the most prevalent (16%), followed by *Leucocytozoon* (14%) and *Plasmodium* species (5.4%), whereas other parasite groups such as *Trypanosoma* sp. and microfilariae of filariid nematodes were also identified (Bennett and Herman 1976). Some years later, Peirce (1984a) recorded the same prevalence pattern in Zambian birds, with *Haemoproteus* and *Leucocytozoon* as the most common parasites. In these comparative studies, the highest haemosporidian prevalence was recorded in Zaire (72%) and the lowest in Kenya (18.3%); in addition, a variation in prevalence across bird families was also found (Bennett and Herman 1976; see also Peirce 1976). The first avian hematozoan survey ($n = 331$) conducted in Cameroon recorded an overall prevalence of 17%, being the most common *Haemoproteus* parasites (11%), followed by *Leucocytozoon* (3%) and *Plasmodium* spp. (2%) (Kirkpatrick and Smith 1988). These authors found low parasitemias (<0.05% in 80% of infected birds), being the highest one for a *Haemoproteus* infection of 0.65%, and 0.7% for a *Plasmodium* infection (Kirkpatrick and Smith 1988). There was a clear tendency for a higher prevalence in colonial compared to solitary bird species, which might be explained by a density effect that provides easier access to blood meals for vectors (Kirkpatrick and Smith 1988).

The first and so far the only major survey of blood parasites in African vultures was conducted in South Africa, where blood samples from 506 individuals of five species of vultures were analyzed. Authors described a new parasite species (*Haemoproteus janovyi*) from the White-backed Vulture (*Gyps africanus*) (Greiner and Mundy 1979; Table 1.1). Overall parasite prevalence was 35.2% for *H. janovyi*, 0.8% for *Leucocytozoon todii*, and 0.6% for *Plasmodium fallax*. There was also a spatiotemporal variation of prevalence. Prevalence varied with bird age, where only 2 out of 133 nestlings were infected with *L. todii* during the dry season, whereas prevalence remained higher and stable through the year in adults. Interestingly, no infections were recorded on the only vulture species that nests on cliffs (Cape Griffon [*Gyps coprotheres*]), but the parasites were found in vulture species that use trees for roosting and nesting (Greiner and Mundy 1979).

More recent studies have analyzed haemosporidians infecting rock pigeons (*C. livia*) in Uganda, detecting high infection prevalence of *Haemoproteus* parasites (76.5%, $n = 34$) and lower prevalence of *Plasmodium* infections (29.4%); prevalence was homogeneous across sampling sites for both parasite genera, and authors detected 29% ($n = 10$) of *Haemoproteus*/*Plasmodium* coinfections (Dranzoa et al. 1999). There was a strong correlation between *Haemoproteus* prevalence and louse fly (*Pseudolynchia canariensis*) abundance across sites, suggesting vector availability as a factor for the high-recorded prevalence. However, *Haemoproteus* infections were considered mild because there was no association between infection and pigeons' body condition, which may be due to the fact that they only found low parasitemia chronic infections (Dranzoa et al. 1999). Also, higher prevalence of *H. columbae* (75–80%, $n = 25–30$) was detected in rock pigeons from Botswana. In addition, these pigeons showed high prevalence of *Toxoplasma gondii* (100%) and also were found harboring several species of nematodes (three species) and ectoparasites (two spp.) (Mushi et al. 1999, 2000). Another study on rock pigeons and

speckled pigeons (*Columba guinea*) in Cape Town, South Africa, detected a significantly higher prevalence of *H. columbae* in rock pigeons (73%) than in speckled pigeons (12%), also showing a higher prevalence of infection in female rock pigeons (Earlé and Little 1993a). The mean prevalence of infection were not different between sexes and between years, but it was significantly higher in rock pigeons than in speckled pigeons. These differences might be explained because *C. livia* is forced to nest in crowded conditions during breeding and to re-use nests for consecutive years, which may increase vector abundance (Earlé and Little 1993a).

An extensive study of 2285 birds of 211 species in South Africa detected a *Haemoproteus* infection prevalence of 11% (n = 254), of 10% (n = 234) for *Leucocytozoon* parasites, whereas *Plasmodium* infections were rare (1.25%). The most prevalently infected birds belonged to the families Fringillidae, Columbidae, and Sylviidae; contrary to other geographical areas, the birds with the lowest prevalence belonged to the Turdidae, Falconidae, Emberizidae, and no infections were reported in Meropidae birds (Earlé et al. 1991a). Furthermore, prevalence of parasites belonging to the different haemosporidian genera varied across localities, mostly due to the heterogeneity of rainfalls across space. For instance, *Leucocytozoon* parasites were more prevalent during late summer and early winter in one location, but more prevalent during winter and early summer at another place (Earlé et al. 1991a; see also Earlé et al. 1991b). The first report of *Leucocytozoon smithi* in South Africa came from domestic turkeys held at a backyard flock (Huchzermeyer and Sutherland 1978). This had important animal health implications because *L. smithi* is a parasite known to create high morbidity and mortality in poultry in the United States, and it was most likely imported into Africa via infected turkeys (Huchzermeyer and Sutherland 1978).

Plasmodium juxtanucleare was recorded infecting the South African native grey-winged francolin (*Scleroptila afra*) in the Eastern Cape Province (11.7%, n = 193); there were no infections in juvenile birds (Earlé et al. 1991c). This study extended the geographical range of this malaria parasite specific to birds of subfamily Phasianinae, because it was formerly restricted to birds from America, Asia, and East Africa. Moreover, authors indicated that the African strain seems to be mild compared to the pathogenic strain from South America (Earlé et al. 1991c). Following this idea, some experimental studies in South Africa demonstrated that the same parasite could be of different virulence in different bird species; for example, *Plasmodium circumflexum* was more virulent for helmeted guineafowl (*Numida meleagris*) and domestic turkeys than for domestic chickens (e.g., Huchzermeyer and Van Der Vyver 1991; see also Huchzermeyer 1993). A survey of 28 red-winged francolins (*Scleroptila levaillantii*) and nine grey-winged francolins showed an infection prevalence of 24.3% (n = 9) by *Leucocytozoon macleani*; there was higher overall prevalence of hematozoan parasites (*Leucocytozoon*, microfilaria, and *Trypanosoma*) in red-winged francolins (64.3%) than in grey-winged francolins (11.1%), and the infection prevalence was significantly higher in commercially grazed and routinely burn grasslands than in grassland with less human management (Jansen et al. 1998).

There seems to be a tendency for game birds of the Galliformes to be mostly infected with parasites of the genus *Leucocytozoon*. That contrasts with the general tendency of distribution of *Haemoproteus* infections, which were reported to be more prevalent in other birds in different regions of Africa (Earlé and Little 1993b; Earlé et al. 1992a). Also in South Africa, *Leucocytozoon tawaki*, originally described in New Zealand from the Fiordland crested penguin (*Eudyptes pachyrhynchus*) was recorded for the first time in Africa infecting the jackass penguin (*Spheniscus demersus*), albeit at a low prevalence (0.75%, $n = 400$) (Earlé et al. 1992b). Authors suggested that the low prevalence was due to the lack of black flies (Diptera: Simuliidae) on offshore islands, limiting blood meals to penguins located on the coast (Earlé et al. 1992b). Three species of black flies (Diptera: Simuliidae) have been involved in the transmission of *L. tawaki* in Fiordland crested penguins from New Zealand South Island, *Austrosimulium dumbletoni*, *Austrosimulium australense*, and *Austrosimulim ungulatum*; ookinetes, oocysts, and sporozoites were observed in the three species of vectors (Fallis et al. 1976). Later studies reported avian malaria infections in wild jackass penguins, but the observed prevalence was lower than that found in captive penguins from North America (Graczyk et al. 1995a).

Previous surveys have shown no hematozoan infections in different penguin species from Antarctic and temperate areas (Jones and Shellam 1999; but see Graczyk et al. 1995c). However, avian malaria is usually more prevalent among captive penguins than in free ranging penguins. In addition, haemosporidians can cause high mortality rates in captive penguins, thus constituting a serious threat for the conservation of endemic and endangered species, as it is the case of wild *S. demersus* (Graczyk et al. 1995b). This penguin species has suffered severe population declines due to oil pollution, declining food sources, and infectious diseases among other factors (Graczyk et al. 1995a, b). Factors such as oil contamination made penguins significantly more susceptible to acquire an avian malaria infection (55%) than healthy ones (31%) (Graczyk et al. 1995b). Thus, avian malaria seems to be a health and conservation issue mostly for captive penguins (Brossy 1992) and occasionally for wild penguin species from tropical regions (Jones and Shellam 1999; but see Graczyk et al. 1995c).

1.2.2 Asia and Oceania

Parasite species originally described in one continent have been reported on other continents, but most likely they represent genetic variants of the same morphospecies that have evolved independently (e.g., *Plasmodium relictum*, Hellgren et al. 2015). For instance, *Plasmodium juxtanucleare* was described from chickens in Brazil, but it was later recorded in Mexico and in domestic fowl from different parts of Malaysia (Bennett and Warren 1966; Mota et al. 2000). However, *P. juxtanucleare* strains from Brazil and Mexico were more similar among themselves in terms of morphology and the course of infections, compared to those found in Malaysia that were more similar to strains obtained from chickens in Sri Lanka (formerly

Ceylon) (Bennett and Warren 1966; Manwell 1966; see Gajanana and Naseema 1980 for the first record of *Plasmodium hexamerium* outside the American continent). Also, in eastern Pakistan some widespread Asian birds have been recorded infected with *Plasmodium circumflexum* and *Plasmodium vaughani*, but authors suggest that most likely many of the recorded infections (22 infected out of 262 surveyed birds from 27 species) were either subspecies or different strains based on variations of morphological traits (Laird and Lari 1958).

Chickens have been thoroughly studied in Southeast Asia given their economic and health importance, particularly for parasites of the genus *Leucocytozoon* that are known to produce high mortality rates in poultry (Fujisaki 1983). Due to its pathogenicity, *Leucocytozoon (Akiba) caulleryi* is of special importance in chickens; however, as in the case of other parasites, its virulence has a geographical component, where strains from Japan are more virulent than those from Taiwan and Malaysia (Fujisaki 1983). Furthermore, a serological survey indicated that most sampled chickens (>95%) had been exposed to *L. caulleryi* and successfully recovered; however, subsequent infections and coinfections with other *Leucytozoon* species and with *P. juxtanucleare* in the same hosts suggested that there was no protective cross species immunity (Fujisaki 1983). Thus, from early haemosporidian studies it is clear that different strains (currently usually named genetic lineages) can have differing health effects on hosts, and virulence also depends on host species and whether infection is single or mixed (Hoare 1943; see Palinauskas et al. 2011, 2018; Ilgūnas et al. 2019a, b; for recent examples).

The major survey of avian haemosporidians in Asia included 55,000 birds from 1132 species in more than 500 sites (McClure et al. 1978). More than half of the bird species recorded in these surveys (55%) were infected by at least one hematozoa parasite. Similar to Africa, the most prevalent were haemosporidians of genus *Haemoproteus* (11.3%), followed by *Leucocytozoon* (2.7%) and *Plasmodium* spp. (0.8%) (McClure et al. 1978). Higher prevalence was recorded in Malaysia and Thailand, maybe due to sample sizes as well as more diverse sampled avian assemblages (McClure et al. 1978). Parasites from the *Haemoproteus* genus were commonly found infecting a large array of bird species, in many of which a high prevalence was recorded. For example, in the province of Loei in Thailand, *Haemoproteus* parasites infected 25 different bird species, whereas no *Plasmodium* infections were detected. In addition, it was also common to observe double and triple coinfections in surveyed birds, including one quadruple infection (Coatney et al. 1960). During recent years, it has become apparent that coinfections in wild birds are more common of what was previously believed (e.g., 86% of infected hawfinches [*Coccothraustes coccothraustes*], Valkiūnas et al. 2003; 7% of infected scarlet rosefinches [*Carpodacus erythrinus*], Synek et al. 2013), which makes microscopic examination of blood films an essential diagnostic method for reliable field studies given that molecular PCR protocols underestimate coinfections (Valkiūnas et al. 2006; but see Martínez et al. 2009; see also Chap. 17 for detailed information about coinfections).

One of the early studies conducted in Indian birds clearly stated that the taxonomy of the genus *Haemoproteus* was basically unknown, in such a degree that the

author decided to name new species based on host species identity and even on the differences in staining of parasites because of the strong contrast that was easily observed in coloration (de Mello 1935a). Furthermore, the *Proteosoma* genus was disregarded (this genus name is a synonym of *Plasmodium*) along with the believe that all infections found in different species belonged to *Proteosoma praecox*, particularly because of the lack of experimental infections proving that *Proteosoma* parasites were not actually other species of the genus *Plasmodium* (de Mello 1935a). The same study highlighted uncertainties regarding the genus *Leucocytozoon*, in particular if parasites of different form and infecting different blood cells (i.e., fusiform form infecting erythroblasts, rounded form infecting mononuclear leucocytes) in the same sample belonged to different species (de Mello 1935a, b). A list of the parasites described from this work and their current taxonomic status is provided in Table 1.1.

Surveys conducted from the period of 1973 to 1988 across India comprised a sample of 1242 birds from 342 species. Blood parasites were found infecting 122 bird species (36% of analyzed bird species), with an overall prevalence of 24% (n = 304). The most common parasite species were of *Haemoproteus* (18%, n = 226), followed by *Leucocytozoon* (1.8%) and *Plasmodium* spp. (1.7%); coinfections were recorded in 64 birds of 31 species (21% of the infected birds) (Nandi 1999). From the 11 states surveyed for avian haemosporidians in India, no infections were found in Tripura (likely due to a small sample, n = 18), the lowest was recorded in Orissa (10.5%), and the highest in Goa (32%); only *Haemoproteus* was recorded in all states (except Tripura) (Nandi 1999). However, both bird species and the number of birds sampled differed across studied states, and hence the observed geographical differences in prevalence should be attributed to host difference and sample size rather than geography or habitat diversity. Nandi (1999) also found that the overall prevalence of parasites belonging to different genera significantly varied among birds of different families. In the case of bird families that had a sample size of at least 10 individuals, no infections were recorded in Ardeidae, Charadriidae, Hirundinidae, Psittacidae and Rallidae; the highest prevalence was recorded in Ploceidae (80%) and the lowest in Alcedinidae (4%) (Nandi 1999). An early survey conducted from 1974 to 1977 in West Bengal, India (Calcutta, Nadia, Sagar Island, Darjeeling), recorded an avian haemosporidian prevalence of 30.8% from a total of 418 birds of 77 species (Nandi et al. 1984). Most infections belonged to *Haemoproteus* parasites (27.7%); *Plasmodium* species had 3.5% prevalence, and *Leucocytozoon* spp. were not recorded in the sample. Again, geographical and between-family host differences were found: the highest prevalence was recorded in Nadia (49%), and the most prevalently infected bird species was the widely distributed baya weaver (*Ploceus philippinus*) (Nandi et al. 1984), which may be a highly tolerant bird to haemosporidian infections. Another survey in the Godavari River Basin Adilabad and Warangal districts (Andhra Pradesh) sampled a total of 146 birds of 65 species, recording a prevalence of 20.5% with most infections from the genus *Haemoproteus* (9.5%), followed by *Leucocytozoon* (2%), and no *Plasmodium* infections (Nandi and Mandal 1984). A survey conducted in India within biosphere reserves recorded a blood parasite prevalence of 22.6% from a total of 146 birds of

93 species; infections were recorded only in 27 species. *Haemoproteus* infections were more common (12%) than *Plasmodium* infections (0.7%), and there were differences in prevalence across sampled sites (Mandal et al. 1989). Another study conducted in Calcutta focused on *H. columbae* infecting rock pigeons, observing 100% prevalence and also showing that female gametocytes were more numerous than male ones (Mandal 1990). Comparing parasitemias in two subsequent years, it was found that parasitemias were higher during the Monsoon season (June to August) reaching a peak in July (Mandal 1990, 1991), and then decreasing during November to May (pre- and post-Monsoon) (Mandal 1990). Furthermore, a survey of the vector of *H. columbae*, *Pseudolynchia canariensis*, was conducted in parallel and showed that fly abundance peaked during June to August and was lowest from January to March (Mandal 1991). From 220 dissected louse flies, a 2.5% were found to carry ookinetes of *H. columbae*, and also 1.8% had epimastigotes of *Trypanosoma hannai* (Mandal 1991). One of the most recent surveys of avian haemosporidians in Asia (including India, Myanmar, and South Korea) screened a total of 699 birds and found an overall prevalence of 34% ($n = 241$) (Ishtiaq et al. 2007). Using molecular methods, they identified 34 distinct *Plasmodium* lineages and 41 *Haemoproteus* lineages; parasites of the last genus presented regional clades, while *Plasmodium* did not have any structure either by geographic region or by host (Ishtiaq et al. 2007). Moreover, prevalence varied among regions (highest in India for this study, also with the highest number of unique *Haemoproteus* lineages), but it remained similar across birds of different families regardless of geographic location. Finally, they found no parasite lineage sharing between India and South Korea, most likely because migratory birds from those two regions belong to different migratory flyways (Ishtiaq et al. 2007).

In the state of Victoria, Australia, a survey of avian haemosporidians was conducted in 316 ducks of five species; the two recorded parasites were *Haemoproteus nettionis* and *Plasmodium relictum*, whereas no infections by *Leucocytozoon* parasites were recorded (Bennett et al. 1977). Taxonomic efforts in Australia include those performed in the re-description of some *Haemoproteus* and *Leucocytozoon* parasites infecting the honeyeaters (Aves: Meliphagidae), specifically using blood smears from 173 noisy miners (*Manorina melonocephala*) (Bennett et al. 1994; see Table 1.1). A survey conducted in Queensland for two species of pigeons (rock pigeon and wonga pigeon [*Leucosarcia melanoleuca*]; $n = 40$) found an infection prevalence with *Haemoproteus columbae* of 58% in rock pigeons ($n = 12$), often associated with high parasitemias, and only one *H. columbae* infection in wonga pigeons, which represented the first record of this parasite in wonga pigeons in Australia (Lederer et al. 1999). The second columbid, after *C. livia*, infected with *H. columbae* in Australia was the superb fruit-dove (*Ptilinopus superbus*) (Lederer et al. 1999). Many species of pigeons are widespread around the globe, and *H. columbae* mirrors the distribution of rock pigeons and presents a consistent morphology across the world (Lederer et al. 1999).

Most of the studies from islands and archipelagos of the southern Pacific come from the Hawaiian archipelago, mainly motivated due to extinctions of endemic birds (honeycreepers: Drepanidinae). In addition, more than 75% of Hawaiian

species are either extinct or endangered (e.g., Hawaiian crow [*Corvus hawaiiensis*], Massey et al. 1996), currently undergoing population declines due to habitat destruction and introduced species, particularly the pathogenic avian malaria, which is known to provoke harmful effects on naïve endemic populations (Warner 1968; van Riper et al. 1986; Atkinson et al. 1995). For example, an experimental study on two avian species, the endemic Iiwi (*Vestiaria coccinea*) and the non-native scaly breasted munia (*Lonchura punctulata*), showed high mortality rates of native juvenile Iiwi, reaching even 100% in groups infected with high parasite doses where declines in food consumption and higher anemia were observed (Atkinson et al. 1995). Moreover, survival of birds with low initial body mass was lower compared to heavier birds, and males were more susceptible than females; clinical signs included tissue lesions and enlargement of spleen and liver (Atkinson et al. 1995). Furthermore, nestlings and juvenile endemics are more susceptible to malaria infections compared to adults (van Riper et al. 1994). In contrast, the non-native scaly breasted munia were resistant to infection, which seems to be a trait shared among invasive avian species (e.g., kalij pheasant [*Lophura leucomelanos*], Lewin and Mahrt 1983; see also Gajanana and Naseema 1980; Ilgūnas et al. 2019b) that makes them better competitors than native birds, particularly during years of food shortages (van Riper et al. 1986; Atkinson et al. 1995) (see Chap. 15 for detailed information about biological invasions of avian malaria parasites). This in turn suggests that the main reservoirs of avian malaria are the same endemic Hawaiian birds that seem to have coevolved with an attenuated avian malaria strain at mid-elevations (900–1500 m asl), which is transmitted by the introduced avian malaria vector *Culex quinquefasciatus* (van Riper et al. 1986). Although there seems to be recent adaptation to avian malaria at low elevations by Hawaiian Amakihi (*Chlorodrepanis virens*) (Woodworth et al. 2005), many of the Hawaiian honeycreepers (e.g., Iiwi, Apapane [*Himatione sanguinea*]) remain at high elevations (>1500 m asl) where mosquito vectors are rarer and lower temperatures interrupt malaria development (Feldman et al. 1995; Atkinson et al. 2000; Yorinks and Atkinson 2000). An important consideration for the conservation of Hawaiian endemic birds is the understanding of the avian malaria vector (*Cx. quinquefasciatus*) genetics and adaptation. For example, it has been determined that there is divergence among mosquito populations across islands and within the main island of Hawaii, where population bottlenecks have been detected; in addition, it has been determined that such Hawaiian mosquito populations are the result of multiple introductions (Fonseca et al. 2000). In contrast to the dramatic example of Hawaiian extinctions provoked by avian malaria, a survey of avian hematozoa parasites on Cook Islands found no infections from 55 birds of nine native species and 24 birds from an introduced bird species; this indicated an absence of parasites given that there were plenty of blood sucking Diptera on the islands (Steadman et al. 1990).

A study of blood parasites from 141 birds (45 species) inhabiting montane forests from Papua New Guinea showed a prevalence of 32.6% (n = 46) for *Haemoproteus* parasites and of 3.5% (n = 5) for *Leucocytozoon fringillinarum* (Jones 1985). Most of the infections were of low parasitemia (<0.001%), and those with higher parasitemia (>0.004%) were from bird species that had high prevalence

(Jones 1985). There was no association between *Haemoproteus* prevalence and altitude (range: 1400–2360 m asl), a result that can be explained by the sharing of bird species across this elevational range. A similar pattern for *Haemoproteus* infection has been recorded in Colombia (González et al. 2014); an elevational effect on prevalence is clearly observed in parasites of the genus *Leucocytozoon*, which can be due to the availability of suitable Simuliidae vectors (e.g., González et al. 2014). Another survey on captive birds of paradise in Papua New Guinea revealed that *Haemoproteus* were the most prevalent parasites (20%), followed by *Plasmodium* (10.2%) and *Leucocytozoon* spp. (6.7%); also, other blood parasites such as microfilariae of filariid nematodes and *Trypanosoma* spp. were recorded (Varghese 1987). From 1982 to 1987, an evolutionary study analyzing sexual selection using ten species of birds of paradise was conducted in the Mount Missim in Papua New Guinea (Pruett-Jones et al. 1990). They found a positive correlation between parasitemia and plumage showiness of male birds across species, and infections were also associated with the degree of sexual dimorphism (Pruett-Jones et al. 1990). When the mating system was promiscuous, species had higher parasite prevalence and males were gaudier than monogamous species; interestingly, males with high parasitemias were not successful at mating (Pruett-Jones et al. 1990).

A study on parasite effects on birds' health (health measured in terms of fluctuating asymmetry, that is the morphometric difference between the right and left sides of, for example, wing and tarsus length) was conducted in Singapore using rock pigeons that were parasitized by two species of chewing lice and by *H. columbae* (Quek et al. 1999). Unexpectedly, no associations were found between parasitemia and fluctuating asymmetry measured from wing, tail, tarsus, and third digit; such a result might be explained due to the low pathogenicity of the examined parasite species, particularly from chewing lice that mostly feed on feathers and skin debris (Quek et al. 1999). An alternative explanation may be related to bird life history traits, such as incubation period that is negatively associated with hematozoan prevalence, probably indicating better immune system maturation during longer embryonic development (Ricklefs 1992), and in the case of Columbidae the feeding of chicks with crop milk that is rich in proteins, fat, antioxidants, immune-enhancing factors, and also antibodies (Gillespie et al. 2012).

In reference to vector research, one of the first studies, analyzing blood meal sources of mosquitoes in Australia, analyzed 1400 samples using the precipitin test on engorged insects (Lee et al. 1954). Sampling was conducted in human dwellings, farms, stables, kennels, and pens, also in habitats surrounding farms, river flats, and creeks. Authors recorded a total of 15 Culicidae species, but the dominant species were *Anopheles annulipes* (feeding mostly on cattle and rabbits), *Culex fatigans* (feeding on humans in the domestic area, on fowl in pens, horses in stables, and dogs in kennels), and *Culex annulirostris* (feeds on humans and most domestic animals, frequently on rabbits). They also found a strong association between collection site and blood source; for example, mosquitoes detected in rivers and creeks were attracted to rabbits (Lee et al. 1954). Another study on mosquito host selection patterns was conducted in Pakistan, showing that all of the 18 species studied, with the exception of *Culex fatigans* (38% blood meals were from humans), were

zoophilic, and human blood meals were rare (range 0.5–14%) (Reisen and Boreham 1979). Researchers did not detect spatial changes in host selection, except for *Cx. fatigans* whose meals depended on the domestic area used in the farm (more human blood meals inside the house and more domestic animal meals at the barn and stables). Moreover, the interaction between host and season was also an important feeding pattern for mosquitoes' preferences, mostly on birds and cows during winter, more on man and cows in spring, and on humans and birds during the summer (Reisen and Boreham 1979). A survey of biting midges (Diptera: Ceratopogonidae) conducted in chicken farms from the Philippine Islands collected a total of 10,067 insects of 17 *Culicoides* species; researchers suggested that the most abundant species (i.e., *Culicoides effuses*, *Culicoides peregrinus*, *Culicoides palpifer*) are potential vectors of *Leucocytozoon caulleryi*, a parasite previously recorded in chickens in the same farms (Abella et al. 1994). In Malaysia, a mosquito (Diptera: Culicidae) survey was conducted in 1981, recording 37 species, 55% of them belonging to the genus *Mansonia* (Cheong et al. 1984). *Anopheles balabacensis* represented a new record for the area in this study (Cheong et al. 1984). Also, sporozoites were found in *Mansonia bonneae* and *An. balabacensis*, and both species had high parous rates and high survival probability, which might imply them as vectors in the transmission of malaria and filariasis (Cheong et al. 1984). In Papua New Guinea, a study was conducted on the islands of Wuvulu and Maron from 1975 to 1978, recording eight mosquito species and investigating their medical relevance in relation to transmission of human malaria, dengue, and filariasis (Rodhain et al. 1980).

1.2.3 America

The first study in the region was that of Aragão (1908), where the sporogonic development of *H. columbae* in hippoboscid flies was discovered and experimentally confirmed; it was the first study demonstrating the involvement of Diptera species (in this case a louse fly) in the life cycle of a haemosporidian parasite from a genus other than *Plasmodium* (see Cox 2010; Santiago-Alarcon et al. 2012a for a historical account). White et al. (1978) conducted the first synthesis of avian haemosporidian research in the Neotropical region, summarizing information on 35,555 birds belonging to 955 species of 80 families. Sixty percent of records belonged to domestic and introduced species (e.g., chickens, *Gallus gallus*; house sparrows, *Passer domesticus*), which had an overall combined hematozoa prevalence of 5.6%. The total prevalence recorded for the region was 10.5% (n = 3743 infections) and increased up to 19.1% when excluding Nearctic migrants. *Haemoproteus* infections represented 7.4% and *Plasmodium* 1.9%; in contrast, they found that only 0.2% of examined birds were infected with *Leucocytozoon,* thus concluding that *Leucocytozoon* species are transmitted to birds in the Neotropics at a very low rate, resulting in low prevalence (White et al. 1978). But the suggestion that *Leucocytozoon* infections are rare in the Neotropical region could be partially based on a markedly spatially dependent distribution of these parasites (e.g., high elevation

environments). The parasites of the genus *Leucocytozoon* have been historically partly undersampled, with scarce information on prevalence and host distribution (Fecchio et al. 2018; Galen et al. 2018). Recently *Leucocytozoon* parasites have been detected in high prevalence mainly at high elevations in Neotropical mountains (e.g., González et al. 2014), and some morphologically and genetically unique new *Leucocytozoon* species have been described (e.g., Lotta et al. 2015; Matta et al. 2014). Furthermore, a recent study reports the presence of *Leucocytozoon* parasites in lowland areas of the Amazonia infecting blue-crowned manakins (*Lepidothrix coronata*), being all infections represented by the novel *Leucocytozoon* lineage LEPCOR08 (Fecchio et al. 2018); but further study is required to confirm if this represents a novel introduction to the region, since leucocytozoids are not characteristic of Amazonian birds. Moreover, another study has recently explored the prevalence and lineage diversity of *Leucocytozoon* parasites across a latitudinal gradient in 69 bird communities from Alaska to Patagonia, revealing that both the prevalence and genetic diversity of *Leucocytozoon* decreased toward the equator (Fecchio et al. 2019). By the year 1978, there was a total of 75 studies on avian haemosporidians, most of them concentrated in Brazil ($n = 25$), Venezuela ($n = 15$), Mexico ($n = 14$), and Argentina ($n = 12$). Thus, further studies are needed in the region before solid conclusions can be drawn in relation to haemosporidian diversity and before valid comparisons with other biogeographical regions are performed.

From the early studies in the neotropics is clear that haemosporidian parasite prevalence was different across birds of different avian families. For example, higher prevalence was detected in Columbidae, Fringillidae, and Turdidae compared to Trochilidae, Charadriidae, and Scolpacidae (White et al. 1978). Moreover, prevalence was also variable across species within a single bird family; such is the case of the Fringillidae, where the house finch (*Haemorhous mexicanus*) presented 56.4% haemosporidian prevalence compared to zero infections for grassland yellow finches (*Sicalis luteola*) (White et al. 1978). Such heterogeneity in infection prevalence across bird families and species has been confirmed in recent studies, where environmental (e.g., proximity to water sources, local vegetation structure) and biotic factors (e.g., insect vector abundance, bird host size and abundance) have a large influence on infection risk (e.g., Knowles et al. 2011, 2014; Renner et al. 2016; Hernández-Lara et al. 2017; Santiago-Alarcon et al. 2012b, 2018, 2019). Thus, generalizations currently are not feasible. However, it is worth to continue studying the variability of the same host-vector-parasite system across different local communities, because as deeper knowledge is gained on the natural history of avian haemosporidians, researchers will be able to extract common patterns away from local contingencies (Schmitz 2010).

Other studies in the region were conducted during the 1980s and early 1990s (see Santiago-Alarcon and Carbó-Ramírez 2015 for a synthesis in Spanish). For example, one study from Chile reported haemosporidian, *Trypanosoma*, and microfilarids prevalence of 14% ($n = 91$) from 23 bird species; the most common were parasites of genus *Haemoproteus* with 10 reported infections (Forrester et al. 1977). A second survey for avian haemosporidians in Chile was conducted in urban rock

pigeons from Santiago, but no Haemosporida infections were detected (Saucedo et al. 1999).

Approximately, 12 morphospecies of avian Haemosporida have been recorded in Jamaican birds: 7 from *Haemoproteus*, 3 from *Plasmodium*, and 2 from *Leucocytozoon* (Bennett et al. 1980). A broad study analyzing a total of 1791 birds from 80 species found an overall prevalence of 7.4% ($n = 133$) for all blood hematozoan groups (including *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, microfilaria, *Atoxoplasma*, *Trypanosoma*). In order of frequency of occurrence of haemosporidians, *Haemoproteus* was the most common parasite (4.2% of prevalence), followed by *Plasmodium* (1.4%) and *Leucocytozoon* (0.2%), with the particularity that *Leucocytozoon* parasites were only found in migratory birds (Bennett et al. 1980). A previous study exploring the distribution of avian hematozoa in North America found that from 182 overwintering Nearctic migrants, only 5.5% ($n = 10$) were infected by hematozoans, which contrasts to the high prevalence observed in Nearctic areas (36.9%) (Greiner et al. 1975). Such results suggested that there is little haemosporidian exchange between migrant and resident birds, where parasites are rather acquired during the breeding season and not during migration (Greiner et al. 1975; White et al. 1978; Bennett et al. 1980). Recent studies support this view, but the picture is more heterogeneous. On the one hand, the European-African migratory route shows high degree of conservatism, where there is little viable parasite exchange between resident and migrant birds (i.e., many European bids are commonly infected with *Haemoproteus* and *Plasmodium* parasites in Africa, but many of those parasites are not transmitted at breeding grounds in Europe; Valkiūnas and Iezhova 1990; Hellgren et al. 2007; García-Longoria et al. 2015; Ricklefs et al. 2017). On the other hand, the North-South American migration route is less restrictive and has a higher degree of haemosporidian exchange between resident and migrant species, which also depends on the phylogenetic relatedness of the migrant and resident bird species (Ricklefs et al. 2017).

A low overall prevalence has been recorded in other neotropical countries such as Brazil (8%, $n = 15{,}574$ birds from 266 species) and Bolivia (5.1%, $n = 641$ birds from 135 species), where parasites of the genus *Haemoproteus* were the most common in both countries (Woodworth-Lynas et al. 1989; Bennett et al. 1991a). A study in Sao Paulo, Brazil, examined a total of 3449 birds from 195 species across 6 years (1967–1972), finding a total hematozoan (i.e., haemosporidians, *Trypanosoma*, microfilarids) prevalence of 7.8%; the most common were *Haemoproteus* parasites (3.5%), followed by *Plasmodium* (1.8%), and *Leucocytozoon* spp. (0.06%). Interestingly, *Leucocytozoon* parasites were recorded only in two Nearctic migrants. Although there were no prevalence differences across years, the lowest prevalence was recorded during the months of February and June (Bennett and de Souza Lopes 1980). Moreover, it was mentioned that coinfections were rare in South America (<10% of infected birds) compared to birds in North America, where approximately 30% of infected birds had more than one blood parasite species of the same or different genera (Bennett and de Souza Lopes 1980).

In Central America, higher prevalence has been recorded in Panama (11%) and Costa Rica (18%) than in El Salvador (4.9%, $n = 246$ birds from 49 species), being

the parasites of genus *Haemoproteus* the most commonly found in all localities (Winchell 1978; Sousa and Herman 1982; Young et al. 1993). The same pattern was observed in northern Mexico, where 12.8% prevalence (n = 196) of blood parasites (i.e., haemosporidians, *Trypanosoma*, microfilarids) was recorded, and *Haemoproteus* parasites represented about half of the reported infections, whereas *Leucocytozoon simondi* was also recorded in ducks (Bennett et al. 1991b). Interestingly, there was no difference in prevalence (excluding ducks) when compared with a sample from 50 years earlier (25% prevalence, n = 102; Beltrán 1942), despite being drastic land use change to agriculture in Mexico (Bennett et al. 1991b).

In Venezuela, several species of Ciconiiformes had high prevalence at the nestling stage by *Plasmodium* parasites (>50%), which contrast with a low 5.8% prevalence in adults (Gabaldon and Ulloa 1980). In addition, the high prevalence in nestlings was characterized by coinfections of species of different *Plasmodium* subgenera (highest parasitemias were seen in species of subgenus *Giovannolaia*), which according to authors can lead to parasite hybridization in vectors and complicate morphological identification of species and even subgenera (Gabaldon and Ulloa 1980; see Valkiūnas et al. 2008b, 2013 for recent hybridization experiments among avian haemosporidians). The reported differences in prevalence of infection between nestlings and adult individuals could be due to the higher pathogenicity of haemosporidians on immune naïve nestlings (Valkiūnas 2005), or a higher mortality associated to coinfections (Marzal et al. 2008). Authors also suggested that detected parasites were native to the Llanos (flatlands) from Venezuela due to high densities of the mosquito *Aedeomyia squamipennis* and sporozoites found in salivary glands of this insect, leading to almost 100% prevalence at the fledgling stage (Gabaldon and Ulloa 1980). In addition, a new haemosporidian species, *Fallisia (Plasmodioides) neotropicalis*, was described in the Llanos region of Venezuela, which is currently the only species from the *Fallisia* genus and *Plasmodioides* subgenus infecting birds (Gabaldon et al. 1985) (Table 1.1). Although this parasite was described from domestic pigeons (*Columba livia*), authors suggested that those are rather incidental infections and that the natural bird hosts are several species from the families Ardeidae, Ciconiidae, and Threskiornithidae (Gabaldon et al. 1985; see also Gabaldon and Ulloa 1980).

Most of the avian haemosporidian research conducted in neotropical archipelagos is very recent (Apanius et al. 2000; Parker 2018). In Mexico, the first study was conducted by Clark and Swinehart in 1969, however, where authors studied a total of 191 birds from 39 species on islands from the northern part of the Pacific Ocean. They reported low overall prevalence (8.9%, n = 17); among infected birds, the authors recorded *Haemoproteus* (prevalence varied 20–56%), *Plasmodium* (2–6%), *Leucocytozoon* (29–42%), *Trypanosoma* (5–43%), *Hepatozoon* (1–15%) species, and microfilariae of filariid nematodes (1–39%). In the Caribbean, a study of avian haemosporidians using microscopic examination was conducted in the Lesser Antilles (St. Lucia, Martinique, and Dominica; Apanius et al. 2000). Prevalence of *Haemoproteus* parasites was 34% in 370 birds of six species (Apanius et al. 2000). Authors detected an interaction effect between island and bird species, indicating that infection dynamics are different across islands for the same host species. These

differences may be due to independent evolution of the host-parasite interaction across islands, despite lack of genetic divergence between island populations of bird hosts (Apanius et al. 2000). Since the study of Apanius et al. (2000), the number of investigations on bird-haemosporidian interactions has increased on Caribbean islands mainly from the work of R.E. Ricklefs group (e.g., Ricklefs and Fallon 2002; Ricklefs et al. 2004, 2017; Ricklefs and Outlaw 2010). The Galapagos Archipelago is the only group of islands in the Pacific that has its entire native avian species; the possible exception is the San Cristobal vermilion flycatcher (*Pyrocephalus rubinus dubius*) (Parker 2018). But some of these species are suffering population declines because of introduced predators and pathogens, and also due to regular El Niño phenomenon that depletes ocean food supplies impacting coastal birds, in particular the endemic Galapagos penguin (*Spheniscus mendiculus*) and flightless cormorant (*Phalacrocorax harrisi*) (e.g., Santiago-Alarcon and Merkel 2018). There are only three parasitological studies conducted on birds from the Galapagos Archipelago before the year 2000. The first one reported infections with filariid microfilariae in flightless cormorants and Galapagos penguins, along with infections with viruses, bacteria, and ectoparasites from both endemic and introduced birds (Harmon et al. 1985). A second study aimed to search for *Trichomonas gallinae* in introduced rock pigeons on human inhabited islands, in which case they found several positive pigeons; fortunately, this parasite has not been found yet in endemic Galapagos doves (*Zenaida galapagoensis*) (Harmon et al. 1987; Padilla et al. 2004). The third study described a new coccidian parasite (*Eimeria palumbi*) from a sample of an infected Galapagos dove (McQuistion 1991). Starting in the year 2001, the number of studies on bird-parasite interactions from the Galapagos fruitfully increased, along with studies on the Diptera insects involved in the transmission of blood parasites and infestation of nests (e.g., *Philornis* spp.); all this research was summarized by Parker (2018).

Different bird species of same families and birds of different families are differentially susceptible to avian haemosporidians; for example, high prevalence is commonly recorded in Columbidae, Vireonidae, Fringillidae, and Turdidae across different geographical regions (i.e., North America, Africa, Southeast Asia, South America) (Greiner et al. 1975; Bennett and Herman 1976; McClure et al. 1978; Bennett and de Souza Lopes 1980). Such phylogenetic component to susceptibility would imply that specific species/family factors, such as behavior (nest type, nesting altitude), morphology (body size), and ecology (abundance), are directly related to infection probability (Scheuerlein and Ricklefs 2004; González et al. 2014; Santiago-Alarcon et al. 2016). Also, some ecological factors influencing vector abundance and distribution can explain differences in distribution of haemosporidian infections among bird species. For example, in birds of some ecological groups such as shorebirds, it is usual to observe zero or quite low avian haemosporidian prevalence, particularly in those from marine coastal habitats compared to inland tropical freshwater wetlands. The reasons of such differences are generally attributed to the adverse conditions in marine coastal environments for the reproduction and/or activity of insect vectors (Mendes et al. 2005). In the case of migratory bird species is known that avian haemosporidians (the majority of which are likely low

parasitemia chronic infections) can have negative, positive, or no impacts at all in terms of, for example, body condition, timing of migration, and reproductive success (D'Amico et al. 2008; Arizaga et al. 2009; DeGroote and Rodewald 2010; Santiago-Alarcon et al. 2013). One of the first studies analyzing the effects of avian haemosporidians on migrating birds was conducted on purple martins (*Progne subis*) that were infected with *Haemoproteus prognei* (synonym of *Haemoproteus hirundinis*) (Davindar and Morton 1993). It was found that yearlings before their first migration had a lower infection prevalence compared to adults. Across a period of 3 years, many birds became infected and kept chronic infections throughout life. However, infection had no apparent effect on the returned frequency from wintering grounds, actually infected adults normally arrived earlier to breeding grounds compared to uninfected ones (Davindar and Morton 1993). Furthermore, there was no difference in clutch size between infected and uninfected birds, and infected females had higher breeding success than uninfected females (Davindar and Morton 1993). Later, experimental and observational studies have revealed detrimental effects of avian haemosporidians in different life history traits of migratory birds such as reproductive success (Marzal et al. 2005), body condition (Marzal et al. 2008), and survival (Marzal et al. 2016; see Chap. 16 for a more thorough discussion in relation to parasitism and migration ecology).

In the case of vector research, one of the first blood-sucking Diptera insect surveys in Brazil was focused on the sandflies (Diptera: Psychodidae) due to their human health importance, particularly in the transmission cycle of leishmaniasis (Lainson et al. 1976). An important result from this study was the effect of seasonality on fly species composition, where anthropophilic species were absent or rare during the dry season, but highly abundant during the rainy season when highest prevalence of leishmaniasis and malaria was recorded (Lainson et al. 1976). Another survey in Brazil found that *Culex saltanensis* was the most commonly captured mosquito (42% of the total 905 captured individuals) using humans and chickens as bait; it was highly ornithophilic (90% were captured on chickens and 10% on people) (Lourenço de Oliveira and de Castro 1991). The sporozoites retrieved from *Cx. saltanensis* (sporozoites were found in the haemocoele of one mosquito) produced infections of *P. juxtanucleare* with detectable gametocytes 41 days post infection. This study represented the first report of a natural vector for *P. juxtanucleare* outside of Asia (Lourenço de Oliveira and de Castro 1991). Although not strictly located in the tropics, important research was conducted in Florida (a subtropical region of North America) on poultry haemosporidians and their vectors. In particular, the life cycle of *Haemoproteus meleagridis* infecting wild turkeys was studied in detail, identifying several species of *Culicoides* (most importantly *Culicoides edeni*) where the parasite was able to complete sporogony (Atkinson et al. 1983; Atkinson 1988). An important outcome of the vector studies in Florida was the vertical sampling of insects given that their abundances and composition change from the lower vegetation strata up to the canopy (Garvin and Greiner 2003; Atkinson 1991). These studies in North America lead to the suggestion that vector abundance parallels the natural transmission of avian haemosporidian parasites (a pattern that has been

corroborated in recent studies), detecting seasonal peaks in northern temperate areas, whereas there is a year-round transmission in subtropical Florida (Atkinson 1991).

1.3 Conclusions and Future Directions

The last century witnessed an enormous increase in the taxonomic work on avian haemosporidians across tropical regions. In some countries (e.g., India, South Africa, Zambia, Brazil, Venezuela, Mexico) large surveys were conducted, and parasites of the genus *Haemoproteus* were the most prevalent at every surveyed location. Our global analysis also highlighted that there were a few publications or even no research on avian haemosporidians in many tropical countries up to the year 2000 (e.g., Bolivia, Paraguay, Nicaragua, Benin, Angola, Ethiopia, Laos, Sri Lanka; Fig. 1.3). Such a pattern has not changed much during the last 20 years; some exceptions include the large amount of information coming out from the Galapagos and Caribbean Islands, from Australia and New Zealand, as well as recent investigations from Madagascar and other smaller islands across the Indic and Pacific Oceans. Furthermore, the good news is that much more research has accumulated on the Diptera vectors transmitting haemosporidians during the last 20 years (see Chap. 6 for a review), which is opening new opportunities for the understanding of the ecology and evolution of these antagonistic interactions.

Prevalence of infection is markedly dependent on sample size and bird composition, which was usually different in the majority of studied sites, complicating comparisons among different locations and regions. Therefore, it would be valuable to study both prevalence and parasitemia of *Haemoproteus*, *Plasmodium,* and *Leucocytozoon* parasites in the same host species across different geographical locations (and countries), making sure to compare samples obtained during the same season and from similar host age classes. Such a detail approach will certainly help in the identification of patterns (e.g., Hernández-Lara et al. 2017). Finally, care should be taken to always compare similar sample sizes across locations, and when that is not possible, it is important to conduct statistical corrections by taking into account sample size.

Although molecular techniques have helped greatly in the discovery of avian haemosporidian genetic diversity, the field is starting to suffer from the lack of taxonomic expertise; there are less and less researchers that have the skills or are interested in classical taxonomy. For the understanding of wildlife haemosporidian diversity across the world, it is essential to acquire microscopic skills and combine them with innovative knowledge, which is provided by current molecular tools. Along with these two approaches, the current availability of bird species distribution maps can help to understand the biogeographical history of different tropical regions, identifying historically unique diversity hotspots (e.g., Prieto-Torres et al. 2018), which can reveal areas where we can potentially find haemosporidian diversity cradles. Given the current rate of habitat transformation and destruction in the

Anthropocene, where extinction rates have never been greatest, the identification of such areas is an urgent need. This is particularly true due to recent discoveries about the impact of haemosporidian parasites in the health of wild birds (Galosi et al. 2019; Groff et al. 2019; Ortiz-Catedral et al. 2019). Haemosporidian genetic lineages are allowing us to conduct evolutionary and ecological research that was out of reach just a few decades ago; however, the issue with leaving behind classical taxonomy resides in that without using both approaches in tandem, we are never sure if we are working with one species or a group of cryptic species. Additionally, it is difficult to determine competent avian hosts and distinguish abortive haemosporidians infections, which are the dead ends for transmission, without observation of gametocytes circulating in peripheral blood (Ortiz-Catedral et al. 2019). Currently used molecular diagnostic tools are insufficiently sensitive for that. Thus, we need to use both methodologies (microscopy and molecules), and sometimes serology is also recommended for diagnostics of latent infections when possible (see Chap. 2), along with novel approaches (e.g., historical biogeography of vertebrate hosts; Prieto-Torres et al. 2018, 2019) to stretch our limited economic resources in order to discover and understand the immense diversity of wildlife haemosporidians across the world.

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Chapter 2
Introduction to Life Cycles, Taxonomy, Distribution, and Basic Research Techniques

Gediminas Valkiūnas and Carter T. Atkinson

Abstract Avian haemosporidian parasites are a closely related group of apicomplexan parasites with important similarities in their life cycles, development, physiology, and reproduction. Current phylogenies based on mitochondrial and nuclear genes reflect more traditional attempts to classify these organisms based on life history characteristics and morphology, but limited sampling from poorly characterized taxa such as the Garniidae from tropical and subtropical regions continues to limit our understanding of their phylogeny and evolution. Recent advances in molecular diagnostics and the ability to barcode these parasites using mitochondrial cytochrome b sequences have revolutionized the field, but traditional methodology based on microscopy of Giemsa-stained blood smears remains essential for diagnostics and understanding life history characteristics and biodiversity of these organisms. The relative strengths and weaknesses of current methods in wildlife haemosporidian research are discussed. We call for a combination of microscopic, PCR-based, and serological diagnostic methodologies for better estimates of true distribution and other aspects of biology of haemosporidians, particularly in studies on virulence, prevalence, and biodiversity.

Keywords Barcoding · Classification · Distribution · Life cycle · Methods of investigation

G. Valkiūnas (✉)
Nature Research Centre, Vilnius, Lithuania
e-mail: gediminas.valkiunas@gamtc.lt

C. T. Atkinson
U. S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, HI, USA

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2.1 Introduction to the Life Cycles of Avian Haemosporidian Parasites

The life cycles of haemosporidian parasites or haemosporidians (Haemosporida) are obligately heteroxenous (Fig. 2.1), with parts that occur within blood-sucking dipteran vectors and parts that occur within vertebrates (Atkinson et al. 2008). All groups of vertebrates (with a rare probability in fish and amphibians) might be infected with haemosporidians after exposure to the bites of infective vectors. Following infection, the parasites may either complete their life cycle or abort their development, depending on how well adapted they are to their hosts. Haemosporidians alternate between vertebrate and invertebrate hosts and differ in mode of multiplication and even patterns of metabolism at different stages of their life cycle. The following developmental stages and their sequence of occurrence are found in all groups of avian haemosporidians.

Infective stages are necessary for the successful completion of haemosporidian life cycles. These stages include gametocytes (Fig. 2.1j) in birds and sporozoites (Fig. 2.1e) in arthropod vectors. However, abortive development is possible in blood-sucking insects and vertebrates if the parasites reach either nonsusceptible or

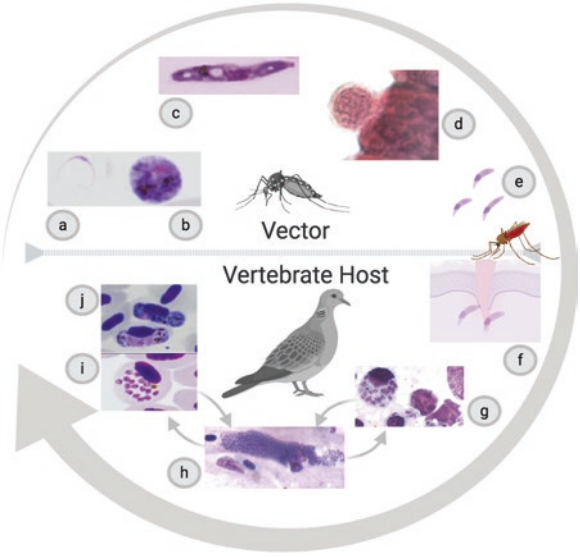

Fig. 2.1 General representation of life cycle of avian haemosporidian parasites: (**a**) microgamete, (**b**) macrogamete, (**c**) ookinete, (**d**) oocyst, (**e**) sporozoites in salivary glands of vector, (**f**) sporozoite in avian host, (**g**) first generation of exoerythrocytic meronts, (**h**) second generation of exoerythrocytic meronts or phanerozoites, (**i**) erythrocytic meront, (**j**) macrogametocyte (top), and microgametocyte (bottom). Double arrows (**i, h**) indicate possible reverse development when merozoites from exoerythrocytic meronts (**h**) or erythrocytic meronts (**i**) initiate secondary exoerythrocytic development resulting in occurrence of phanerozoites

partially susceptible hosts (Valkiūnas et al. 2013a, 2013b; Moens et al. 2016; Ortiz-Catedral et al. 2019). As a result, the presence of parasite DNA and positive PCR amplification of that DNA does not always indicate that the host is fully susceptible and capable of supporting development of infective stages that can reach a new host. Observations of invasive stages in vectors (sporozoites) and birds (gametocytes) are essential for demonstrating that the life cycle can be completed. According to current knowledge, only species of blood-sucking dipteran insects (Diptera) are involved in haemosporidian parasite transmission (Table 2.1).

In vectors, both sexual processes (gametogenesis and fertilization) and asexual multiplication occur (Fig. 2.1a–d). Development of gametes (Fig. 2.1a,b), fertilization, and meiosis occur only in vectors, and they are considered to be final or definitive hosts as a result. Sporozoites are formed in oocysts (Fig. 2.1d) by means of mitotic (agamous) division. Sporogony and development of sporozoites (Fig. 2.1e) are the final, cumulative stage of development in vectors. Vertebrates are intermediate hosts, in which only asexual multiplication occurs (Fig. 2.1g–i), finally leading to development of intracellular gametocytes (Fig. 2.1j) that are essential for parasite transmission to vectors. It is important to note that haemosporidians exist and multiply exclusively outside of host cells in arthropod vectors, but multiplication and growth occur only inside host cells in vertebrates. Due to direct exposure to oxygen in vectors, the mitochondrial oxidative phosphorylation metabolic pathway predominates during development in arthropods. However, glycolysis is the main metabolic pathway during development of haemosporidians in vertebrates due to abundance of glucose and lack of direct exposure to oxygen (Hall et al. 2005; Jacot et al. 2016). This might explain why parasite phylogenies based on mitochondrial genes are highly informative for determining associations among haemosporidians and their arthropod vectors, but might be less valuable for understanding evolutionary relationships among Haemosporida parasites in general (Borner et al. 2016; Bukauskaitė et al. 2018; Pacheco et al. 2018; Toscani Field et al. 2018; see Chaps. 3 and 4 for an introduction to phylogenetic and systematic methods and a review of current molecular methods applied to avian haemosporidians).

Schizogony is a form of mitotic, asexual multiplication that leads to production of numerous genetically identical cells in both vertebrate hosts and vectors. Three types of schizogony are recognized in haemosporidians. First, merogony occurs in tissues (Fig. 2.1g,h) and blood cells (Fig. 2.1i) and leads to production of uninuclear merozoites that are responsible for spread of infection in vertebrates. Second, gametogony or production of microgametes and macrogametes (Fig. 2.1a,b) takes place in the midgut of vectors (gametogenesis). Third, sporogony (Fig. 2.1d) leads to the development of sporozoites (Fig. 2.1e) in vectors. To avoid confusion, it is preferable to specify the type of schizogony (gametogony, sporogony, or merogony) when describing reproduction of these parasites.

Birds become infected with haemosporidians when vectors release viable sporozoites into susceptible avian hosts while taking a blood meal (Fig. 2.1f). The sporozoites initiate merogony in cells of fixed tissues and develop into tissue meronts or exoerythrocytic meronts (Fig. 2.1g,h). This part of the life cycle takes place during

Table 2.1 Main characters of different life cycle stages of the genera (*Plasmodium*) and subgenera (*Haemoproteus, Parahaemoproteus, Leucocytozoon, Akiba,* and *Plasmodioides*) of avian haemosporidian parasites

Character	*Plasmodium*	*Haemoproteus*	*Parahaemoproteus*	*Leucocytozoon*	*Akiba*	*Plasmodioides*
Development in dipteran insects						
Taxonomic status of vectors	Culicidae	Hippoboscidae	Ceratopogonidae	Simuliidae	Ceratopogonidae	Unknown[a]
Rate of complete sporogony (~ 20 C, days)	>10	>10	<10	<10	<10	Unknown
Number of germinative centers in oocyst	Numerous	Numerous	One	One	One	Unknown
Maximum diameter of mature oocyst (μm)	>20	>20	<20	<20	<20	Unknown
Maximum number of sporozoites in oocyst	>100	>100	<100	<100	<100	Unknown
Maximum length of mature sporozoites (μm)	>15	<15	<15	<15	<15	Unknown
Development in avian hosts						
Taxonomic status of avian hosts	Aves	Columbidae, marine Laridae,[b] Fregatidae	Aves	Aves	*Gallus*	*Columba livia*,[c] Ciconiiformes
Merogony in hepatocytes	Absent	Absent	Absent	Present	Absent	Unknown
Merogony in cells of hemopoietic system	Present	Absent	Absent	Absent	Absent	Unknown
Thin-walled elongate meronts in brain capillaries	Present	Absent	Absent	Absent	Absent	Present
Megalomeronts	Absent	Present	Present	Present	Present	Unknown
"Central body" in megalomeronts	Absent	Absent	Absent	Present	Absent	Unknown
Extracellular development of megalomeronts	Absent	Absent	Absent	Absent	Present	Unknown
Prominent (more than threefold in comparison to controls) enlargement of host cell nuclei	Absent	Absent	Absent	Present	Absent	Absent
Merogony in blood cells	Present	Absent	Absent	Absent	Absent	Present
Pigment granules (hemozoin) in blood stages	Present	Present	Present	Absent	Absent	Absent

[a]Available limited data indicate that species of Culicidae might be involved in transmission
[b]One species was reported in swallow-tailed gull *Creagrus furcatus* (Levin et al., 2012)
[c]Experimental host

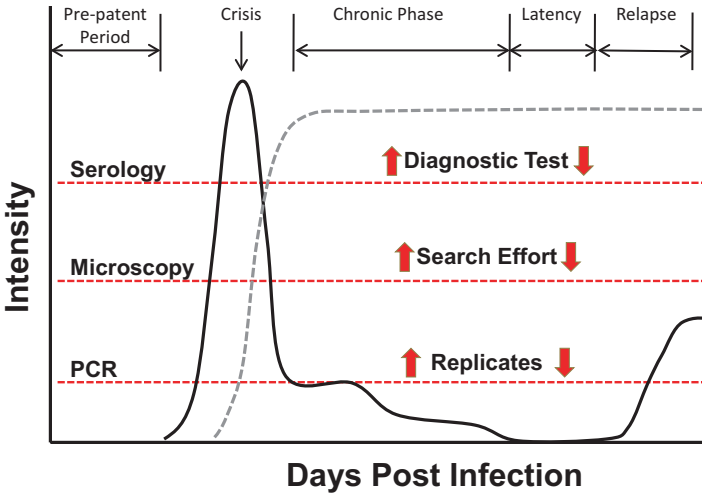

Fig. 2.2 Temporal dynamics (days post infection) and changes in parasitemia (intensity) of erythrocytic infections (solid line) and antibody titer (dashed line). Microscopy, PCR, and serology vary in their ability to detect infections depending on the stage of infection, intensity of parasitemia (solid line), and antibody titer (dashed line). For example, as search effort by microscopy increases, the ability to detect low-intensity infections increases and the limit of detection (red line) shifts to a lower position. Similarly, as the number of PCR replicates increases, the ability to detect low-intensity infections increases and the limit of detection (red line) shifts to a lower position. The ability to detect different antibody titers is test dependent, and the limit of detection (red line) will shift up or down depending on methodology. When infections become latent and disappear from the circulation, serology is the only method that can detect evidence of infection. None of the three methods will detect infections during the prepatent period (initial development in organs), before parasites enter circulating erythrocytes

the prepatent period when parasites multiply before they invade blood cells (Fig. 2.2). Several (at least two) generations of exoerythrocytic merogony occur before development of merozoites that are capable of invading blood cells. The first appearance of parasites in blood cells marks the beginning of the patent period of infection. The latter usually can be provisionally classified into the following stages (Fig. 2.2): (a) the acute stage when parasites appear in blood cells and intensity of parasitemia increases, (b) the crisis when the parasitemia reaches a peak, and (c) the chronic phase when parasitemia markedly decreases to intensities that may be barely detectable. In relapsing haemosporidian infections, the chronic phases might be followed by a latent stage when parasites disappear from the circulation, but persist as tissue stages (Fig. 2.2). The latter might initiate additional cycles of development within blood cells and lead to relapses associated with secondary parasitemia in the peripheral circulation.

Once infected with haemosporidians, birds usually remain infected for many years or even for their lifespan. Prolonged infections are important for parasite survival during unfavorable periods for transmission, particularly in temperate

ecosystems where vectors may disappear during significant portions of the year. However, this mode of persistence remains insufficiently understood and might vary in different host–parasite associations. Persistent tissue meronts have been reported in avian haemosporidians, but hypnozoites (sleeping unicellular parasites produced by sporozoites or merozoites) have not yet been reported, but might exist. Preliminary observations indicate that some species of tropical avian malaria parasites might persist due to light and long-lasting erythrocytic merogony (Valkiūnas et al. 2016a, b), as is the case of *Plasmodium malariae* in humans.

Relapses should be distinguished from recrudescences. The latter follows an increase in parasitemia from multiplication of persistent circulating blood stages, while the former originates from persistent exoerythrocytic meronts. Strictly speaking, recrudescences are possible only in haemosporidians that multiply in blood cells (i.e., species of Plasmodiidae and some species of Garniidae). By contrast, relapses are possible from parasites in the families Haemoproteidae and Leucocytozoidae as well as Plasmodiidae when activation of exoerythrocytic merogony can lead to increases in erythrocytic parasitemia.

Uninuclear merozoites (Fig. 2.1h,i) are formed in meronts by means of mitotic (agamous) division. The merozoites are asexual stages responsible for spread of infection within the vertebrate host. After several generations of exoerythrocytic merogony, numerous merozoites develop and the number of identical copies of parasites increases exponentially prior to invasion of erythrocytes. Some merozoites from exoerythrocytic meronts invade blood cells and develop into sexual stages (gametocytes or gamonts), while others continue merogony in the circulating blood cells (only in species of Plasmodiidae and some Garniidae). Gametocytes (Fig. 2.1j) are asexual developmental stages, which possess sexual potency – they produce gametes in vectors. Each macrogametocyte produces one macrogamete, and each microgametocyte normally produces eight microgametes. Macro- and microgametocytes have readily recognizable sexual dimorphic characters under the light microscope (Fig. 2.1j). Sexual dimorphism is the most easily recognizable feature of haemosporidians, which readily distinguishes them from other intracellular blood parasites. In the great majority of haemosporidian species, macrogametocytes can be distinguished from microgametocytes on Giemsa-stained slides by their intensely staining bluish cytoplasm and compact nucleus with distinctive boundaries. By contrast, microgametocytes have a more pinkish cytoplasm and larger, usually poorly defined boundaries of nuclei. The presence of mature gametocytes in the circulation is essential for infection of vectors.

During development of the avian species of *Plasmodium*, merozoites from erythrocytic meronts (Fig. 2.1i) might initiate secondary exoerythrocytic development and produce secondary exoerythrocytic meronts or phanerozoites (Fig. 2.1h). However, this aspect of the life cycle is poorly known, and it remains unclear how commonly this occurs.

Mature gametocytes produce gametes (Fig. 2.1a,b) in the midgut of vectors shortly after they feed on infected birds. Gametocytes rapidly (usually within several minutes) escape from host cells, gametes develop, and the sexual process of the oogamy type occurs where larger, nonmotile female roundish gametes are fertilized

by smaller, motile elongate male gametes. An important stimulus for the production of gametes is the exposure to air, when parasites leave the peripheral circulation during the blood meal and reach the midgut of arthropod vectors. Fertilization occurs extracellularly, and the resulting zygotes develop into worm-like motile ookinetes (Fig. 2.1c). Haemosporidians are haploid at all stages of development, except the zygote stage, which possess a diploid (2n) set of chromosomes. Meiosis occurs in the zygote before initial stages of ookinete development. Each ookinete possesses four haploid nuclear copies, which are covered by a single nuclear envelope. No further nuclear division occurs until ookinetes transform into oocysts.

Ookinetes move toward the epithelial layer of the midgut, migrate through the layer, and round up extracellularly under the basal lamina, giving rise to roundish oocysts (Fig. 2.1d). In all haemosporidians, a capsule-like wall of host origin surrounds the parasite at this stage of development. Nuclear division occurs by means of mitosis. Oocyst differentiation (sporogony) terminates by the development of numerous uninuclear elongate sporozoites (Fig. 2.1e), which are released into the haemocoele and are distributed throughout the vector body. Some of them reach the salivary glands of the vector, where they complete maturation. The sporozoite is an essential developmental stage for natural transmission. They gain entry to new vertebrate hosts when they are injected with the saliva via the mouth parts during the blood meal (see Chap. 5 for the taxonomy, systematics and biology of Diptera vectors).

General characteristics of the life cycle are similar in haemosporidian parasites belonging to different families and genera, but there are some differences. The main life cycle characteristics are summarized in Table 2.1.

2.2 Taxonomy and Classification of Avian Haemosporidians

The taxonomic position of haemosporidian parasites varies among classifications (Ruggiero et al. 2015; Cavalier-Smith 2018). Here, we provide the classification by Valkiūnas (2005) and Krylov and Frolov (2007), with minor modifications. Haemosporidians are protists belonging to the order Haemosporida in the subphylum Sporozoa, which is placed in the phylum Apicomplexa together with the subphylum Dinozoa. Organisms belonging to the phyla Apicomplexa and Ciliophora are placed in the superphylum Alveolata. All members of this superphylum possess a unique character – they are covered by pellicle constructed of a plasmalemma and cortical alveoli in at least one stage of their life cycle (Fig. 2.3). The shape of the cortical alveoli is markedly variable in different groups of Alveolata. In apicomplexan parasites, the alveoli are thin and long, and formerly, they usually were described as an inner interrupted membrane layer (Carter and Graves, 1988; Valkiūnas, 2005; Fig. 2.3).

The following characteristics are common among all groups of Haemosporida: obligate-heteroxenous parasites with merogony in cells of the blood and/or fixed tissues of vertebrate hosts; gametocytes with sexually dimorphic characters develop

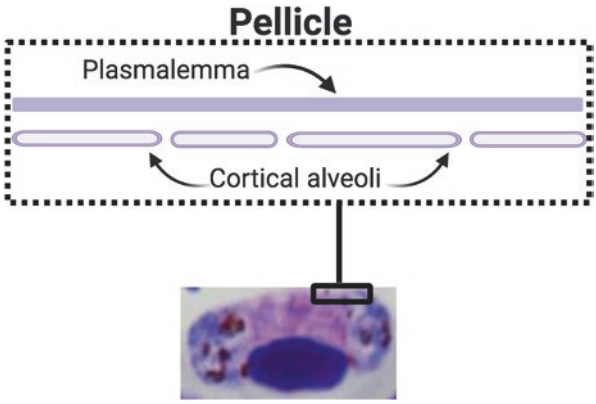

Fig. 2.3 Diagrammatic representation of the structure of pellicle in species of superphylum Alveolata. Plasmalemma is continuous and numerous cortical alveoli are located beneath the plasmalemma (see Valkiūnas 2005 for microstructural details in Haemosporida species)

and locate in blood cells; sexual reproduction and sporogony occur in blood-sucking dipteran insects; gametogenesis occurs outside of cells without formation of syzygy; a microgametocyte produces eight microgametes and a macrogametocyte produces one macrogamete; sexual process of oogamy type; zygote transforms into motile ookinete; oocyst changes in size and does not possess spores; sporozoites present in salivary glands; and infection of vertebrates usually occurs actively by a biting vector (Fig. 2.1). All haemosporidians can be distinguished from other apicomplexan parasites based on ultrastructural organization: the presence of conoid in ookinetes and its absence in sporozoites (Valkiūnas 2005).

Four families have been traditionally recognized in the order Haemosporida. These are Haemoproteidae, Plasmodiidae, Leucocytozoidae, and Garniidae. Avian haemosporidians have been classified into one separate genus or subgenus in each family. The majority of recent phylogenies based on genetic data support the validity of the Haemoproteidae, Plasmodiidae, and Leucocytozoidae, but relationships between these groups as well as the relationships among their genera and subgenera remain insufficiently understood (Ricklefs and Outlaw 2010; Perkins 2014; Borner et al. 2016; Toscani Field et al. 2018; Pacheco et al. 2018). Regrettably, the species of the family Garniidae as well as other haemosporidians belonging to many genera and subgenera described in reptiles (*Asiamoeba, Ophidiella, Sauramoeba, Saurocytozoon*, and others) remain uncharacterized from the genetic point of view. Limited sampling from these poorly characterized groups and species-rich taxa continues to limit our understanding of the evolution of the major groups of avian haemosporidians.

Subgeneric classification has been traditionally used in the taxonomy of avian haemosporidians (Corradetti et al., 1963), and it is currently worth maintaining. Until more taxonomic data are available, it is preferable to group closely related species into subgenera within families rather than raising the taxonomic rank of subgenera to poorly differentiated genera with questionable or unclear phylogenetic relationships. Subgeneric classification simplifies the usage of binominal nomenclature and identification of parasites, especially when life cycles and phylogenetic relationships remain insufficiently studied. In other words, the application of

subgeneric classification is practical and convenient for the practice of faunistic work. Importantly, it does not create obstacles for current applied and theoretical research since the numerous taxonomic questions that remain (Perkins 2014) are moved from the generic level to the subgeneric level. This has significantly less influence on nomenclature and seems preferable at present. This is a particularly sensitive issue due to necessity to maintain proportional (subgeneric) classification in the entire group of Haemosporida, particularly in the genus *Plasmodium*, which includes human and other mammalian malaria parasites belonging to different widely accepted subgenera.

Bird haemosporidians can be classified into the following way:

Superphylum Alveolata (Cavalier-Smith, 1991)
Phylum Apicomplexa (Levine, 1970)
Subphylum Sporozoa (Leuckart, 1879)
Class Coccidea (Leuckart, 1879)
Order Haemosporida (Danilewsky, 1885)
Family Haemoproteidae Doflein, 1916
Genus *Haemoproteus* Kruse, 1890
Subgenus *Parahaemoproteus* Bennett, Garnham, and Fallis, 1965
Subgenus *Haemoproteus* Kruse, 1890
Family Plasmodiidae Mesnil, 1903
Genus *Plasmodium* Marchiafava and Celli, 1885
Subgenus *Haemamoeba* Grassi and Feletti, 1890
Subgenus *Giovannolaia* Corradetti, Garnham, and Laird, 1963
Subgenus *Novyella* Corradetti, Garnham, and Laird, 1963
Subgenus *Bennettinia* Valkiūnas, 1987
Subgenus *Huffia* Corradetti, Garnham, and Laird, 1963
Family Garniidae Lainson, Landau, and Shaw, 1971
Genus *Fallisia* Lainson, Landau, and Shaw, 1974
Subgenus *Plasmodioides* Gabaldon, Ulloa, and Zerpa, 1985
Family Leucocytozoidae Fallis and Bennett, 1961
Genus *Leucocytozoon* Berestneff, 1904
Subgenus *Leucocytozoon* Berestneff, 1904
Subgenus *Akiba* Bennett, Garnham, and Fallis, 1965

Phylogenies based on more genomic data, particularly genes that are responsible for basic metabolic processes, are needed for better understanding relationships between subgenera and genera of haemosporidians. Phylogenies based on mitochondrial genomes clearly group haemosporidian parasites by groups that parallel classification of their dipteran vectors. However, true evolutionary relationships among haemosporidian groups may be obscured, in part, because of the importance of nonoxidative phosphorylation metabolic pathway (glycolysis) during development within their vertebrate hosts (Hall et al. 2005; Jacot et al. 2016; Bukauskaitė et al. 2018). Accumulation of additional genomic data is needed for better understanding the evolution of haemosporidians.

According to the current classification, the avian haemosporidians can be readily identified and attributed to certain families and genera. Members of the Haemoproteidae and Plasmodiidae do not digest hemoglobin completely when inhabiting red blood cells, resulting in the presence of residual malarial pigment or pigment granules (hemozoin). By contrast, the blood stages of species of Leucocytozoidae and Garniidae do not possess pigment granules. Multiplication in blood cells is a characteristic of species of Plasmodiidae and Garniidae, but not of Haemoproteidae and Leucocytozoidae. Some other differences of avian haemosporidian parasites belonging to different genera are summarized in Table 2.1.

It is important to note that blood stages of haemoproteids belonging to the subgenera *Haemoproteus* and *Parahaemoproteus* are indistinguishable by their gametocytes. Phylogenies based on the cyt b gene place parasites of these subgenera to readily distinguishable clades, simplifying subgeneric identification of these infections when sequence information is available (Ferreira-Junior et al., 2018; Pacheco et al., 2018). Additionally, the described avian haemoproteids belonging to subgenus *Parahaemoproteus* are widespread among major groups of birds, but species of the subgenus *Haemoproteus* are more restricted in their vertebrate host distribution (Table 2.1). This provides some important clues for identifying these parasites when only blood samples are available.

The subgenus *Papernaia* was created for *Novyella*-like avian malaria parasites, whose erythrocytic meronts do not possess globules, structures of nuclear origin and function (Landau et al. 2010). However, recent experimental observations show that malaria parasites, which do not possess globules in their natural hosts, might develop these structures after passage of infected blood through abnormal avian hosts. This indicates that this feature should not be used in the taxonomy of avian *Plasmodium* parasites at the subgeneric level. As a result, *Papernaia* is now considered to be a synonym of the subgenus *Novyella* (Valkiūnas and Iezhova 2018).

2.3 Basic Discoveries on Biology of Avian Haemosporidians in Tropical Regions

After the discovery of avian intracellular blood parasites and the documentation of high prevalence in numerous species of wild birds in the territory of the current Ukraine (Danilewsky 1884, 1889), these pathogens gained much attention as possible model organisms for better understanding human malaria infections. *Haemoproteus columbae*, the widespread parasite of domestic pigeons in countries with warm climates, was among the first described and named avian haemosporidians; it was discovered by Kruse (1890) in southern Italy. The first prominent studies on avian malaria in the tropics were carried out by the military doctor Ronald Ross (1898) in India. He used naturally infected passeriform birds as malaria parasite donors to infect mosquitoes and proved that malaria is a vector-borne disease. The first Nobel Prize in Medicine was awarded for this discovery. Being an

excellent writer and avian malaria research enthusiast, Ronald Ross attracted the attention of researchers in human medicine to the use of avian parasites as models for human malarial infections. Numerous studies on the biodiversity of avian blood parasites were launched, particularly in tropics of South Asia, Africa, and South America. These led to the discovery of many species belonging to genera *Plasmodium, Haemoproteus,* and *Leucocytozoon* in tropical birds (Table 2.2; see also Chap. 1). Early contributions by I. F. de Mello (1916–1937) in India and J. Tendeiro (1947) in West Africa were particularly valuable in early faunistic surveys of avian haemosporidian parasites. Between 1905 and 1953, numerous studies were carried out and their results published (Table 2.2). It was proved that avian haemosporidians are not only prevalent and diverse in countries with warm climates, but also cause diseases of veterinary importance (Hewitt 1940; Mohammed 1958; Garnham 1966; see Chaps. 1 and 17).

After a short period of waning interest in avian haemosporidians (between 1950 and 1970) as rodent and primate models for studies of human malaria became available, renewed interest in the biodiversity of these parasites led to the establishment of the International Centre for Avian Malaria Parasites in 1968 (from 1975, International Reference Centre for Avian Haematozoa, IRCAH) at Memorial University of Newfoundland, Canada. Prof. Marshall Laird launched this center under the aegis of the World Health Organization, and Prof. Gordon F. Bennett headed it for many years, coordinating collection and investigation of numerous blood samples, which arrived from all over the world, particularly from Southern Asia and sub-Saharan Africa. Prominent knowledge of the distribution of avian haemosporidians by host and geographic region was gained through research activities of the center (McClure et al. 1978; Laird 1998; Bennett et al. 1992; see Chap. 1 for further information on tropical research during the twentieth century), and numerous new haemosporidian species, which are mainly of tropical and subtropical transmission, were discovered and described (Table 2.2). Importantly, type specimens of the majority of discovered parasites were established and are available for research at Queensland Museum, Australia, where the collection is currently housed. G. F. Bennett and his colleagues created the foundations for current faunistic studies of avian haemosporidians. Biodiversity research has been actively pursued since then, with many new agents of haemosporidiosis discovered in tropical countries, many of them in remote areas (Table 2.2).

Among the milestone studies in the field of tropical haemosporidian research, the following discoveries were particularly important. Aragão (1908) discovered, described, and illustrated tissue stages of *H. columbae*. This finding suggested the possible broad existence of exoerythrocytic development in the life cycles of haemosporidians. Shortly afterward, Adie (1915) described the complete sporogonic development of this parasite in louse flies belonging to the Hippoboscidae, providing the first evidence that Diptera other than mosquitoes can be involved in transmission of these pathogens. The role of biting midges in the family Ceratopogonidae (Fallis and Wood 1957; Akiba 1960) and black flies in the family Simuliidae (O'Roke 1930; Skidmore 1931) in transmission of haemoproteids and leucocytozoids was discovered after that (see Chap. 6). Lainson et al. (1971) discovered

Table 2.2 Species of avian haemosporidian parasites that are transmitted mainly in the tropics and subtropics. Migratory birds from the Holarctic and Neartic can acquire some of these infections at their wintering sites in the tropics and bring them to breeding grounds at northern latitudes where vector-borne transmission may be interrupted

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
Haemoproteidae				
Haemoproteus				
Parahaemoproteus				
H. aegithinae	*Aegithina tiphia* (Passeriformes, Aegithinidae)	India	Not available	Mello (1935b)
H. africanus	*Mandingoa nitidula* (Passeriformes, Estrildidae)	Tanzania	Not available	Bennett and Peirce (1991)
H. antigonis	*Grus virgo* (Gruiformes, Gruidae)	India	Not available	Mello (1935b)
H. apodus	*Chaetura andrei* (Apodiformes, Apodidae)	Brazil	Not available	Bennett et al. (1986a)
H. balearicae	*Balearica pavonina* (Gruiformes, Gruidae)	West Africa	Not available	Peirce (1973)
H. bennetti	*Chrysophlegma flavinucha* (Piciformes, Picidae)	India	Not available	Greiner et al. (1977)
H. bilobata	*Psilopogon pyrolophus* (Piciformes, Megalaimidae)	Malaya	Not available	Bennett and Nandi (1981)
H. borgesi	*Campethera punctuligera* (Piciforme, Picidae)	Guinea-Bissau	Not available	Tendeiro (1947)
H. bubalornis	*Bubalornis albirostris* (Passeriformes, Ploceidae)	Kenya	Not available	Bennett and Peirce (1991)
H. bucconis	*Nystalus chacuru* (Piciformes, Bucconidae)	Brazil	Not available	Bennett et al. (1986a)
H. bucerotis	*Tockus erythrorhynchus* (Bucerotiformes, Bucerotidae)	Botswana	Not available	Bennett et al. (1995)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. bukaka	*Cracticus louisiadensis* (Passeriformes, Artamidae)	Papua New Guinea	KX100323	Goulding et al. (2016)
H. burhini	*Burhinus capensis* (Charadriiformes, Burhinidae)	South Africa	Not available	Bennett et al. (1995)
H. centropi	*Centropus sinensis* (Cuculiformes, Cuculidae)	India	Not available	Mello (1935b)
H. ciconiae	*Ciconia ciconia* (Ciconiiformes, Ciconiidae)	Lithuania (transmission in Africa)	Not available	Valkiūnas et al. (2016a)
H. circumnuclearis	*Mionectes olivaceus* (Passeriformes, Tyrannidae)	Colombia	Not available	Bennett et al. (1986b)
H. clamatori	*Clamator jacobinus* (Cuculiformes, Cuculidae)	Kenya	Not available	Peirce and Adlard (2005)
H. contortus	*Numenius phaeopus* (Charadriiformes, Scolopacidae)	Philippine Islands	Not available	Bennett (1979)
H. coraciae	*Coracias benghalensis* (Coraciiformes, Coraciidae)	India	KU297278.1**	Mello and Afonso (1935)
H. cornuata	*Psilopogon asiaticus* (Piciformes, Megalaimidae)	Western Bhutan	Not available	Bennett and Nandi (1981)
H. cracidarum	*Ortalis ruficauda* (Galliformes, Cracidae)	Venezuela	Not available	Bennett et al. (1982)
H. cublae	*Dryoscopus cubla* (Passeriformes, Malaconotidae)	Zambia	Not available	Peirce (1984a)
H. cuculis	*Eudynamys scolopacea* (Cuculiformes, Cuculidae)	Australia	Not available	Peirce and Adlard (2005)
H. cyanomitrae	*Cyanomitra olivacea* (Passeriformes, Nectariniidae)	Ghana	FJ404696	Iezhova et al. (2010)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. dicaeus	*Prionochilus percussus* (Passeriformes, Dicaeidae)	Malaysia	Not available	Bennett and Bishop (1990)
H. dicruri	*Dicrurus macrocercus* (Passeriformes, Dicruridae)	India	Not available	Mello (1935b)
H. elani	*Elanus caeruleus* (Accipitriformes, Accipitridae)	India	Not available	Mello (1935b)
H. enucleator	*Ispidina picta* (Coraciiformes, Alcedinidae)	Uganda	DQ659592**	Bennett et al. (1972)
H. erythrogravidus	*Zonotrichia capensis* (Passeriformes, Passerellidae)	Colombia	KT698209, KF537315, KF537329	Mantilla et al. (2016)
H. eurylaimus	*Serilophus lunatus* (Passeriformes, Eurylaimidae)	Thailand	Not available	Bennett et al. (1991a)
H. eurystomae	*Eurystomus orientalis* (Coraciiformes, Coraciidae)	Malaysia	Not available	Bishop and Bennett (1986)
H. formicarius	*Dysithamnus mentalis* (Passeriformes, Thamnophilidae)	Brazil	Not available	Bennett et al. (1987)
H. forresteri	*Atelornis crosslevi* (Coraciiformes, Brachypteraciidae)	Madagascar	Not available	Savage and Greiner (2004)
H. furnarius	*Automolus leucophthalmus* (Passeriformes, Furnariidae)	Brazil	Not available	Bennett et al. (1987)
H. gallinulae	*Gallinula chloropus* (Gruiformes, Rallidae)	India	Not available	Mello (1935b)
H. gavrilovi	*Merops apiaster* (Coraciiformes, Meropidae)	Southern Kazakhstan	KP462688	Valkiūnas and Iezhova (1990)
H. goodmani	*Atelornis pittoides* (Coraciiformes, Brachypteraciidae)	Madagascar	Not available	Savage and Greiner (2004)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. fuscae	*Halcyon smyrnensis* (Coraciiformes, Alcedinidae)	India	EU810722	Mello and Fonseca (1937)
H. halcyonis	*Halcyon smyrnensis* (Coraciiformes, Alcedinidae)	India	Not available	Mello (1935b)
H. handai	*Psittacula cyanocephala* (Psittaciformes, Psittaculidae)	Pakistan	Not available	Maqsood (1943)
H. herodiadis	*Ardea intermedia* (Pelecaniformes, Ardeidae)	India	Not available	Mello (1935a)
H. hirundinis	Species is nonidentified (Passeriformes, Hirundinidae)	Algeria	KJ499183	Sergent and Sergent (1905)
H. homobelopolskyi	*Ploceus melanocephalus* (Passeriformes, Ploceidae)	Uganda	HQ386240, HQ386241	Iezhova et al. (2011)
H. homohandai	*Ara chloropterus* (Psittaciformes, Psittacidae)	Surinam	KY783725	Valkiūnas et al. (2017)
*H. homoleiothrichus***	*Trochalopteron erythrocephalum* (Passeriformes, Leiothrichidae)	India	KY623721	Ishtiaq et al. (2018)
H. indicator	*Indicator indicator* (Piciformes, Indicatoridae)	Uganda	Not available	Bennett et al. (1986a)
H. janovyi	*Gyps africanus* (Accipitriformes, Accipitridae)	Zimbabwe	Not available	Greiner and Mundy (1979)
H. khani	*Dicrurus forficatus* (Passeriformes, Dicruridae)	Madagascar	Not available	Savage and Greiner (2005)
H. killangoi	*Zosterops senegalensis* (Passeriformes, Zosteropidae)	Uganda	JN661945	Bennett and Peirce (1981)
H. lairdi	*Merops variegatus* (Coraciiformes, Meropidae)	Uganda	Not available	Bennett (1978)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
*H. leiothrichus***	*Trochalopteron erythrocephalum* (Passeriformes, Leiothrichidae)	India	KY623720	Ishtiaq et al. (2018)
H. macrovacuolatus	*Dendrocygna autumnalis* (Anseriformes, Anatidae)	Colombia	KJ499987, KJ175078. KJ592828	Matta et al. (2014b)
H. madagascariensis	*Vanga curvirostris* (Passeriformes, Vangidae)	Madagascar	Not available	Savage et al. (2004)
H. manwelli	*Merops orientalis* (Coraciiformes, Meropidae)	India	KP462687	Bennett (1978)
H. megapodius	*Megapodius freycinet* (Galliformes, Megapodiidae)	India	Not available	Nandi and Mandal (1980)
H. meropis	*Merops orientalis* (Coraciiformes, Meropidae)	India	Not available	Zargar (1945)
H. micronuclearis	*Quelea quelea* (Passeriformes, Ploceidae)	Uganda	HQ386235, Q386236, HQ386237, HQ386238, HQ386239	Iezhova et al. (2011)
H. minchini	*Corythaeola cristata* (Musophagiformes, Musophagidae)	Tanzania	KU160476	Chavatte et al. (2017)
H. monarchus	*Monarcha cinerascens* (Passeriformes, Monarchidae)	New Guinea	Not available	Bennett et al. (1991b)
H. montezi	*Tauraco porphyreolophus* (Musophagiformes, Musophagidae)	Mozambique	Not available	Travassos Santos Dias (1953)
H. neseri	*Cossypha dichroa* (Passeriformes, Muscicapidae)	South Africa	Not available	Bennett and Earlé (1992)
H. nipponensis	*Cyanoptila cyanomelana* (Passeriformes, Muscicapidae)	Japan	Not available	Bennett et al. (1991b)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. nucleocondensus	*Acrocephalus arundinaceus* (Passeriformes, Acrocephalidae)	Lithuania (transmission in Africa)	JX026901	Križanauskienė et al. (2012)
H. nucleofascialis	*Malimbus rubricollis* (Passeriformes, Ploceidae)	Uganda	HQ386243, HQ386244	Iezhova et al. (2011)
H. nucleophilus	*Melanocharis nigra* (Passeriformes, Melanocharitidae)	New Guinea	Not available	Bennett and Bishop (1990)
H. orizivorae	*Lonchura oryzivora* (Passeriformes, Estrildidae)	Indonesia	Not available	Anschütz (1909)
H. ortalidum	*Ortalis ruficauda* (Galliformes, Cracidae)	Venezuela	KX171627	Gabaldon and Ulloa (1978)
H. otocompsae	*Pycnonotus jocosus* (Passeriformes, Pycnonotidae)	India	Not available	Mello (1935b)
H. pachycephalus	*Pachycephala pectoralis (Passeriformes,* Pachycephalidae)	Philippine Islands	Not available	Bennett et al. (1991b)
H. paranucleophilus	*Malimbus rubricollis* (Passeriformes, Ploceidae)	Uganda	HQ386242	Iezhova et al. (2011)
H. paraortalidum	*Aburria jacutinga* (Galliformes, Cracidae)	Brazil	MH036944	Ferreira-Junior et al. (2018)
H. payevskyi	*Acrocephalus scirpaceus* (Passeriformes, Acrocephalidae)	Lithuania (transmission in Africa)	AF254968	Valkiūnas et al. (1994)
H. pelouroi	*Bostrychia hagedash* (Pelecaniformes, Threskiornithidae)	Guinea-Bissau	Not available	Tendeiro (1947)
H. philippinensis	*Hemixos flavala* (Passeriformes, Pycnonotidae)	Malaysia	Not available	Rahal et al. (1987)
H. pittae	*Pitta arcuate* (Passeriformes, Pittidae)	Borneo	Not available	Bennett et al. (1991a)
H. plataleae	*Platalea leucorodia* (Pelecaniformes, Threskiornithidae)	India	Not available	Mello (1935b)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. porzanae	*Porzana pusilla* (Gruiformes, Rallidae)	Tunisia	Not available	Galli-Valerio (1907)
H. pratasi	*Numida meleagris* (Galliformes, Numididae)	Guinea-Bissau	Not available	Tendeiro (1947)
H. psittaci	*Psittacus erithacus* (Psittaciformes, Psittacidae)	Tropical Africa	Not available	Bennett and Peirce (1992)
H. ptilotis	*Caligavis chrysops* (Passeriformes, Meliphagidae)	Australia	KP721986	Cleland and Johnston (1909)
H. queleae	*Quelea erythrops* (Passeriformes, Ploceidae)	Equatorial Guinea	Not available	Marullaz (1912)
H. rileyi	*Pavo cristatus* (Galliformes, Phasianidae)	India	Not available	Malkani (1936)
H. rotator	*Gallinago stenura* (Charadriiformes, Scolopacidae)	Philippine Islands	Not available	Bennett (1979)
H. sanguinis	*Pycnonotus jocosus* (Passeriformes, Pycnonotidae)	India	DQ847194.1**	Chakravarty and Kar (1945a)
H. sequeirae	*Nectarinia coccinigaster* (Passeriformes, Nectariniidae)	Guinea-Bissau	Not available	Tendeiro (1947)
H. souzalopesi	*Cnemotriccus fuscatus* (Passeriformes, Tyrannidae)	Brazil	Not available	Bennett et al. (1986b)
H. stellaris	*Pseudhirundo griseopyga* (Passeriformes, Hirundinidae)	Uganda	Not available	White and Bennett (1978)
H. telfordi	*Lissotis melanogaster* (Otidiformes, Otididae)	Zaire	Not available	Bennett et al. (1975)
H. thereicerycis	*Psilopogon zeylanicus* (Piciformes, Megalaimidae)	India	Not available	Mello (1935b)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. timalus	*Turdoides rubiginosus* (Passeriformes, Leiothrichidae)	Kenya	Not available	Bennett et al. (1991b)
H. trochili	*Eutoxeres aquila* (Apodiformes, Trochilidae)	Colombia	Not available	White et al. (1979)
H. trogonis	*Harpactes duvaucelii* (Trogoniformes, Trogonidae)	Malaysia	Not available	Bennett and Peirce (1990)
H. undulatus	*Urocolius indicus* (Coliiformes, Coliidae)	South Africa	Not available	Bennett and Earlé (1992)
H. upupae	*Upupa epops* (Bucerotiformes, Upupidae)	India	Not available	Mello (1935b)
H. uraeginthus	*Uraeginthus bengalus* (Passeriformes, Estrildidae)	Chad	Not available	Bennett and Peirce (1991)
H. vacuolatus	*Andropadus latirostris* (Passeriformes, Pycnonotidae)	Ghana	EU770153	Valkiūnas et al. (2008)
H. valkiunasi	*Fregata andrewsi* (Suliformes, Fregatidae)	Christmas Island, Australia	GQ404559	Merino et al. (2012)
H. vangii	*Cyanolanius madagascarinus* (Passeriformes, Vangidae)	Madagascar	Not available	Savage et al. (2004)
H. wenyoni	*Orthotomus sutorius* (Passeriformes, Cisticolidae)	India	Not available	Mello et al. (1916)
H. witti	*Trochilus polytmus* (Apodiformes, Trochilidae)	Jamaica	KF537304	White et al. (1979)
H. xantholaemae	*Megalaima haemacephala* (Piciformes, Megalaimidae)	India	Not available	Zargar (1945)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
H. zosteropis	*Zosterops palpebrosus* (Passeriformes, Zosteropidae)	India	EU810756**	Chakravarty and Kar (1945b)
Haemoproteus				
H. columbae	*Columba livia* (Columbiformes, Columbidae)	Italy	AF495554	Kruse (1890)
H. iwa	*Fregata minor* (Suliformes, Fregatidae)	Hawaii	JF833050	Work and Rameyer (1996)
H. jenniae	*Creagrus furcatus* (Charadriiformes, Laridae)	Galapagos	JN827318	Levin et al. (2012)
H. multipigmentatus	*Zenaida galapagoensis* (Columbiformes, Columbidae)	Galapagos	GU296216, GU296215, GU296224	Valkiūnas et al. (2010)
H. multivolutinus	*Turtur tympanistria* (Columbiformes, Columbidae)	Uganda	JX275888	Valkiūnas et al. (2013a)
H. paramultipigmentatus	*Columbina passerina* (Columbiformes, Columbidae)	Socorro Island	JN788934, JN788939	Valkiūnas et al. (2013b)
H. pteroclis	*Pterocles alchata* (Pteroclidiformes, Pteroclidae)	Iraq	Not available	Shamsuddin and Mohammad (1980)
Plasmodiidae				
Plasmodium				
Haemamoeba				
P. coturnixi	*Coturnix coturnix* (Galliformes, Phasianidae)	Pakistan	Not available	Bano and Abbasi (1983)
P. gallinaceum	*Gallus gallus* (Galliformes, Phasianidae)	Ceylon	AY099029	Brumpt (1935)
P. griffithsi	*Meleagris gallopavo* (Galliformes, Phasianidae)	Burma	Not available	Garnham (1966)
P. lutzi	*Aramides cajaneus* (Gruiformes, Rallidae)	Brazil	KC138226	Lucena (1939)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
P. parvulum	*Schetba rufa* (Passeriforme, Vangidae)	Madagascar	Not available	Savage et al. (2005)
P. tejerai	*Meleagris gallopavo* (Galliformes, Phasianidae)	Venezuela	JX272844, HQ591361	Gabaldon and Ulloa (1977)
Giovannolaia				
P. anasum	*Anas clypeata* (Anseriformes, Anatidae)	Taiwan	Not available	Manwell and Kuntz (1965)
P. durae	*Meleagris gallopavo* (Galliformes, Phasianidae)	Kenya	Not available	Herman (1941)
P. fallax	*Syrnium nuchale* (Strigiformes, Strigidae)	Congo	Not available	Schwetz (1930)
P. formosanum	*Arborophila crudigularis* (Galliformes, Phasianidae)	Taiwan	Not available	Manwell (1962)
P. gabaldoni	*Columba livia* (Columbiformes, Columbidae)	Venezuela	Not available	Garnham (1977)
P. garnhami	*Upupa epops major* (Bucerotiformes, Upupidae)	Egypt	Not available	Guindy et al. (1965)
P. gundersi	*Strix woodfordii* (Strigiformes, Strigidae)	Liberia	Not available	Bray (1962)
P. hegneri	*Anas crecca* (Anseriformes, Anatidae)	Taiwan	Not available	Manwell and Kuntz (1966)
P. homocircumflexum	*Lanius collurio* (Passeriformes, Laniidae)	Lithuania (transmission in Africa)	KC884250	Palinauskas et al. (2015)
P. leanucleus	*Passer domesticus* (Passeriformes, Passeridae)	China	Not available	Huang (1991)
P. lophurae	*Lophura igniti* (Galliformes, Phasianidae)	Borneo	Not available	Coggeshall (1938)
P. octamerium	*Vidua macroura* (Passeriformes, Viduidae)	Africa (no other data)	Not available	Manwell (1968)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
P. pinottii	*Ramphastos toco* (Piciformes, Ramphastidae)	Brazil	Not available	Muniz and Soares (1954)
Novyella				
P. ashfordi	*Acrocephalus arundinaceus* (Passeriformes, Acrocephalidae)	Bulgaria	AF254962	Valkiūnas et al. (2007)
P. bertii	*Aramides cajaneus* (Gruiformes, Rallidae)	Venezuela	Not available	Gabaldon and Ulloa (1981)
P. columbae	*Columba livia* (Columbiformes, Columbidae)	Brazil	Not available	Carini (1912)
P. delichoni	*Delichon urbicum* (Passeriformes, Turdidae)	Lithuania (transmission in Africa)	KU529943	Valkiūnas et al. (2016b)
P. dissanaikei	*Psittacula krameri manillensis* (Psittaciformes, Psittaculidae)	Ceylon	Not available	Jong (1971)
P. forresteri	*Strix varia* (Strigiformes, Strigidae)	Florida	Not available	Telford et al. (1997)
P. globularis	*Andropadus latirostris* (Passeriformes, Pycnonotidae)	Ghana	EU770151	Valkiūnas et al. (2008)
P. homonucleophilum	*Locustella naevia* (Passeriformes, Locustellidae)	Lithuania (transmission in Africa)	KC342643	Ilgūnas et al. (2013)
P. lucens	*Cyanomitra olivacea* (Passeriformes, Nectariniidae)	Cameroon	FJ389156	Valkiūnas et al. (2009)
P. megaglobularis	*Cyanomitra olivacea* (Passeriformes, Nectariniidae)	Ghana	EU770152	Valkiūnas et al. (2008)
P. multivacuolaris	*Andropadus latirostris* (Passeriformes, Pycnonotidae)	Cameroon	FJ389157	Valkiūnas et al. (2009)
P. parahexamerium	*Alethe diademata* (Passeriformes, Muscicapidae)	Cameroon	FJ389155	Valkiūnas et al. (2009)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
P. paranucleophilum	*Tachyphonus* sp. (Passeriformes, Thraupidae)	South America (no other data)	KX159490	Manwell and Sessler (1971)
P. unalis	*Turdus fuscater* (Passeriformes, Turdidae)	Colombia	KC771248	Mantilla et al. (2013)
Bennettinia				
P. juxtanucleare	*Gallus gallus* (Galliformes, Phasianidae)	Brazil	AB250415, AB302893	Versiani and Gomes (1941)
Huffia				
P. hermani	*Meleagris gallopavo* (Galliformes, Phasianidae)	Florida	Not available	Telford and Forrester (1975)
P. huffi	*Ramphastos toco* (Piciformes, Ramphastidae)	Brazil	Not available	Muniz et al. (1951)
Garniidae				
Fallisia				
Plasmodioides				
F. neotropicalis	*Columba livia* (Columbiformes, Columbidae)	Venezuela	Not available	Gabaldon et al. (1985)
Leucocytozoidae				
Leucocytozoon				
Leucocytozoon				
L. artamidis	*Cracticus nigrogularis* (Passeriformes, Artamidae)	Australia	Not available	Peirce et al. (2005)
L. balmorali	*Dryoscopus cubla* (Passeriformes, Malaconotidae)	Zambia	Not available	Peirce (1984b)
L. centropi	*Centropus superciliosus* (Cuculiformes, Cuculidae)	South Africa	Not available	Fantham et al. (1921)
L. colius	*Colius striatus* (Coliiformes, Coliidae)	South Africa	Not available	Bennett et al. (1993)
L. dizini	*Crinifer piscator* (Musophagidae, Musophagidae)	Guinea-Bissau	Not available	Tendeiro (1947)

(continued)

Table 2.2 (continued)

Family, genus, subgenus, and species	Type avian host	Type locality	Barcode DNA sequence*	Reference of original description
L. eurystomi	*Eurystomus gularis* (Coraciiformes, Coraciidae)	Congo	Not available	Kerandel (1913)
L. maccluri	*Zoothera marginata* (Passeriformes, Turdidae)	Thailand	Not available	Greiner (1976)
L. neavei	*Numida meleagris* (Galliformes, Numididae)	Sudan	Not available	Balfour (1906)
L. nycticoraxi	*Nycticorax nycticorax* (Pelecaniformes, Ardeidae)	Iraq	Not available	Shamsuddin and Mohammad (1980)
L. nyctyornis	*Nyctyornis athertoni* (Coraciiformes, Meropidae)	India	Not available	Nandi (1986a)
L. pterotenuis	*Grallaria ruficapilla* (Passeriformes, Grallariidae)	Colombia	KM610046	Lotta et al. (2015)
L. quynzae	*Heliangelus amethysticollis* (Apodiformes, Trochilidae)	Colombia	KF479480, KF309188, KF309189	Matta et al. (2014a)
L. schoutedeni	*Gallus gallus* (Galliformes, Phasianidae)	Congo	DQ676823, DQ676824	Rodhain et al. (1913)
L. sousadiasi	*Vanellus tectus* (Charadriiformes, Charadriidae)	Guinea-Bissau	Not available	Tendeiro (1947)
L. squamatus	*Picus squamatus* (Piciformes, Picidae)	India	DQ451432**	Nandi (1986b)
L. struthionis	*Struthio camelus* (Struthionidae, Struthionidae)	South Africa	Not available	Walker (1912)
L. vandenbrandeni	*Anhinga rufa* (Suliformes, Anhingidae)	Belgian Congo	Not available	Rodhain (1931)
Akiba				
L. caulleryi	*Gallus gallus* (Galliformes, Phasianidae)	Vietnam	AB302215	Mathis and Léger (1909)

*Unified codes of genetic lineages are available in MalAvi database (Bensch et al., 2009; http://mbio-serv2.mbioekol.lu.se/Malavi/). These are often different from codes, which were provided in original descriptions (see corresponding references)

(continued)

Table 2.2 (continued)

**Molecular characterization needs confirmation because morphological data were insufficient during parasite identification

***Taxonomic status of *H. leiothrichus* and *H. homoleiothrichus* requires confirmation with regard to *H. timalus*. Gametocytes of *H. leiothrichus* and *H. homoleiothrichus* are similar morphologically (Ishtiaq et al., 2018), and they are barely distinguishable from gametocytes of *H. timalus* because of overlapping diagnostic characters. Additionally, all three parasites develop in closely related species in the family Leiothrichidae. Molecular characterization of *H. timalus* has not been done. Because *H. timalus* has priority in nomenclature, either *H. leiothrichus* or *H. homoleiothrichus* might be synonyms of *H. timalus*. Molecular characterization of *H. timalus* is needed to answer this question

parasites of new family Garniidae in South American reptiles, and Gabaldon et al. (1985) found that representatives of this family also parasitize birds. These discoveries significantly broadened the biodiversity and evolutionary history of haemosporidians.

During the past 15 years, over 40 new avian haemosporidian parasites have been discovered in tropical Africa, South America, Asia, and Australia (Table 2.2). Importantly, molecular characterization of avian haemosporidians was done in parallel with morphological investigations, resulting in establishment of barcodes for detection of over 50 agents of infections (Table 2.2). This has provided new opportunities to apply molecular markers to investigations of the pathology of these organisms both in blood-sucking insects during sporogonic development (Bukauskaitė et al. 2018) and in birds during exoerythrocytic development (Ortiz-Catedral et al. 2019). Recent molecular studies combined with histopathology investigations showed that avian *Plasmodium* and *Haemoproteus* parasites are more virulent that has been formerly believed. However, molecular characterization of the majority of described avian haemosporidian species has not been done (Table 2.2), and it is an important task for current researchers working in tropical countries. Molecular studies demonstrated that genetic diversity of these pathogens is large, being markedly greater than their morphological diversity, suggesting the need for developing better faunistic, taxonomic, and conceptual understandings of these pathogens (Nilsson et al. 2016). The development of molecular markers provides new opportunities for better understanding biology of haemosporidian infections, particularly during abortive development, which often causes severe disease in both insects and avian hosts, but which remains insufficiently studied (Valkiūnas and Iezhova 2017; Ortiz-Catedral et al. 2019; see Chap. 4).

It is important to note that diseases and even mortality of birds, particularly of poultry, have been reported, mainly in countries with warm climates. The most significant haemosporidian pathogens reported from the tropics include *P. gallinaceum, P. griffithsi, P. tejerai, P. durae, P. gabaldoni, P. juxtanucleare, H. mansoni* (possible syn. *H. meleagridis*), *L. struthionis,* and *L. (Akiba) caulleryi* (Table 2.2, Atkinson et al. 2008).

The tropics and subtropics of all zoogeographical regions remain insufficiently investigated with regard to the systematics and biology of haemosporidians. This has led to significant gaps in our knowledge that should be a high priority for future

research in both tropical parasitology and evolutionary biology. First, representatives of the family Garniidae are restricted to tropical and subtropical countries and have not been investigated with molecular methods. These parasites are particularly diverse in reptiles of South America (Lainson et al. 2012) where they also parasitize birds (Gabaldon et al. 1985). The lack of genetic information about species of the family Garniidae, as well as parasites of reptiles belonging to *Asiamoeba, Ophidiella, Saurocytozoon*, and some other genera, is a major obstacle in understanding phylogenetic relationships among all haemosporidians. Additional sampling, preferably at the type localities of certain parasite species, is needed. Second, molecular barcodes have not been determined for the great majority of described parasites, but are essential for disease diagnostics (Table 2.2). Third, most research has focused on small passeriform birds and small birds from other avian families because they are relatively easy to catch with mist nets. This has led to significant gaps in knowledge about parasites of nonpasserine birds and bird species inhabiting higher vertical strata (mid- and canopy layers) that do not fall in understory mist nets – information that is essential for understanding the biology of haemosporidians (Table 2.2). Fourth, vector studies remain scarce in the tropics, and the role of species belonging to endemic insect genera and subgenera remains unclear in parasite transmission, but are essential for epidemiological research (see Chaps. 5 and 6 for discussions regarding Diptera vectors). Fifth, the frequency of occurrence of abortive haemosporidian infections in abnormal hosts and the virulence of such infections should be determined, particularly with regard to avian health. It will be difficult to develop a more complete picture of the biology, virulence, transmission, evolution, and systematics of haemosporidians until these gaps in the current knowledge are filled.

2.4 Methods for the Study of Avian Haemosporidians

Much has been written about laboratory and field techniques for studying haemosporidian parasites, ranging from details about fixing and staining blood smears for microscopic study to molecular methods for detecting infections by PCR and high-throughput sequencing. Given the extensive body of literature that is already available about these methods (Hewitt 1940; Shute and Maryon 1966; Garnham 1966; Bensch et al. 2004; Valkiūnas 2005; Valkiūnas et al. 2006; Atkinson et al. 2008; Santiago-Alarcon and Carbó-Ramírez 2015; see Chap. 4 for a discussion on modern molecular methods applied to haemosporidian research), we focus here on their relative strengths and weaknesses and how a combination of methodology can lead to better estimates of true distribution of haemosporidians, particularly of their prevalence and biodiversity.

The detection and diagnosis of avian haemosporidians is often difficult because of their complex life cycles, intracellular development, and variable intensities of infection in both vertebrate and invertebrate hosts. In spite of over 100 years of research on these parasites, there is no single best method for determining infection status of either wild birds or arthropod vectors. When intensity of infection is

relatively high, both microscopy and PCR amplification of parasite DNA can accurately establish that birds carry developmental stages of haemosporidian parasites in their circulating blood. However, neither microscopy nor PCR is completely satisfactory for detecting low-intensity erythrocytic infections, and both will miss preerythrocytic and latent infections that are limited to deep tissues. Neither method can determine whether birds have recovered from past infections. As a result, false-negative detections may be much more widespread than generally recognized.

Similarly, intensity of infection in arthropod vectors may be highly variable, and it may be difficult to distinguish abortive infections that are limited to the blood meal from successful sporogonic development and invasion of salivary glands by sporozoites without time consuming and tedious dissections. Abortive infections can also be difficult to determine in vertebrate hosts by PCR since amplification of parasite DNA does not distinguish gametocytes from asexual parasite stages. Since most ecological studies of these parasites rely on either microscopy or PCR amplification of parasite DNA for diagnosing infections, it is important to recognize their limitations and develop a better understanding of how multiple methods can be used together to establish more accurate measures of parasite prevalence and diversity (Fig. 2.2).

The ability to make thin, undistorted blood smears that are quickly and thoroughly dried and fixed under difficult field conditions and then optimally stained with Giemsa is an essential skill for the study of haemosporidian parasites (Valkiūnas et al. 2006; Santiago-Alarcon and Carbó-Ramírez 2015). A well prepared, fixed, and stained thin blood smear provides a permanent record that can be archived in suitable museum collections and can serve as a physical type specimen for new species descriptions. A well-prepared blood smear also provides key morphometric and other morphological details about the parasite that are critical for identification to the level of species, information about presence or absence of gametocytes that is important for determining whether the host is a reservoir for infecting arthropod vectors, information about intensity of infection, information about coinfection with other hematozoan parasites, and detailed hematological information, including red and white blood cell differential counts, that is important for assessing the physiological condition of the host. While this technique has many strengths, it is limited in several ways. Infection intensities that fall below 0.01% (<1 in 10,000 erythrocytes) may be missed by inexperienced microscopists, leading to underestimation of prevalence, (2) low intensities of infection may limit detection of meronts and/or gametocytes that are key for determining subgenus and species of the parasites, and (3) coinfections, particularly of parasites belonging to same subgenus, may be difficult or impossible to distinguish in low-intensity infections.

Ecological studies of avian haemosporidians that were done prior to the late 1990s relied exclusively on stained blood smears for determining infection in wild birds. Many of the limitations of this methodology were rarely discussed or clearly identified for novices in the field, particularly investigators without an extensive background in parasitology. Most of these studies probably underestimated prevalence of infection and frequency of coinfection or limited identifications to the generic or subgeneric level because naturally occurring infections are frequently

chronic and the number of parasites in the peripheral circulation is low. This is particularly true for samples collected during seasons when transmission is interrupted in wildlife (e.g., winter time at high latitudes, dry season in tropical areas).

The advent of molecular techniques in the late 1990s led to a revolution in the field by making possible to use PCR to amplify parasite nuclear and mitochondrial genes for DNA sequencing, and for the first time to barcode individual parasite lineages and associate them with species that were formerly defined strictly by morphology and life history characteristics (Bensch et al. 2004, 2009). Molecular methods have a number of advantages over microscopy. They can accurately determine genetic lineages in low-intensity infections that may be missed or impossible to identify by microscopy, and they can also be used to accurately quantify low-intensity infections using quantitative PCR. However, PCR amplification of parasite DNA is not completely fool proof, and the technique may still miss extremely low-intensity infections and often fail to detect coinfections if one parasite lineage amplifies preferentially over another. In addition, PCR protocols using general primers are usually unable to distinguish coinfections of haemosporidians belonging to same subgenus. Some of these issues can be resolved through the use of high-throughput sequencing methods that can identify populations of different lineages within an infected host (Jarvi et al. 2013), but this methodology has not yet been widely used in ecological studies (see also Chap. 4). Most importantly, the use of molecular methods alone may make it difficult to associate specific parasite lineages with known morphological species of avian haemosporidians, particularly when the method is used in previously unexplored geographic areas where morphological and genetic diversity of the parasites is unknown and new parasite species may be present.

The combination of microscopy and molecular approaches for diagnosis of haemosporidian infections is essential and becoming increasingly common and has important advantages over use of either methodology alone. A combined approach improves sensitivity of detection, makes it possible to associate specific genetic lineages with morphological species, allows the identification of coinfections and cryptic diversity within morphological species that may be associated with specific host associations or life history characteristics, and provides a combination of morphological and genetic information for defining new species.

While PCR and microscopy can complement each other in ecological studies, neither is able to detect latent infections where the parasite resides intracellularly within fixed, noncirculating tissues of the host or determine stage of infection (acute vs. chronic) based on collection of a single blood sample (Fig. 2.2). While information about latency and recovery from past infections is not critical for faunistic investigations, it becomes extremely important for determining infection status in epidemiological investigations of haemosporidian parasites. Vertebrate hosts produce strong humoral and cellular immune responses to infection with haemosporidians (van Riper et al. 1994; Doolan et al. 2009), making it possible to use antibody titers to identify individuals with chronic low-intensity infections that might be missed by microscopy or PCR, to identify latent infections that have retreated to the tissues, or to provide evidence of prior infection, especially when recaptured birds

can be resampled over time (Fig. 2.2). Serological methods have been used effectively for investigations of malaria in wild and captive penguins (Graczyk et al. 1993, 1995), native Hawaiian forest birds (Atkinson et al. 2001; Atkinson and Samuel 2010), and diagnosis of *L. (Akiba) caulleryi* in domestic chickens (Ito and Gotanda 2005), but the methodology is not universally applicable to a wide range of host–parasite associations without substantial work to find adequate sources of parasite antigens, determine cross reactions with other organisms, and validate tests using experimental studies. Most work in this area has focused on parasites of domestic poultry (i.e., *L. (A.) caulleryi*) or generalist parasites such as *Plasmodium relictum* and *Plasmodium elongatum* that can complete their life cycles readily in available domesticated hosts (e.g., canaries, Pekin ducklings). The absence of convenient experimental models for most avian haemosporidians has limited widespread use of serology, but the application of whole-genome sequencing and development of species or genus-specific recombinant antigens may make wider application of these approaches feasible in the future.

2.5 Conclusion

Recent ecological studies are providing evidence that diversity of avian haemosporidian parasites is greatest in the tropics, but there are significant gaps in our knowledge about the life cycles, vectors, virulence, genetics, and many other issues about their biology in these regions of the world. This is particularly true in remote tropical areas where collection of samples for combined experimental, microscopic, PCR-based, and serological studies remains challenging, but essential for a better understanding the biology of these organisms. Future work on molecular barcodes, vectors, host range, and virulence of rare or poorly studied taxa will improve our understanding of phylogenetic relationships among all haemosporidians.

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Chapter 3
Phylogenetics and Systematics in a Nutshell

Alejandro Espinosa de los Monteros

Abstract During the last 50 years, phylogenetic systematics has suffered a substantial transformation in philosophy and methods. Systematics has gone from been a merely descriptive discipline to a scientific theory encompassing solid evolutionary principles capable of inferring robust and replicable historical hypothesis about the interrelationships of taxa. This chapter provides the basic concepts in the field of systematic biology (e.g., terminology, characters codification, tree description) and phylogenetic reconstructions (e.g., alignments, reconstruction methods, support measurements). A particular emphasis is given to nucleotide data. It will provide a guide on how sequences can be used to detect natural selection, adaptation, recombination, and to evaluate substitution saturation. In particular, this chapter seeks to provide the novice with all basic concepts necessary to understand and interpret phylogenetic hypotheses: for instance, to understand nucleotide substitution models, what a molecular clock is, tree selection methods (e.g., Maximum Parsimony, Maximum Likelihood, Bayesian), how to interpret node support values, and testing tree topologies (e.g., Kishino-Hasegawa). Finally, a short review is presented on the current phylogenetic knowledge of avian Haemosporida.

Keywords Avian Haemosporida phylogeny · Bayesian inference · Maximum Likelihood · Molecular clock · Parsimony · Phylogenetic reconstruction

3.1 Introduction

It is possible that taxonomy and systematics are the earliest biological subjects developed by mankind. The need to organize and classify natural resources was indubitably an essential issue for the survival of human populations. In 1735, the Swedish naturalist Carl von Linné published *Systema Naturae* formalizing the rules

A. Espinosa de los Monteros (✉)
Laboratorio de Sistematica Filogenetica. Departamento de Biología Evolutiva, Instituto de Ecología, Xalapa, Veracruz, Mexico

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for binomial nomenclature. Although Linné's system has been challenged, he is still considered as the father of modern taxonomy. The domain of taxonomy (i.e., naming taxa and classification) is different from that of systematics. Systematics is the basis to understand the evolutionary interrelationships of taxa in an attempt to generate historical scenarios for how lineages have changed and diversified throughout time. In other words, systematics is the method that scientists used for reconstructing the pattern of events that have assembled the current biodiversity on Earth.

Phylogenetic systematics is the way that biologists infer hypotheses that explain the evolutionary history of life, both living and extinct. During the last 50 years, this discipline has suffered radical changes due mainly to the consolidation of evolutionary theory (e.g., models for molecular evolution), the development of new technologies (e.g., better computer microprocessors), and the access to new sources of data (e.g., molecular markers). Furthermore, phylogenetic principles have been refined and robust statistical methods have been implemented to select among phylogenetic trees. The phylogenetic thought has transformed systematics from a simple descriptive subject to a robust predictive and interpretative science. According to Cracraft (2002), systematics attempts to answer seven great questions: What is a species? How many species are there? What is the tree of life? What has been the history of character transformations? Where are Earth's species distributed? How have species distributions changed over time? And how is phylogenetic history predictive? It is possible that phylogenetic systematics currently is one of the most integrative subjects in the biological sciences. Phylogenies have become the base for analyses of behavior, biogeography, development, ecology, paleontology, etc., that are enclosed within an evolutionary framework. At the end, phylogenetic trees have become the backbone to infer evolutionary hypothesis.

This chapter is written mainly for nonsystematists; therefore, the goal is to explain how systematists propose hypotheses about the evolutionary history of lineages (i.e., phylogenies). In a nutshell, this chapter presents a brief, but comprehensive description of the theory and methods currently used in systematic biology. The reader interested in a more thorough explanation of specific subjects must refer to the original sources listed in the references or to specialized books on phylogenetic inference (e.g., Hillis et al. 1991; Nei and Kumar 2000; Felsenstein 2004; Cadotte and Davies 2016).

3.2 Basic Phylogenetic Background

Any biological interaction that we observe is shaped by two components: ecologic and historic. In nature, the organisms are not isolated, they are constantly interacting with other members of the population, with individuals of other species, and are affected by environmental factors (see Chaps. 7 and 9). In essence, the biodiversity of a place is assembled through the interaction of natural forces like mutation, genetic drift, migration or gene flow, and selection. At the end, such forces modify the reproductive output of the individuals, and, during the course of generations,

they affect the frequency and distribution of characters within and among populations. It should be possible, therefore, to unveil a historical pattern of character transformation. Phylogenetic trees are hypotheses, based on character analysis, for explaining the historical component of lineage evolution.

Speciation is a fundamental process in evolutionary theory, in which a lineage (population) splits giving origin to two or more new species (Mayr 1993). The general speciation model (Fig. 3.1a) states that new species are originated when a barrier rises splitting an ancestral population into two or more descendant populations. The barrier must suppress or at least reduce the gene flow among population. Then, the evolutionary forces (i.e., mutation, genetic drift, selection) act upon the individuals transforming, removing, or fixing characters differentially. Based on the Biological Species Concept (Mayr 1982, 2000), new species appear once reproductive isolation barriers are formed among independent lineages. In phylogenetic theory, nonetheless, reproductive isolation is irrelevant. Species differentiate once characters are fixed and lineages become fully diagnosable units (Cracraft 1983; Wheeler and Meier 2000). Those fixed characters, therefore, are the essential

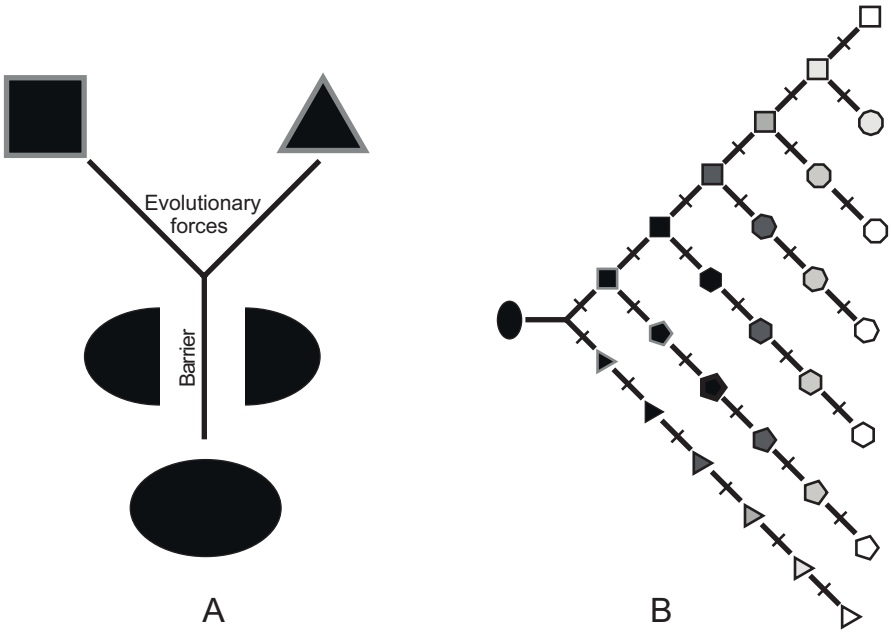


Fig. 3.1 General speciation model. (**a**) A barrier appears fragmenting a panmictic population; the barrier disrupts gene flow while the evolutionary forces act differentially on the isolated populations favoring character transformation. The speciation process starts with the fluctuation of the character-states frequency and ends once the states are differentially fixed in the daughter populations giving birth to new diagnosable independent lineages. (**b**) Through time, some lineages may experience character transformation without losing their historical identity (i.e., anagenesis; not changing symbol), whereas other lineages diversify (i.e., changing symbol) incrementing the biodiversity

phylogenetic markers that allow systematists to infer the evolutionary interrelationships of lineages (Fig. 3.1b).

The use of characters as the core for phylogenetic analysis is based on the principle of homology. In biological terms, homologous characters are those features that have a common origin independently of their current form or function (e.g., scales, feathers, and hairs). In phylogenetic theory, characters shared by different taxa are homologous if these traits were inherited from a common ancestor. In contrast, two or more taxa might share a similar character (in anatomical position, form, or function) that has evolved independently, in other words, it was not inherited from a common ancestor (e.g., wings in insects, bats, and birds; eyes in vertebrates and mollusks) this is known as homoplasy. A character is any feature own by an organism that can be observed, measured, diagnosed, identified, etc. (e.g., eye coloration, body length, number of cervical vertebra, etc.). Among lineages, characters may be constant (show no variation), or variable. Such variation is what is called character states (e.g., eyes coloration: blue/brown/red, number of chromosome pairs: 23/24, vacuoles: presence/absence, position of gametocyte: central/polar/subpolar, etc.). In a transformation series, the ancestral character state is called "plesiomorphic", whereas the derived trait that has evolved from the plesiomorphic character is called "apomorphic" state. Thus, a "synapomorphy" is a derived character state shared by two or more lineages, while a "symplesiomorphy" is an ancestral character state shared by two or more lineages. Symplesiomorphies are traits inherited from an ancestor older than the most common recent ancestor of the lineages that share such trait. In phylogenetic systematics, only the synapomorphies can be used to establish hypotheses about common ancestry between taxa or lineages. Characters appropriate for phylogenetic analysis must be variable among taxa/lineages, and must be coded in the genome so that they can pass from ancestors to descendants. Molecular markers (e.g., nucleotide sequences, enzymes, amino acids) fit both conditions, this is one of the reasons for their massive use in phylogenetics (Avise 2000, 2004).

3.3 Recovering Phylogenies

Phylogenetic inference takes place in three stages. The first stage is character sampling. The selection of taxa will depend on the question that the phylogeny will try to answer. Ideally, each taxon (a.k.a. Operational Taxonomic Unit, or OTU) should be represented by a series of individuals. This is necessary to ensure that all the variations encompassed within that unit can be registered. Then characters are sampled depending on the variation observed (a "potential" phylogenetic informative character must show more variation among OTUs than within an OTU). Variable characters are gathered in a matrix for phylogenetic analysis (Fig. 3.2). The structure of the matrix is very simple: columns contain each individual character, and rows start with the taxon name followed by the respective character states. As a convention, nonmolecular characters are labeled with integers starting with 0 for the

	Taxon \ Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Outgroup	1	2	0	1	0	0	0	1	0	2	0	0	2	0	1	0	0	2	0	0
2	Haemoproteus columbae	1	0	0	0	1	0	0	1	2	2	0	2	0	0	0	2	2	1	1	0
3	Haemoproteus iwa	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
4	Haemoproteus lanii	1	0	0	0	1	0	0	1	1	0	2	0	0	0	0	0	0	1	0	0
5	Haemoproteus pallidus	1	0	0	0	1	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0
6	Leucocytozoon lovati	1	1	0	0	0	1	1	1	0	2	1	2	0	1	0	0	0	0	0	1
7	Leucocytozoon majoris	1	1	0	0	0	1	1	1	0	2	2	0	0	0	0	1	1	2	0	0
8	Leucocytozoon toddi	1	1	0	1	0	1	1	1	2	1	0	1	0	0	0	0	?	2	0	2
9	Plasmodium globularis	1	2	1	0	0	1	0	0	0	2	0	1	1	0	0	0	1	?	0	2
10	Plasmodium lucens	1	2	0	2	0	1	0	0	1	0	1	1	1	1	0	0	2	2	0	2

Fig. 3.2 Character matrix for phylogenetic analysis. Rows are taxa, whereas columns are characters. Standard characters, as convention, are coded by numbers (starting with zero). The numerical sequence does not necessarily indicate the transformation order. Character 1 is nonvariable; characters 3 and 19 are autapomorphies for *Plasmodium globularis* and *Haemoproteus columbae*, respectively. Those are examples of uninformative characters; the remaining characters in the matrix are potentially informative characters. Binary characters are those that only present two alternative states (e.g., Ch5 to Ch7). Traits with more than two states are known as multistate characters (e.g., Ch2, Ch10, Ch15). The lack of information in a taxon for a specific character is generally coded as a question mark and is known as a missing data (e.g., Ch17 for *Leucocytozoon toddi*)

first state observed for that character. In most methods for character analysis, the labels do not indicate a sequential order (i.e., transforming from state 0 to state 3 does not require to go through states 1 and 2, it only implies one evolutionary change). There is no limit in the number of characters or taxa that the matrix may encompass, studies suggest that more data results in a more robust and consistent phylogeny (Huelsenbeck et al. 1996; Graybeal 1998; Barker and Lutzoni 2002; Purvis and Agapow 2002).

The second stage of phylogenetic inference is the reconstruction *per se*. In essence, this is a very simple procedure. The procedure of reconstruction consists on inferring all the possible evolutionary interrelationships among the taxa encompassed in the analysis. Those interrelationships can be drawn in two forms of trees: unrooted (a.k.a. nets or networks; Fig. 3.3a) and rooted (a.k.a. cladograms or phylogenetic trees; Fig. 3.3b). A tree is a branching diagram that most of the time shows a bifurcated pattern; however, any tree might display reticulation and/or splitting events originating more than two branches (i.e., polytomies). In graphic theory, splitting locations are called nodes, and in evolutionary terms represent speciation events. Therefore, the single line before the node represents the hypothetical ancestor, whereas the two (or more) lines after the node are the descendant species. The species that originate from a single speciation event (node) are called sister lineages (Fig. 3.3). Any phylogenetic tree depicts a hierarchical structure because the speciation process yields to sets of successive descendants from a line of more inclusive ancestors. A cluster of lineages recovered in the phylogeny that includes the hypothetical ancestor and all its descendants is known as a monophyletic group. The number of monophyletic groups encompassed in a fully resolved (dichotomous) phylogeny is equal to the number of nodes in that tree. An unrooted tree is a

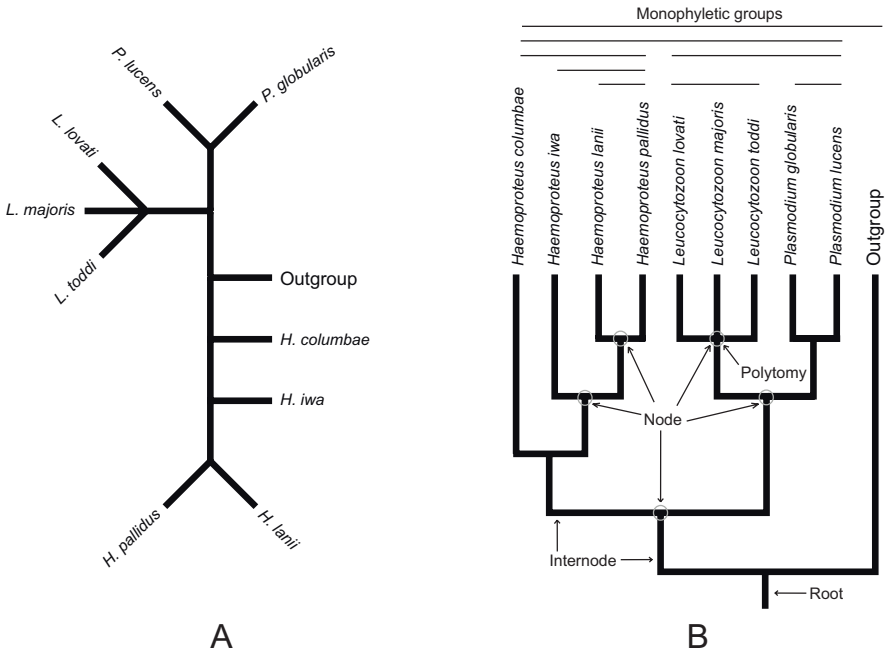


Fig. 3.3 Anatomy of a phylogenetic tree. (**a**) Unrooted cladogram also called net or network; (**b**) rooted cladogram. The difference between the two phylogenetic trees is that the rooted phylogeny has a dimension of relative time where the past is indicated by the root, and the present by the tips if they represent extant taxa. Internodes represent hypothetical ancestors; therefore, two lineages that originate in a single speciation event (node) share the same most recent common ancestor. Those lineages are called sister taxa (e.g., the genus *Plasmodium* is the sister lineage of the genus *Leucocytozoon*; *Haemoproteus lanii* is the sister taxa of *H. pallidus*)

hypothesis of genealogical relationships that is not polarized in time. Nets are useless to infer the evolutionary history of characters. Unrooted phylogenies do not tell us which state is plesiomorphic, nor which is apomorphic. Instead, a rooted tree shows temporal direction, being the past at the base (root) of the tree, and the present at the tip of the terminal branches. Such time, nonetheless, is relative. An upper node is a more recent speciation event than that depicted by a lower node, but when exactly these events took place is unknown. Several methods for rooting phylogenetic trees have been proposed (e.g., fossils, ontogeny, assumption of character transformation); however, the most commonly used strategy is the outgroup method. The outgroup is any taxon or taxa that do not belong to the group involved directly in the systematic problem (the ingroup). The best outgroup should be the sister taxon of the ingroup. Though, due to the fragmented knowledge that we have about the tree of life that is not always a feasible option. Outgroup selection might be a crucial decision for reconstruction. The use of distantly related taxa as outgroups might produce a negative effect for inferring the phylogeny. The outgroup taxa play the same role as the other taxa during the process of phylogenetic reconstruction.

This reconstruction can be achieved by three alternative techniques. The exhaustive reconstruction implies the inference of all possible topologies involving the complete taxon sampling. For instance, in the case of three taxa, there is only one unrooted tree, but three different rooted trees (Fig. 3.4). For four taxa, there are three unrooted trees, but 15 rooted trees. On the one hand, this technique has the advantage that the full universe of possible trees is evaluated; thus, it finds the best-fitted evolutionary hypothesis. On the other hand, it has the disadvantage that the number of possible trees increases exponentially with respect to the number of taxa (Table 3.1). The total evolutionary interrelationships for 20 taxa are 8,200,794,532,637,891,559,375 trees! Therefore, even for a moderate number of taxa, the exhaustive reconstruction is hardly implemented. An alternative for the reconstruction is the technique called Branch & Bound. Here, four taxa are selected at random and the three unrooted trees are assembled. Each one is evaluated and the best-fitted tree is selected. Then, randomly, a new taxon is drafted and integrated to the best-fitted tree selected during the previous step to construct the possible topologies. Once again, these trees are evaluated, the best-fitted one is selected, and remaining ones are discarded. This process is iterated until all the taxa have been integrated to a final phylogeny. Although Branch & Bound also ensures to find the

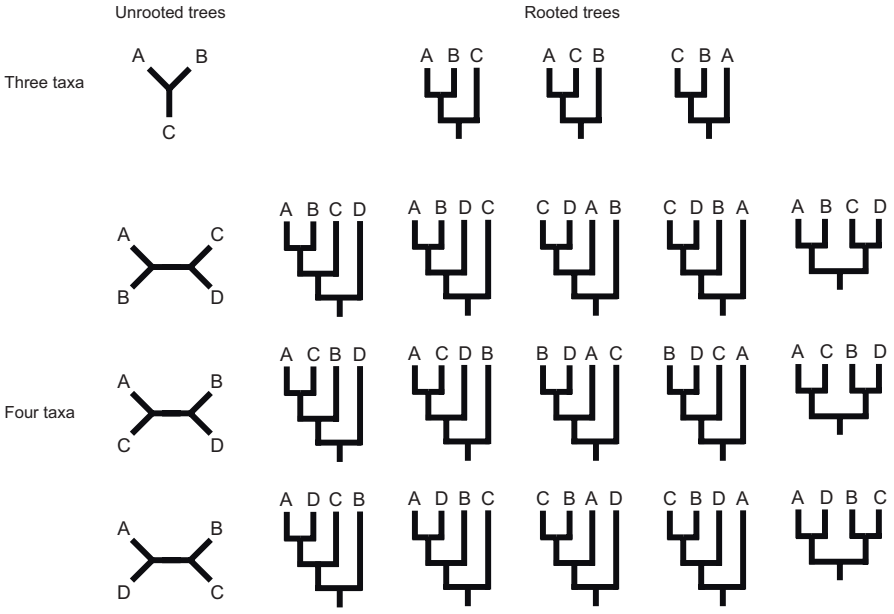


Fig. 3.4 Exhaustive reconstruction for three and four taxa. The number of possible different phylogenies increases rapidly as with the number of taxa involved in the analysis (see Table 3.1). Although exhaustive reconstructions are the only way to find the entire optimal phylogenetic hypothesis, the time required to run this kind of analysis makes them an impractical option even for a small number of taxa. For that reason, most current phylogenetic software does not include exhaustive searching algorithms

Table 3.1 Number of unrooted and rooted trees

Number of taxa	Unrooted trees[a]	Rooted trees[b]	Reconstruction
3	1	3	Exhaustive
4	3	15	Exhaustive
5	15	105	Exhaustive
6	105	945	Exhaustive
7	945	10,345	Exhaustive
8	10,395	135,135	Exhaustive
9	135,135	2,027,025	Exhaustive
10	2,027,025	34,459,425	Exhaustive
20	2.2×10^{20}	8.2×10^{21}	Branch & Bound
30	8.6×10^{36}	4.9×10^{38}	Heuristic
40	1.3×10^{55}	1.0×10^{57}	Heuristic

Based on Felsenstein (1977)
[a]Number of unrooted trees for n taxa Nu = $(2n-5)!/[2n-3*(n-3)!]$
[b]Number of rooted trees for n taxa Nr = $(2n-3)!/[2n-2*(n-2)!]$

best-fitted tree, the number of possible evaluation paths might be practically "infinite." Thus, most analyses achieve reconstruction by a heuristic technique. This procedure involves the inference of a starting tree that can be obtained by randomly assembling one, or with the aid of some distance algorithm [e.g., Wagner (Farris 1970), neighbor-joining (Saitou and Nei 1987)]. The probability to find the best-fitted tree at random is $1/((2n-5)!/(2n-3 * (n-3)!))$, where "$n$" is the number of taxa. Most of the time, distance algorithms recover a much better tree; nonetheless, this is not necessarily the best-fitted tree. The heuristic reconstruction can be improved by assembling the starting tree multiple times (thousand and even millions of times). At the end of these iterations, the better-fitted tree is retained as the final solution. A complementary strategy is the "branch swapping" technique. Here, the starting tree is bisected and the detached branch is attached to a different section of the tree. The new tree is retained if it is better than the previous one (which is rejected).

The third and final stage of phylogenetic inference is the use of an optimality criterion. Usually, the second and third stages are performed simultaneously. In general, the different optimality criteria provide a measure of the fit between the character matrix and a particular topology. Based on the principle of homology, each character in the matrix represents an individual hypothesis of relationship among the taxa (Fig. 3.5a–d). Species sharing the same character state should be more closely related to each other than to those sharing an alternative state. The assumptions outlined in the optimality criterion are used to validate or invalidate this primary hypothesis of evolutionary relationship. For many years, the optimality criterion most commonly used was parsimony. "*Entia non sunt multiplicanda sine necessitate"* this is a philosophical and methodological principle postulated by the English Franciscan friar William of Ockham (1285–1347). In science, the "principle of economy" (a.k.a. Occam's Razor) can be stated as: if multiple hypotheses can explain the existing data, the simplest possible hypothesis should be accepted. In

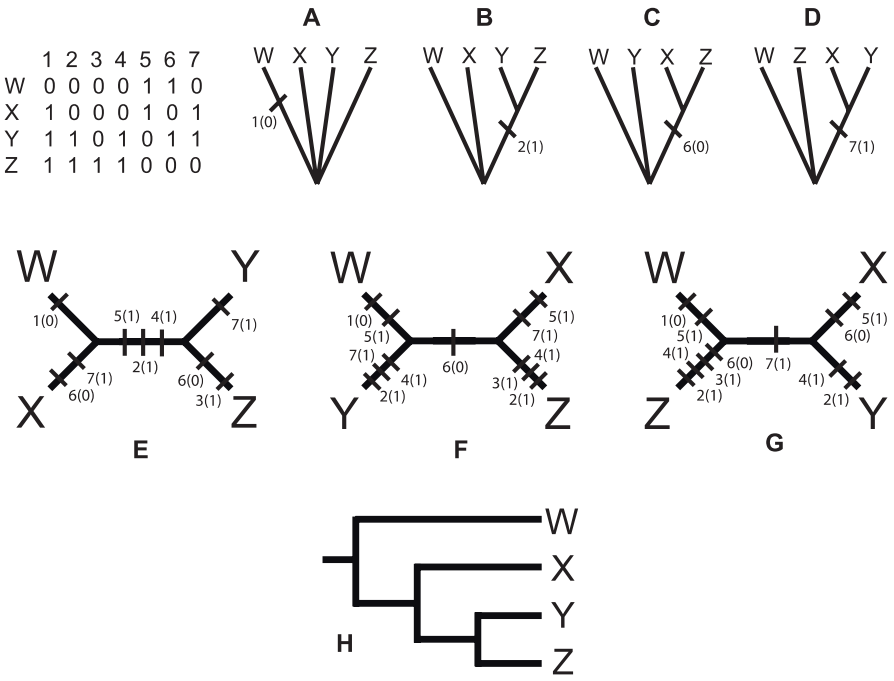


Fig. 3.5 Phylogenetic inference process. A set of taxa is selected, and a set of variable characters is sampled to assemble the phylogenetic matrix. Each character of the matrix is an independent hypothesis of phylogenetic relationships. (**a**) Autapomorphies differentiate terminal units but do not depict putative interrelationships among taxa; for this reason, autapomorphies are considered noninformative phylogenetic characters. Characters 2, 6, and 7 are potentially phylogenetic informative characters; nonetheless, they describe mutually excluding hypothesis of relationship. Character 2 implies a close relationship between taxa Z and Y (**b**), character 6 infers that the sister taxon of Z should be X (**c**), and character 7 contradicts the previous hypothesis by suggesting that X and Y share a more recent ancestor with each other than with any other taxa (**d**). The way to solve the conflictive information provided by the individual characters is to evaluate the congruence of all the characters in each phylogeny. The exhaustive reconstruction produces three different phylogenetic hypotheses (**e**–**g**) on which the character transformation is optimized. Based on the maximum parsimony criterion, the optimal phylogeny is (**e**) (9 steps against 11 steps for **f** and **g**). Finally, to obtain the best-fit phylogenetic hypothesis, the network is rooted on the outgroup (**h**)

systematics, this criterion maximizes the congruence of synapomorphies; therefore, it minimizes evolutionary convergence and parallelism. Thus, based on the parsimony criterion, the best-fitted phylogeny must maximize homology and minimize homoplasy. To accomplish this, the characters are optimized on each alternative tree (Fig. 3.5e–g). The optimization consists in finding the minimum possible number of times that a character state appears on the tree. For instance, let us consider a matrix of 10 taxa; for the first character, taxon A and E are the only ones sharing state 1; only on the trees holding these two taxa as sister groups, the state 1 will be a synapomorphy (would appear once in the evolutionary history of those taxa). In any other tree, the state 1 would have appeared twice (an evolutionary parallelism). The

number of times that the state appears in the tree is called the length of the character or the evolutionary steps of the character. The length of the tree, therefore, is the number of evolutionary steps (changes) required to explain the complete character matrix. The most parsimonious phylogeny is then the shortest tree. At this point, the result of the phylogenetic inference is an unrooted tree. Finally, the root of the tree is inferred by choosing any member (or group of members) of the outgroup and setting the base of the tree at that point (Fig. 3.5h).

3.4 A Century of Molecular Data

Systematists have used morphological characters to classify the biodiversity even before biological sciences adopted an evolutionary thought. However, very little is known about the rate of transformation of these kind of characters, the processes leading to its expression, and if the observed character estate is even coded in the genome or simply is the outcome of environmental factors. Molecular markers are the most recent source of data incorporated in phylogenetic inference. Surprisingly, the evolutionary knowledge about molecules is more extensive than to any other kind of characters used before. The used of molecules to understand the possible biological interrelationships among the living beings is not as recent as many think. Nuttall (1904) published one of the early studies on this regard. With the aim of estimating a measurement of relatedness between different species, he compared the intensity of the immunological reaction of one organism to blood serum extracted from other organisms. Very few papers were published during those initial years. It was after the middle of the twentieth century that molecules began to be frequently used. This was due to the development of simple isolation methods, low cost technology, and the consolidation of phylogenetic theory. In a few decades, molecular studies have gone form single genes (e.g., Espinosa de los Monteros 2000) to multiloci studies (e.g., Borner et al. 2016), and complete genomes data sets (e.g., Johnson et al. 2018).

In a broad sense, the age of molecular markers might be divided in the period of macromolecules (protein and amino acids) and the period of nucleotides (AFLPs, sequences, microsatellites). Allozyme and isozyme studies for population genetics and systematics were common from the early 1960s until the end of the 1990s. The variation contained in these macromolecules is adequate to undertake problems at population level like genetic differentiation (e.g., Baker 1982; Rockwell and Barrowclough 1987) and species delimitation (e.g., Avise et al. 1982; Gutierrez et al. 1983). However, the information provided by these macromolecules is not sufficient to answer higher-level systematics questions. Unfortunately, studies based on amino acid sequencing never really took off. Although, there are 20 essential amino acids, and proteins might be formed by several hundreds of them, selection pressure has constricted variation in proteins. Therefore, to gather enough variable characters is too expensive and the effort is not worth it. The first attempt for implementing nucleotide data was the DNA-DNA hybridization technique. This method

is based on denaturing the double helix of several taxa and using the strand of a reference taxon to hybridize the complementary strands of the other taxa. Denaturing the DNA consists in breaking the hydrogen bonds between complementary nucleotides (i.e., three between G–C pairs, and two between T–A). In the laboratory, the simplest way for denaturing DNA is with heat. Then, once the hybrid DNA helixes are formed, heat is applied to denature them. The more closely related the target species is to the reference taxon, less difference exists between the nucleotides in the hybrid helix. Therefore, more heat is required to denature those hybrid helixes. The amount of heat is recorded and used as a measure of relatedness between taxa; then, a distance matrix is assembled and a neighbor-joining algorithm is used to construct the phylogeny. In some sense, this technique could be considered as the first phylogenomic method; nonetheless, the distance obtained in DNA-DNA hybridization is not appropriate for phylogenetic inference (Lanyon 1992). During the 1980s, restriction site analyses were implemented for phylogenetic inference. This method is based on digesting genomic DNA with specific restriction enzymes and to score the resulting band pattern. The enzymes identify precise DNA sequences (i.e., restriction sites) that usually are 4–15 base pairs in length. For instance, the enzyme HpaI recognizes the sequence GTTAAC; if this sequence is present anywhere in the genome, the enzyme cuts the DNA on that site producing as many fragments of DNA as restriction sites exist. If in one taxon, the restriction site is missing, the result would be a DNA fragment of a size equal to the combined length of the two fragments that would be produced in the species where the site is present. After digesting the genome with many restriction enzymes, a matrix of presence/absence for the sites is assembled.

Many alternative molecular markers have been identified for phylogenetic use. By far the data sources based on PCR (Polymerase Chain Reaction) are definitely the most extensively used. Next some of the most popular molecular markers are presented:

1. RAPDs – The Random Amplification of Polymorphic DNA was one of the initial PCR methods. It is similar to the restriction site technique, but instead of enzymes for RAPDs, general oligonucleotides (a.k.a. primers) are used to amplify random DNA fragments. The PCR products are subjected to electrophoresis in agarose gels, and a semiunique pattern of amplified fragments can be observed. Again, a presence/absence matrix is assembled suitable for phylogenetic inference. The problem with RAPDs data is that primers bind somewhere in the genome, but it is not certain exactly where. Therefore, the amplified fragments although might have the same size not necessarily belong to the same part of the genome. Another problem is that the PCR experiments show very low repeatability results.
2. AFLP – In contrast, Amplified Fragment Length Polymorphism is a highly specific and reproducible technique. First restriction enzymes are used to digest genomic DNA; then, a particular DNA sequence (adaptor) is ligated to one end of the restriction fragments. Complementary primers to the adaptor are used to amplify the restriction site and several adjacent nucleotides. During primer

synthesis, a fluorescent marker is added that allows visualizing the AFLP amplification via automated capillary sequencing.

3. Microsatellites – These are repetitive DNA motifs. They are formed by 1–6 base pairs repeated usually from 5 to 50 times. Microsatellites may occur at thousands of places in the genome. These molecular markers have one of the highest mutation rates in the DNA, which allows finding differences between closely related individuals in a population. Microsatellites are also named as short tandem repeats (STRs), or as simple sequence repeats (SSRs). Microsatellites are generally used in genetics to infer interactions between populations such as gene flow, endogamy, genetic structure (F_{st}), and geographic structure.
4. Direct DNA Sequencing – Frederick Sanger and colleagues developed this method in 1977. Several modifications have been done through time, but in essence, it is the major source for phylogenetic data. After DNA isolation and purification, a first PCR amplification is performed using flanking primers for the target fragment. This first step is necessary to produced abundant template for the subsequent nucleotide labeling. This is the second step in the sequencing method. At the beginning, this labeling was done using radioactive sulfur (S_{35}) or phosphorous (P_{32}); therefore, enforcing special safety lab precautions and regulations. Fluorescent labeling, thus, was a major breakthrough. A second PCR is performed adding to the base mixture a set of dideoxynucleotides. These are modified nucleotides missing the –OH group at carbon 3 and a fluorescent-labeled base (each base has a distinct label). Once one of the dideoxynucleotides is incorporated during the PCR, the DNA extension is stopped due to the lack of the 3′-OH. The random incorporation of dideoxynucleotides produces DNA fragments ranging from 1 bp to the full length of the target segment. Finally, the PCR products are subjected to capillary electrophoresis separating DNA fragments by length. The polymer inside the capillary tube acts as a mesh allowing the shorter fragments to move faster than the longer ones. At present, molecular data is been produced massively around the world; literally, millions of base pairs are sequenced every day.

One final consideration, before attempting any analysis with molecular data, it is important to have some level of confidence about the identity of the data. Otherwise, a great source of error is introduced in the phylogeny. Many potential sources of contamination exist. If the PCR uses universal primers (i.e., primers that hybridize and amplify a wide range of taxa), there is always the risk that some products might belong to alien genomes (see Chap. 4 for details on contamination and guidelines for molecular analyses in reference to avian haemosporidians). It is not rare that during DNA isolation, tissue belonging to endosymbionts (Smith et al. 2012), endoparasites (Biedrzycka et al. 2016), or ectoparasites (Campana et al. 2016) is extracted along with the host species. Also, translocated DNA fragments (i.e., pseudogenes) are very common features in both plant and animal genomes (Tutar 2012). Informatics tools such as BLAST (Altschul et al. 1990) provide basic information about the data such as gene identification and taxon relationship.

3.5 Sequence Alignment

As for any other phylogenetic analysis in molecular systematics, the first task is inferring homology hypothesis within characters. This is not trivial for molecular data. One difference between nucleotide data and other character sources is that the former always and only has four alternative states (five if the in-Del is considered as an alternative state). Then, the primary hypothesis of homology is set by the alignment of the sequences (Fig. 3.6). Sometimes, building a reliable alignment can be

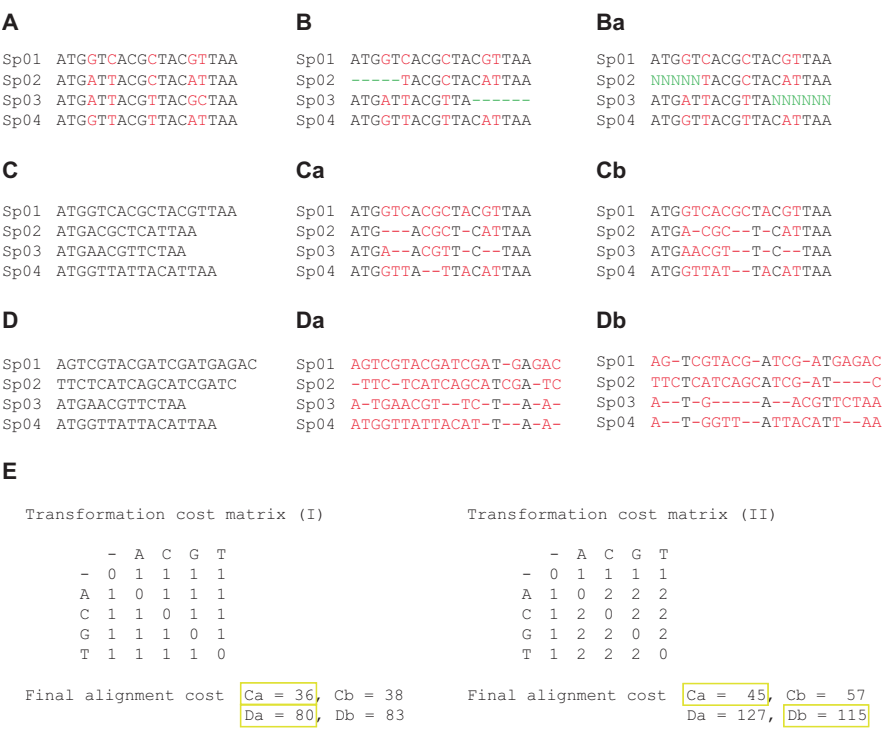


Fig. 3.6 Multiple sequence alignment. In molecular characters, the homology is established by the alignment of data. Some data sets can be align by eye simply by adding In-Dels (–) or "N" to fill out the sections with missing data (**A**, **B**); complex data sets (**C**, **D**), however, require the use of alignment software. The final alignment is selected after applying transformation cost matrices (**E**) to the alternative alignments produced by the software. To compute the cost, each position is evaluated for all possible changes produced by the alignment. In alignment **C**a, the first three positions are constant (zero cost each); however, the fourth position implies five changes (G to –, G to A, – to A, – to G, and A to G). Thus, by applying the cost scheme of transformation matrix I, the fourth position implies a cost of 5. The final cost for alignment **C**a will be the sum of the cost of each position (36 based on matrix I). Finally, alternative alignments are evaluated based on the same parameters (e.g., **C**b), and the least expensive alignment is chosen. If matrix I is used, **C**a (36) is preferred over **C**b (38) and **D**a (80) is preferred over **D**b (83). Changes in the cost matrix may recover different results. If the cost scheme of transformation matrix II is used, **C**a is again preferred over **C**b, but this time **D**b is preferred over **D**a

simple. For instance, mitochondrial protein–coding genes have continuous reading frames. So, to align the 1143 base pairs (bp) of the cytochrome-*b* gene (this is the standard length of the avian cytochrome *b*), the initial methionine codon (i.e., ATG) must be localized for identifying the first three positions of the sequence (Fig. 3.6A). However, sequences could have different lengths, making the alignment process more complex. Difference in length can be the result of technique problems (e.g., data is unreadable at the beginning or the end during capillary electrophoresis, Fig. 3.6B). In such cases, the alignment can be done by eye simply by moving the sequences in the matrix to the front or to the end until the nucleotide matching is maximized. In many other cases, the difference in length is intrinsic to the genome region (Fig. 3.6C, D). Sections encompassing noncoding genes (e.g., transfer RNA, ribosomal RNA, interspace sequences) and intron-exon coding genes have such attribute. The fixation rate of mutations in noncoding regions can be very high. When the mutation implicates multiple nucleotide insertions or deletions, the difference in length might involve hundreds of base pairs. Aligning those sections might be particularly hard because multiple possible alignments can be produced (Fig. 3.6Ca–Db). Therefore, the alignment process is usually done with the aid of alignment software (e.g., MUSCLE, Edgar 2004; CLUSTAL, Larkin et al. 2007). The way these alignment algorithms work can differ. In general, they use a matrix of costs based on nucleotide transformation and in-Del addition (Fig. 3.6E). Pairs of sequences are aligned trying to maximize the nucleotide match between them. The costs of the alignments are computed. The least expensive pairs of alignments are reassembled into a single alignment, and the process is iterated until all the sequences have been added. Unfortunately, there is no rule for assigning the cost values in the matrix, and the user modifies those values according to their personal preferences. In many cases, the default settings in the software are used without change. Furthermore, because the amount of initial combination of sequence-pairs increases very fast with respect to the number of the sequences involved, the algorithms not necessarily recover the best possible alignment. Systematists, therefore, usually amend by eye the alignment assembled by the computer software. Repeating multiple independent times, the alignment procedure is a way for testing the alignment. Alternatively, for coding regions, further corroboration could be achieved by translating the nucleotide sequence to the amino acid sequence, in which the characters must show a better match (proteins are more conserved than nucleic acids).

3.6 Nucleotide Sequence Analyses

As mentioned previously, the last step during phylogenetic inference is the use of an optimality criterion for tree selection. All the different criteria are based on particular assumptions about the evolutionary model for character transformation. Therefore, the selection of the best-fit model for the data set is decisive in the final result (Buckley 2002; Kolaczkowski and Thornton 2004; Lemmon and Moriarty

2004; Piontkivska 2004; Goloboff et al. 2018). The main assumption in Maximum Parsimony is that any character state can transform to any other state with the same probability independently from the character kind or the number of states in the character (Fig. 3.7a–d). Therefore, the obvious question is: Do molecular markers comply with such evolutionary model? Figure 3.7e shows an alignment of eight sequences. After close examination, it is obvious that not every nucleotide position changes. The fragment encompasses 92 nucleotides from which 23 (25%) are variable positions. That variation is not random, a pattern of nucleotide transformation can be observed. Most of the changes occurred in third position (20 changes), a few in first position (3 changes), and none in second position. These sequences belong to the mitochondrial gene cytochrome oxidase subunit I (COI), which is a protein-coding gene. Each amino acid is codified by a set of three nucleotides (codon); thus, the genetic code contains a total of 64 codons. This implies that some of the 20 essential amino acids are codified by more than one codon. For instance, Threonine is codified by four codons (i.e., ACU, ACC, ACA, and ACG). For this example, a change in third position does not produce an amino acid change. These silent mutations are easily fixed in the genome because they do not have an effect on protein structure or function; therefore, they are not subjected to natural selection. Some

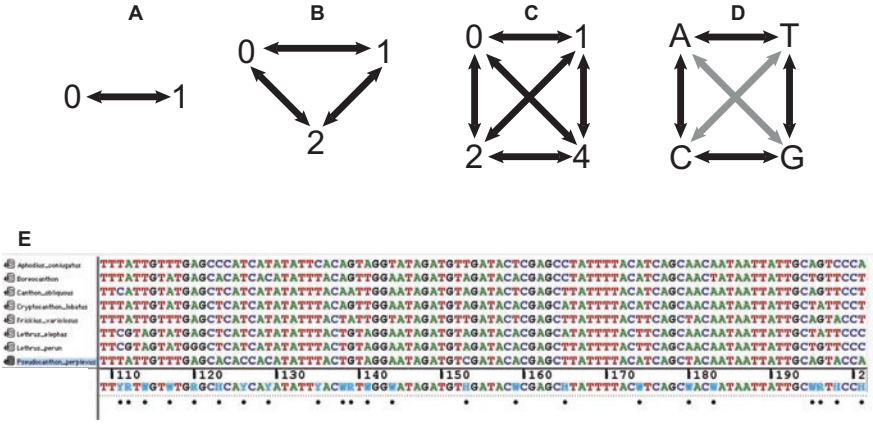


Fig. 3.7 Scenarios for character state transformation. Arrows indicate the possible paths of state transformation. Unrestricted scenarios allow the transformation between any states without the need of going through intermediate states. The number of paths rapidly increases with the number of states: 2 for a binary character (**a**), 6 for a three state character (**b**), 12 for a four state character (**c**), 20 for five states, etc. Nucleotides are a four states character (**d**); however, the transformation probability may change depending on the specific path (e.g., transition [gray path] vs. transversion [black path]). Multiple sequence alignment (**e**) showing alternative nucleotide transformations. A consensus sequence is shown below the numbers, variable positions are identified by a black dot; Blue letters: R = transition between purines G–A; Y = transition between pyrimidines T–C; W = transversion between A–T; H = transformation among A–C–T; other transformation codes can be: M = A–C, K = G–T, S = C–G, B = C–G–T, V = A–C–G, D = A–G–T, and N = A–C–G–T

codons also show this "buffer" if the change takes place on first position (e.g., CGG and AGG codify for Arginine, UUA and CUA for Leucine). Instead, any change in second position results in an amino acid change. Depending on the molecule region, some structural genes such as transfer-RNA or ribosomal RNA also have a differential fixation rate of mutations. The secondary structure of these markers forms paired sections (stems) and unpaired ones (loops), being the former more conserved than the latter (Espinosa de los Monteros 2003). Even in genome regions that are strictly noncoding frames, the fixation rate is not homogeneous.

The genome size is highly variable. For instance, in average, mycoplasmas have 5×10^5 nucleotides in their genome, whereas *Homo sapiens* has 3.4×10^9 bases, and *Amoeba dubia* has 6.7×10^{11} nucleotides (Li 1997). In some way, the genome has been considered as an infinite source of data. If the probability of state transformation would be constant and equal for any character (position in the case of nucleic acids), it would be expected that in such "infinite model", the frequency of each state would be equal to the inverse of the number of character states. For nucleotide sequences, therefore, the base proportion should be 0.25 for that neutral and infinite scenario. Again, by examining the alignment in Fig. 3.7e, the nucleotide frequency of *A* is 0.32, *C* is 0.16, *G* is 0.15, and *T* is 0.37. There is a clear bias toward TA (more than twice of GC). Such nucleotide bias is a common feature in protein-coding genes and in some structural noncoding sequences (Singer and Hickey 2000; Albu et al. 2008; Wang 2010). Natural selection and genetic drift are the two main evolutionary forces responsible for such bias (Albu et al. 2008).

Furthermore, the transformation among the different nucleotide states is not symmetrical. The nucleic acids are conformed by two different kinds of nucleotide bases: purines and pyrimidines. The molecular structure of purines (adenine and guanine) is formed by two-carbon nitrogen rings, whereas pyrimidines (thymine and cytosine) have only one-carbon nitrogen ring. For a four state character, there are six general possible transformation paths (Fig. 3.7c). A mutation that implicates the same kind of molecule is known as "transition" (G–A and T–C), while a transformation between alternative kinds is called "transversion" (A–C, A–T, G–C, and G–T; Fig. 3.7d). The dynamics of nucleotide substitution together with assumptions regarding the probability of transformation from one nucleotide to another are the models of nucleotide substitution (Table 3.2). Many of these probabilistic models have been proposed in the literature (Zharkikh 1994; Whelan et al. 2001; Posada and Buckley 2004; Yang and Rannala 2012). The simplest model assumes no bias in the path of transformation between bases; therefore, the mutations occur randomly among the four nucleotides. If α is the transformation probability in each of the three possible paths leaving any base, then the substitution rate for each nucleotide would be 3α. Because only one probability (α) is assumed, this is known as the one-parameter model (Jukes and Cantor 1969). When nucleotide data fit the Jukes-Cantor model, Maximum Parsimony and other probabilistic methods converge on the same phylogenetic tree. This is not surprising because the evolutionary assumptions in Maximum Parsimony are the same as in the Jukes-Cantor model. In many cases, however, the assumption that all nucleotide transformations occur at the same rate is unlikely. The substitution matrix for the sequences

Table 3.2 Examples for models of nucleotide substitution

Jukes-Cantor (nst 1)					Hasegawa-Kishino-Yano (nst 2)				
	A	T	C	G		A	T	C	G
A	–	α	α	α	A	–	βf_T	βf_C	αf_G
T	α	–	α	α	T	βf_A	–	αf_C	βf_G
C	α	α	–	α	C	βf_A	αf_T	–	βf_G
G	α	α	α	–	G	αf_A	βf_T	βf_C	–
Kimura 2-parameter (nst 2)					Tamura-Nei (nst 6)				
	A	T	C	G		A	T	C	G
A	–	β	β	α	A	–	βf_T	βf_C	αf_G
T	β	–	α	β	T	βf_A	–	γf_C	βf_G
C	β	α	–	β	C	βf_A	γf_T	–	βf_G
G	α	β	β	–	G	αf_A	βf_T	βf_C	–
General reversible model (nst 6)					Unrestricted model (nst 6)				
	A	T	C	G		A	T	C	G
A	–	φf_T	δf_C	αf_G	A	–	χ	ε	ϕ
T	φf_A	–	γf_C	λf_G	T	φ	–	φ	μ
C	δf_A	γf_T	–	βf_G	C	δ	γ	–	η
G	αf_A	λf_T	βf_C	–	G	α	λ	β	–

Greek letters represent alternative substitution rates
f_A, f_C, f_G, f_T are the nucleotide frequencies for the respective nucleotides
nst number of substitution types

in the alignment of Fig. 3.7e is: 0.15 A–C, 0.12 A–G, 0.45 A–T, 0 C–G, 0.27 C–T, 0 G–T. Kimura (1980) was one of the first in considering such common unbalance in nucleotide transformation. He observed that transitions occurred more frequently than transversions. He proposed a two-parameter model of nucleotide substitution in which the transition rate at each nucleotide site is α per unit of time, whereas the transversion rate is β (Table 3.2). Nucleotide substitution models increase in complexity until every transformation path has its own particular rate. The extreme is reached in the "unrestricted model" where the mutation rate between two nucleotides is different depending on the direction of the transformation (e.g., the rate of going from A to C is not the same as of going from C to A).

The proportion of invariant sites in a sequence depends mainly on gene function. However, the positions that have a change probability greater than zero keep accumulating mutations through time. If sufficient time has passed, two sequences may have the same nucleotide not necessarily because they inherited that character state from a common ancestor, instead, this could be the result of multiple successive mutations. In the four states system, any base has at most three chances of transforming into a different nucleotide; however, any other change would result in the fixation of a nucleotide that was already present in that position. This process of molecular convergence is known as the saturation effect. Saturation not only obscures the amount of differences between taxa, if the wrong evolutionary criterion for tree selection is applied, saturation might mislead the phylogenetic signal.

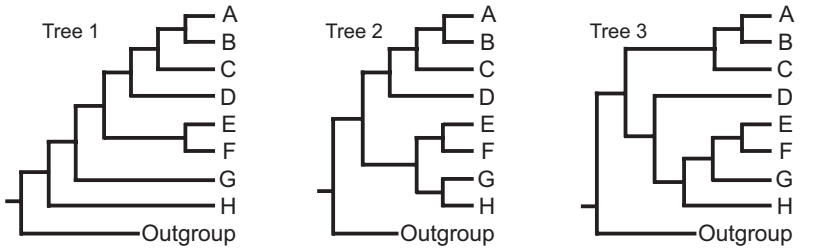
The fixation rate depends not only on the intrinsic properties of the genetic markers; it is affected by several factors like metabolic rate, generation time, behavior, and even the specific place where the population lives. Therefore, closely related lineages may have different fixation rates. Two distant lineages with high fixation rates might accumulate multiple spurious similarities to each other due to nucleotide saturation. Thus, if the branch length were proportional to the amount of mutations accumulated in that part of the tree, the two lineages with high fixation rate would have longer branches than any other section within the phylogeny. If the chosen optimality criterion enforces character state congruence (such as in Maximum Parsimony), the selected trees would be those having the two long-branch taxa as sister groups. In phylogenetic analyses, this phenomenon is known as the "long-branch attraction effect." It is possible that the long-branch attraction is the most persistent problem in systematic studies based on molecular data (Gribaldo and Philippe 2002; Baldauf 2003). This potential hazard is minimized by applying a tree selection criterion that fits the adequate nucleotide substitution model incorporating differential rates of replacement among lineages (e.g., Maximum Likelihood).

Because the aim of this chapter is to offer a general review of the current phylogenetic methods for nonsystematists, I will not go into the mathematical and statistical theory behind the algorithms that implement the criterion of Maximum Likelihood for tree selection. Instead, I will try to explain details that are not necessarily obvious or easy to understand. In practical terms, the criterion of Maximum Likelihood differs from Maximum Parsimony not only in using particular transformation rates adequate for each branch of the tree. Likelihood takes into account all the possible states that the hypothetical ancestor may have and how the transformation model affects the probability that the descendants have their specific observed nucleotide sequence. In the simplest example of two taxa and their ancestor, if the two terminals have the same state (e.g., G), the most parsimonious scenario would be that the ancestor had that state (G) and simply passed it to their descendants without implying any evolutionary change. Alternatively, if the terminals have different states (G and T), the most parsimonious scenario would be that the ancestor had one of the alternative states (G or T), and in time, the other lineage suffered a mutation (to T or G) adding one evolutionary step to the phylogenetic history. For the likelihood criterion, however, four alternative scenarios must be taken into account in any of those examples. For instance, if the terminals have different states (G and T), the final likelihood would be the probability for the scenario in which the ancestor has G affected by the transformation rate maintaining G in the first terminal times the transformation rate leading to T in the second terminal, plus the probability for the ancestor having T affected by the transformation rate changing to G in the first terminal times the transformation rate maintaining T in the second terminal, plus the probability for the ancestor having A affected by the transformation rate changing to G in the first terminal times the transformation rate changing to T in the second terminal, plus the probability for the ancestor having C affected by the transformation rate changing to G in the first terminal times the

transformation rate changing to T in the second terminal. Now, in the simplest non-trivial unrooted tree (i.e., four terminal and two ancestors), the number of calculations increases significantly because there are 16 potential scenarios for the combination of all the possible states in the ancestors. This is the likelihood for one character only. The likelihood for the full tree is computed as: $L = L_{(1)} * L_{(2)} * L_{(3)} * \ldots L_{(n)}$ where n is the number of positions in the nucleotide alignment. The value of L for each character is smaller than one; therefore, the likelihood of the tree (i.e., the product of the likelihoods at each site) is a very small number. Therefore, to avoid such tiny numbers, a mathematical trick is applied. The L for each site is transform to the natural log of that value ($\ln L$). According to the logarithm properties, the product of two numbers is equal to the sum of their logarithms. Thus, the $\ln L$ of the tree becomes a large number. Many algorithms do one more correction; the final $\ln L$ is multiplied by -1, so the value reported looks like $-\ln L$. This is basically a "user-pleasing" step; in this way, the best likelihood is the smallest number evoking the most parsimonious value. In the early 2000s, due to the amount of computations involved in the calculation of the Maximum Likelihood of a tree, a single search took several days. Therefore, for many years, this kind of analysis was avoided. However, current software such as GARLI (Zwickl 2006), RAxML (Stamatakis 2014), or IQ-Tree (Nguyen et al. 2015) can run several hundred iterations in a few hours.

3.7 Consensus Trees

In any phylogenetic analysis, the selection criterion may identify more than one optimum tree. If the results involve two or very few trees, it would be possible to present those trees as available alternative hypothesis. However, in parsimony analyses, it is not rare to recover several thousand, or more, equally parsimonious trees. In any case, the phylogenetic signal is summarized using the Consensus Tree method. Different types of consensus trees have been proposed (Swofford 1991); however, the most commonly used are the strict (Sokal and Rohlf 1981) and the majority-rule consensus tree (Margush and McMorris 1981). To construct these two varieties of consensus, it is necessary to identify all the monophyletic groups recovered in each of the alternative optimum trees (Fig. 3.8). If the alternative trees are not identical, some monophyletic groups would be recovered in several trees and some other would be exclusive of one particular tree. Then, the frequency that each monophyletic group is recovered with among the optimum trees is recorded. The strict consensus shows only those monophyletic groups that are present in 100% of the optimum trees; instead, the majority-rule consensus presents the monophyletic groups that are recovered in some fraction of trees (usually >50%). For instance, the 75% majority-rule consensus is the tree formed by clades that are recovered in more than 75% of the alternative trees. Consensus trees can be also used to summarize the results from different phylogenetic reconstructions, for inferring robustness in



	Tree 1	Tree 2	Tree 3	%
(A B)	X	X	X	100
(A B C)	X	X	X	100
(A B C D)	X	X		66
(E F)	X	X	X	100
(A B C D E F)	X			33
(G H)		X		33
(E F G H)		X	X	66
(A B C D E F G)	X			33
(A B C D E F G H)	X	X	X	100
(A B C D E F G H Og)	X	X	X	100
(E F G)			X	33
(D E F G H)			X	33

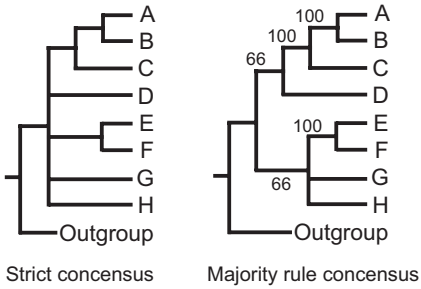


Fig. 3.8 Consensus trees. When multiple optimal trees are recovered for a single data set, the phylogenetic signal is usually summarized in a consensus tree. A table for monophyletic groups is assembled; each group is identified on the individual tree and recorded on the table; once all the groups are assigned, the percentage of recovery is calculated. The strict consensus tree shows only those groups that were recovered in all the trees. The majority rule consensus tree shows those monophyletic groups that are above a percentage threshold

statistical tests like Jackknife and bootstrap, or for accessing phylogenetic uncertainty as in Bayesian or coalescent inference. Nonetheless, the use of some consensus methods has been criticized because in some instances, the resulting consensus can show groups that are not present in any of the competing trees (Felsenstein 2004), or by inconsistencies during the construction of supertrees (Goloboff and Szumik 2016).

3.8 Phylogeny Support

Something that must be clear is that phylogenies are hypothesis of evolutionary interrelationships among taxa. There is no way to be certain that the phylogeny is even close to the "true" evolutionary history of the lineages included in the analysis. The optimum phylogeny is so only for the specific taxa sampling, the data matrix,

and the evolutionary model assumed. Consequently, a possible test for the consistency of the phylogenetic hypothesis is to change the composition of taxa or the use of alternative character sources. However, phylogenetic studies do not use such strategies because systematists include in their analyses all the information they can gather. Most of the time the incongruence between different studies is due to the differences introduced by the researcher in taxa and character composition. Perhaps, the bootstrap method (Felsenstein 1985) is the most popular test for assessing the robustness of the phylogenetic hypothesis. It is a statistical technique that basically involves resampling the characters from the data set with replacement until a bootstrap matrix is created. This matrix has exactly the same taxa and the number of character as the original matrix; however, because characters are sampled at random, the specific composition of characters differs. In the bootstrap matrix, some characters could be overrepresented and some other characters could be absent. Once the bootstrap matrix is generated, a normal phylogenetic reconstruction algorithm is applied to infer the best tree (or trees) for that matrix. The resulting tree is stored in memory; the procedure is repeated, creating a new bootstrap matrix and its optimal tree. This process is iterated ad libitum (minimum 100 times). Then, a majority-rule consensus tree is built using all the trees stored in the memory. It is possible that some of the monophyletic groups recovered in the original phylogeny do not appear in the bootstrap consensus tree. In that case, such clades would receive null support; otherwise, the frequency for the monophyletic groups recovered in the consensus tree is the bootstrap support. There is no agreement for a threshold in bootstrap value to consider a clade as well supported; thus, the closest to 100% the better. In fact, bootstrap values do not give a certainty of the evolutionary history; the bootstrap test provides an evaluation of the internal consistency of the characters in the matrix. Nodes supported by three or more unique synapomorphies score high bootstrap values, whereas nodes with one or several homoplasious synapomorphies usually score poorly in the test. Therefore, some systematists consider a futile exercise the use of these kinds of tests to evaluate the veracity of the phylogeny (Carpenter 1992).

The Kishino-Hasegawa algorithm (Hasegawa and Kishino 1989; Kishino and Hasegawa 1989) implements a likelihood-based statistical method to determine if there are significant differences in the topology of two alternative phylogenetic hypotheses. The test estimates the confidence intervals and standard errors for the difference in the log-likelihoods between the conflicting cladograms/phylogenies. A central consideration is that the test is developed for evolutionary hypotheses that were specified a priori. Therefore, the alternative trees would have been inferred independently of the nucleotide sequences that will be used to estimate their likelihoods. Otherwise, the test should not be used, or particular modifications to the algorithm must be implemented (Goldman et al. 2000; Felsenstein 2004). Although several corrections have been proposed (Shimodaira 1998; Goldman et al. 2000; Emerson et al. 2001), the basic test computes the statistic δ, which is the difference between the likelihood of the two phylogenies ($\delta = L_1 - L_2$). If δ is greater than zero,

the phylogenies are incongruent and the one having the best likelihood should be accepted. However, likelihood values usually are extremely similar; thus, their difference in not necessarily significantly different from zero. To estimate the confidence intervals and standard errors for δ, a bootstrap is implemented. A null distribution for δ is inferred by computing the δ values for the bootstrap trees. A threshold value for the significant level is decided (usually 0.05), and if δ falls within the confidence interval, then the alternative hypothesis that the two phylogenies are incongruent should be rejected.

3.9 Bayesian-Based Inference

Since the beginning of the twenty-first century, the development of powerful computer microprocessors and algorithms such as the Markov Chain Monte Carlo (MCMC) has favored the implementation of Bayesian-based methods as an alternative in phylogenetic reconstruction (Huelsenbeck et al. 2001). In simple terms, the Bayes' theorem infers how the prior probability of an event (P_A) is modified by the probable occurrence of an alternative event (P_B). Mathematically, it is represented by the ratio of a set of conditional probabilities for A, B, and their interaction (see Matthiopoulos 2011 for an introduction to probability theory as applied to ecology and evolution). Without realizing it, everybody applies this theorem on everyday decisions. Let us assume that going from home to the movie theater takes 10 minutes by car; however, most people do not leave home 10 minutes before the beginning of the movie. The departure time is modified by factors like rain, traffic, time of the day, etc. In a very light way, that is Bayesian inference. According to the Bayesian approach, in phylogenetic inference, the posterior probability ($P_{A/B}$) for the distribution of optimal trees is the result of the prior probability of a tree (P_A) affected by the likelihood of the sequence alignment (P_B). The full process to produce the Markov chains is computer intensive. In general, algorithms designed for MCMC methods are based on at least two subroutines: Metropolis-Hastings (Hastings 1970), and Metropolis-coupling (Geyer 1991) procedures (see Dobrow 2016 for an introduction to stochastic processes).

The original Metropolis algorithm (Metropolis et al. 1953) was designed to randomly sample probability values that follow a symmetrical distribution. In 1970, Wilfred K. Hasting modified the procedure, so the new algorithm was efficient for sampling a more complex multidimensional distribution. To generate the posterior probability for the distribution of optimal trees, the algorithm first selects at random one tree (T_0) from the universe of all possible trees. Based on the best-fit model of evolution, the likelihood of T_0 is calculated. Then, an alternative but topologically close tree (T_1) is drawn, and its likelihood is computed. Next, based on the likelihood values, an acceptance ratio ($\alpha = \log L\ T_1/\log L\ T_0$) is computed. If α is equal or higher than 1, T_1 becomes the preferred tree, and it is stored in memory. However, if

α is smaller than 1, then a random number between 0 and 1 is generated; if this number is higher than α, T_0 remains as the optimal tree. At this point, the algorithm has completed one cycle (generation). A new generation starts by drawing T_2, followed by calculating a new α using the log$L$ of T_2 divided by the log$L$ of the tree retained in the previous generation. This algorithm is run until the MCMC reaches a stationary distribution, a requirement that can take several million generations.

A common problem during phylogenetic reconstruction is that the universe of all possible trees could have local peaks of suboptimal values (a.k.a. tree islands, see Nixon 1999) in which heuristic-search algorithms easily get stuck. Geyer's Metropolis-coupled MCMC algorithm was designed specifically to deal with that issue. If tree islands are common, the MCMC could fail from obtaining an accurate posterior density. To escape from local optima, the algorithm runs multiple parallel chains; in fact, MrBayes (Ronquist et al. 2012) is able to run several parallel analyses with multiple parallel chains each. In generation zero, each chain independently draws a starting tree. The chain recovering the best tree becomes the cold chain, and its tree turns out to be the reference tree (T_0) during generation 1 for the other chains (hot-chains). T_1 is independently assembled in all chains according to a perturbation parameter called "heat." The higher the heat, the higher the chance of assembling very different T_1's at every chain. If a new optimal tree is selected, its chain would become the cold chain and the tree would be the new reference tree. The best tree discovered at each generation is stored in memory for assembling the target distribution of optimal trees. High perturbation facilitates the escape from suboptimal tree island; however, that also increases dispersion in the results, making harder for the MCMC to reach the stationary distribution.

Once the stationary values Log$L$ are reached, the MCMC stops. In most runs, the initial generations recover suboptimal trees; then, an initial fraction of the data stored in the memory must be discarded (burn-in fraction). Now, the remaining trees can be used to generate a majority-rule consensus tree. A specific phylogeny or a particular monophyletic group might be recovered multiple times in different generations, that recurrence is a good estimate of their posterior probability. Thus, the frequencies obtained in the majority-rule consensus tree become the posterior credibility probabilities for those monophyletic clades. These posterior probability values have a straight forward interpretation, the closer to 1 the more robust is the monophyly for the clade. Besides doing a more thorough exploration of tree space, Bayesian analyses allow the systematists to get an idea of the uncertainty associated to the phylogenetic hypothesis. This phylogenetic uncertainty is inferred from the multiple alternative trees recovered. All this information might be used posteriorly in analyses of ancestral character reconstruction, or in biogeographic inference (e.g., dispersion-vicariance-extinction). Finally, an important fact must be taken into account; phylogenetic studies usually report bootstrap and posterior probability values as a statistical support measure. In most studies, posterior probabilities are significantly higher than their bootstrap counterparts. Therefore, the exclusive use of the former as a way of statistical robustness might result in blind acceptance of dubious evolutionary interrelationships (Garcia-Sandoval 2014).

3.10 Molecular Clock and Coalescence Analyses

Nuttall (1904) observed that the immunological reaction intensity of an individual to blood serum from other organisms was inversely proportional to their relatedness. Siblings showed null or mile reaction between them, but when a subject was inoculated with serum from a different species, the reaction was so violent that in many instances led to death. The conclusion was that such reaction was due to differences in the elements that form the blood serum, and the incompatibility between any two organisms was proportional to the time since they last shared a common ancestor. Reports like this gave origin to the idea for the existence of some sort of a biological molecular clock (Kumar 2005). Zuckerkandl and Pauling (1962) trying to understand the evolutionary history of the hemoglobin gene family concluded that there is a uniform rate of protein evolution, and implemented for the first time an incipient molecular clock. However, the theoretical foundations for the molecular clock were set after the elaboration of the neutral theory of molecular evolution (Kimura 1968, 1983). The molecular clock hypothesis assumes that proteins and nucleic acids evolve at a constant mutation rate over time. If such hypothesis were accurate, it would be a major breakthrough for estimating absolute time in molecular phylogenies. Implementing a strict molecular clock is very simple. First, the distance between two sequences (e.g., number of nucleotide differences) is calculated. That distance is divided in half, because those differences have been accumulated by the sister lineages since they split from a common ancestor. Finally, a fix substitution rate is applied, and the genetic distance is transformed into time. However, there are a few requisites to implement this strict clock: fixation rate of mutations must be constant through time; fixation rate must be the same in all lineages; the phylogeny must be true; the nucleotide transformation pattern must be correct; and, a reliable calibration point must be known. Perhaps, the last one is the only assumption that it is not always violated. The calibration point is a node in the phylogeny from which the absolute age of divergence is known; it is used to calculate a truthful fixation rate. Fossil evidence or well-accepted geological events are used for this purpose. Nonetheless, for many lineages, such information is nonexistent, the confidence range for its date is broad or unreliable (Hipsley and Müller 2014). All other assumptions for validating a strict clock are easily violated. Dickerson (1971) clearly proved that proteins could evolve at significant different rates. Also, there is evidence that a protein might have different evolutionary rates among lineages (Nei et al. 2001). Fixation rate is not constant; it has an inverse relationship with body size, life-history traits, generation time, and metabolic rate (Martin and Palumbi 1993; Bromham 2002). Similarly, nucleotide saturation, phylogenetic uncertainty, unfit evolutionary models, etc. minimize the validity of the assumptions that support the clock hypothesis. Consequently, even though a strict biological molecular clock is an appealing concept, in practice, it is unfeasible (see Chap. 12 for more on molecular evolution of haemosporidian parasites).

Besides all those inconsistencies, molecular markers show acceptable linear relationship to divergence time; therefore, there should be some way for inferring acceptable assessments of evolutionary time. At population level, life-history traits, generation times, and metabolic rate do not show significant variation. Therefore, genealogical analyses based on coalescent theory (Kingman 1982a, b) have relaxed the need for a strictly constant rate. Applying a relaxed molecular clock consists in allowing the fixation rate to vary among lineages and over time but restricting such fluctuation to certain confidence limits around a prior value. Those limits are set using a standard Bayesian procedure (Liang et al. 2009). During these analyses, the algorithm uses local evolutionary models to correct the branch length between sister lineages, leading to the recovery of pseudo-ultrametric trees (i.e., a rooted tree in which branches grow with constant rate; in other words, a tree with equal distances from the root to every branch tip). Once the analyses have reached stationary results, the trees stored in memory are used to estimate the goodness-of-fit for the coalescent model, average splitting time for each node in the phylogeny, and the 95% confidence limits for these dates. Coalescent analyses can also estimate demographic parameters like change of effective population size through time (Ho and Shapiro 2011).

There is no doubt that phylogenetic inference has been transformed after the strengthening of the theory of biological molecular clocks. Until recently, the lack of hard evidence (e.g., fossils) for dating historical events was a major setback for evolutionary biology. Molecular clocks finally allowed the assignation of absolute time, within a reliable confidence interval, to key evolutionary episodes such as species origin, populations splitting, gene duplication, the origin of sequence variations, etc. Thus, by being aware of the assumptions and limitations when using molecular clocks, the field of evolutionary biology has accomplished advances once considered unattainable.

3.11 Phylogenetic Interrelationships of Avian Haemosporida

Many different parasites can be found in the blood of the vertebrates; however, *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, and *Fallisia* are the only four genera of the order Haemosporida identified in birds (Valkiūnas 2005). They are transmitted by blood-sucking dipterans (Santiago-Alarcon et al. 2012): the *Plasmodium* genus is the only one responsible for the avian malaria disease. Their minuscule sizes, as well as the lack of complex morphological features are some of the reasons that inferring the phylogenetic interrelationships of this group of organisms becomes not an easy endeavor. Traditional classifications are based on specific characters observed during stages in blood cells, life-history traits, and host taxa (e.g., young gametocytes, growing gametocytes, fully grown micro- and macrogametocytes, trophozoites, and meronts; Martinsen et al. 2007). However, when looking through the

microscope, three-dimensional features cannot be observed, and blood smear preparation could produce deformations in parasite structures and host cells. Nonmolecular interrelationship hypotheses therefore set their classifications mainly based on the vertebrate host (species, genus, family) and the parasite's life history as diagnostic traits (Martinsen et al. 2008). Using exclusively morphological information and Maximum Parsimony as the optimality criterion, Barta (1989) recovered one of the first phylogenies for the class Sporozoa. The matrix encompassed 26 characters that covered ontogenetic stages and ultrastructural features. Unfortunately, the phylogenetic signal in the data set was weak, and the most parsimonious tree showed many unresolved nodes. The lack of resolution obtained with morphology has compelled to the use of molecular markers to explore the evolutionary interrelationships of Haemosporida. The early studies were based on a single gene sequences from a limited numbers of taxa (Escalante et al. 1998; Bensch et al. 2000; Perkins and Schall 2002). Surprisingly, these attempts were not much better than the previous morphological ones. More recently, multilocus sequence analyses have been done. Borner et al. (2016) used sequences from 26 genes that encompassed nuclear, mitochondrial, and apicoplast (a unique organelle of Apicomplexan parasites) genomes. Galen et al. (2018) reconstructed the phylogeny assembling a data set of 21 nuclear genes. So far, the largest data set was analyzed by Pacheco et al. (2018), who gathered mitochondrial genomes for more than 100 species of haemosporidians collected from birds, reptiles, and mammals. The results from these studies report conflicting outcomes among them; however, the phylogenetic signal suggests that *Leucocytozoon* is the sister taxon to the other haemosporidians, and that *Haemoproteus* is not a monophyletic group. Therefore, it has been suggested that this genus should be split into at least two independent genera: *Parahaemoproteus* and *Haemoproteus* (see also Chap. 12).

Nonetheless, the phylogenetic information contained in the morphology has not been disregarded. Hernández-Lara et al. (2018) based on a data set of 133 morphological characters from 93 species recovered the evolutionary interrelationships of these parasites. In this study, they compared the topological congruence obtained from morphology, mitochondrial DNA sequences, and the combined data set. Once more, the individual data sets showed lack of resolution in some nodes, but the total evidence analysis (i.e., morphology plus mitochondrial DNA sequences) recovered a fully resolved tree. Apparently, each individual partition contributed to the phylogenetic signal at a different level in the cladogram; therefore, when they were combined in a single matrix, they complemented each other. According to the authors, their total evidence matrix showed results with a resolution and support similar to that obtained using full mitochondrial genome sequences. Their total evidence cladogram recovered the genus *Leucocytozoon* (*Leucocytozoon*) as the sister clade of all the other Haemosporida lineages, whereas at the most apical monophyletic clade, *Haemoproteus* and *Parahaemoproteus* formed a sister relationship between them (Fig. 3.9). By optimizing the morphological characters on their optimum tree, they concluded that all the main lineages (genus and most subgenera) can be

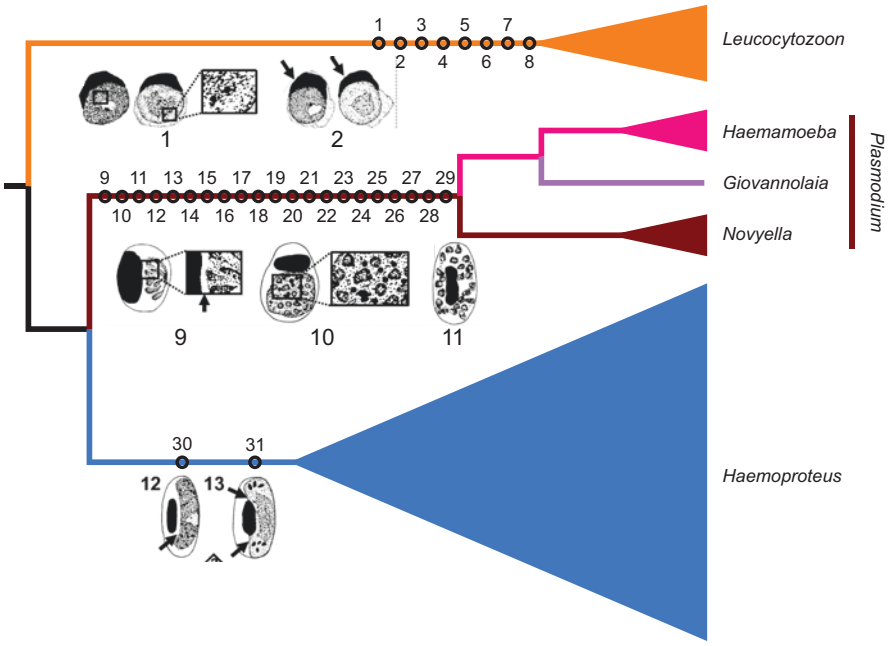


Fig. 3.9 Optimization of morphological characters on the phylogenetic hypothesis inferred by Hernández-Lara et al. (2018) for the avian Haemosporida. The monophyly of the genera *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* is supported. Each genus can be diagnosed by a unique set of morphological characters. Illustrated morphological characters are as follows (see Table 3.3 for a full list): (1) Fully grown macrogametocyte with evident volutin granules. (2) Grown gametocytes appressed to the envelope of the erythrocyte. (9) Erythrocytic meronts do not encircle the erythrocyte nucleus and do not touch erythrocyte's nucleus. (10) Rounded pigment granules in the erythrocytic meront. (11) Meront encircling erythrocyte's nucleus and not clear or evident influence on erythrocyte's morphology. (30) Growing macrogametocyte adheres to the envelope but not to the erythrocyte's nucleus. (31) Fully grown microgametocyte slightly enclosing the erythrocyte's nucleus. Within *Plasmodium*, only three subgenera are recovered as monophyletic. The subgenus *Huffia* is recovered within *Haemamoeba*, whereas the subgenus *Bennettinia* is recovered within *Novyella*

identified by unique sets of character states (Table 3.3), and that the hypothesis that many similar morphological traits observed in the different avian Haemosporida are the result of convergent evolution might be false. Finally, the study by Hernández-Lara et al. (2018) demonstrated the importance and taxonomic value of morphological characters. There is no doubt that DNA barcoding for species recognition has been a major innovation in modern taxonomy (see Chap. 4 for the case in avian haemosporidians); nonetheless, the usefulness of traditional morphological features must be also acknowledged especially when the immediate identification of blood parasites is a priority, or in field conditions where molecular technology might not be available (see Chap. 2).

Table 3.3 Synapomorphies recovered for the genera of avian Haemosporida

Genus	Character[a]	Synapomorphy
Leucocytozoon	1	Fully grown macrogametocyte with valutine granules
	2	Grown macrogametocyte appressed to the envelope of the enucleated erythrocyte
	3	Roundish host cell of the fully grown macrogametocyte
	4	Cap-like nucleus of the fully grown macrogametocyte
	5	Nucleus of the host cell extends up to half of the circumference around the fully grown gametocyte
	6	Micromacrogametocyte with evident valutine granules
	7	Roundish host cell of the microgametocyte
	8	Vacuole from 1 to 2.5 μm in the microgametocyte
Plasmodium	9	Erythrocytic meront does not encircle the erythrocyte nucleus
	10	Rounded pigment granules in the erythrocytic meront
	11	Influence not evident on the erythrocyte by the meront
	12	Pigment granules in the erythrocytic meront randomly scattered in its cytoplasm
	13	Small (<0.5 μm) pigments in the erythrocytic meront
	14	Trophozoite in mature erythrocyte
	15	Trophozoite does not adhere to the erythrocyte nucleus
	16	Trophozoite does not or slightly displace to the erythrocyte nucleus
	17	Subpolar position of the trophozoite
	18	Absence of a long outgrowth that exceeds the length of the main body of the trophozoite
	19	Vacuolated trophozoite
	20	Trophozoite with pigment granules
	21	Rounded pigment granules in the trophozoite
	22	Pigments aggregated in clumps in the trophozoite
	23	Dark brown pigment granules in the trophozoite
	24	Meront present in mature erythrocytes
	25	Outgrowth <2 μm in the growing erythrocytic meront
	26	Merozoites randomly arranged in the erythrocytic meront
	27	Meront may not adhere to the erythrocyte nucleus
	28	Vacuoles absent in the erythrocytic meront
	29	Erythrocytic meront does not influence the host nucleus
Haemoproteus	30	Growing macrogametocyte adheres to the envelope but not to the erythrocyte nucleus
	31	Fully grown microgametocyte slightly enclosing the erythrocyte nucleus

After Hernández-Lara et al. (2018)
[a]As illustrated in Fig. 3.3

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Chapter 4
The Use of Molecular Methods in Studies of Avian Haemosporidians

Staffan Bensch and Olof Hellgren

Abstract During the last two decades, molecular methods to study mitochondrial DNA sequence variation have become an important part in the studies of avian haemosporidians. Up until recently, these methods have primarily been used for identification of the parasites and for tentative phylogenetic reconstructions, allowing researchers not trained in traditional parasitology to compare data across the globe. However, with the introduction of genome and transcriptome sequencing, studies are emerging that go deeper into the genetics and molecular biology of the parasites. In this chapter, we describe and summarize the common methods used for genetic barcoding of the parasites and give an introduction of what to take into account when designing a molecular study of avian haemosporidians. This chapter further discusses why nuclear genetic data are needed in order to answer several important ecological and evolutionary questions and which methods to use in order to overcome the obstacles of obtaining nuclear data of the parasites. Finally, this chapter highlights the challenges and opportunities that come with the use of molecular methods, such as how to study and interpret prevalence, the challenge of aborted developments, and how to obtain data for more robust phylogenies and population structure studies of the parasites.

Keywords Barcoding · Genomics · Haemosporida · Molecular parasitology · Molecular phylogenies · Transcriptomics

4.1 Introduction

The prevailing molecular methods for identification of haemosporidian parasites of birds are based on DNA sequence variation within the mitochondrial genome. One region in particular, a 479 bp fragment of the cytochrome b gene, has been the

S. Bensch (✉) · O. Hellgren
Department of Biology, Ecology Building, Lund University, Lund, Sweden
e-mail: staffan.bensch@biol.lu.se

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target in several hundred publications and, thus by sheer number, has become the "barcoding" region for avian haemosporidians (Bensch et al. 2009). Unique haplotypes of this region are called "lineages". Published records of haemosporidian lineages are stored and available for comparison via BLAST (Altschul et al. 1990) or for download in the database "MalAvi", along with associated information such as host species and geographic locality (Fig. 4.1). The MalAvi database presently (June 2020) contains information on 1577 lineages of *Haemoproteus*, 1287 of *Plasmodium*, and 1138 of *Leucocytozoon*. This barcoding region of the *cytb* gene, for simplicity called the MalAvi region, can be amplified by a number of primer pairs (Table 4.1) with the only requirement for identification of lineages that the sequenced amplicons cover most (~90%) of this domain. The coordinated efforts of barcoding have made a huge impact in the field of avian haemosporidian research (Rivero and Gandon 2018). However, to correctly interpret results from *cytb* studies, that is, whether the lineages represent good species or intraspecies variation, there is a need for more genetic information, especially from genes not linked to the mitochondrial genome.

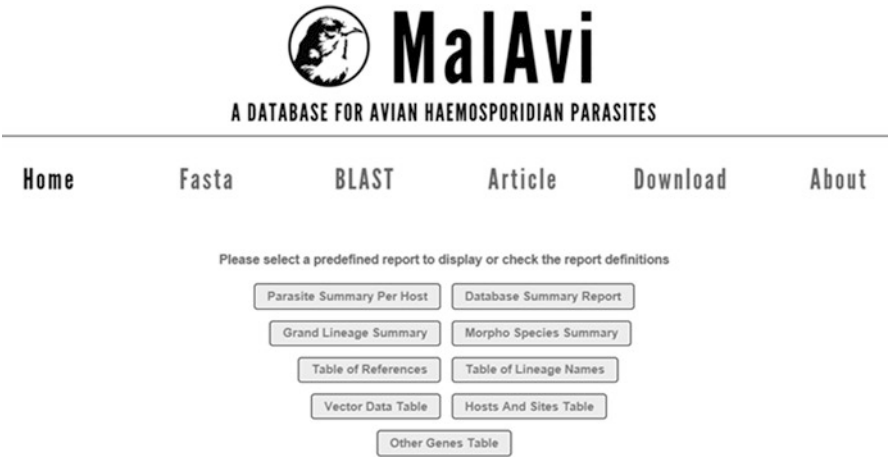

Fig. 4.1 The MalAvi database. Unique *cytb* haplotypes are called "lineages" and are named, typically by a 5–6-letter acronym corresponding to the scientific name of the first recorded host species combined with a two-digit number. For example, the lineage ACAED02 was the second lineage to be found in *Acrocephalus aedon*. To check whether a lineage is already present in the MalAvi database, one can use the BLAST function on the website, or download all of the lineages as a FASTA file for examination locally on the computer (click FASTA => All sequences). All the report tables can be checked online or downloaded in Excel format. For example, previous records of a lineage (host species or locations) can be found in the "Hosts And Sites Table." The database is curated by Staffan Bensch and updated online every 2–3 months. To submit data to the MalAvi database, one can use the Excel form provided at the main web page (http://mbio-serv2.mbioekol.lu.se/Malavi/) and email this (along with questions) to staffan.bensch@biol.lu.se

Table 4.1 A selective list of primers that amplify haemosporidian mitochondrial DNA from the genera *Haemoproteus* (H), *Plasmodium* (P), and *Leucocytozoon* (L)

Primer combinations[a]	mtDNA Region	Main use	Length[b] (bp)	H	P	L	MalAvi Overlap (%)	Reference
(1) HAEMF × HAEMR2	*cytb*	Identification	479[b]	-	-		100	Bensch et al. (2000)
(1) HAEMNF × HAEMNR2 (2) HAEMF × HAEMR2	*cytb*	Identification / screening	479[b]	-	-		100	Waldenström et al. (2004)
(1) HAEMNFI × HAEMNR3 (2) HAEMF × HAEMR2	*cytb*	Identification / screening	479[b]	-	-		100	Hellgren et al. (2004)
(1) HAEMNFI × HAEMNR3 (2) HAEMFL × HAEMR2L	*cytb*	Identification / screening	479[b]			-[c]	100	Hellgren et al. (2004)
(1) DW2 × DW4 (2) DW1 × DW6	*cytb*	Phylogeny	1138[b]	-	-	-	100	Perkins and Schall (2002)
(1) 621F × 983R	*cytb*	Identification / screening	229[b]	-	-		15	Fallon et al. (2003a)
(1) 3760F × 4292Rw2	*cytb*	Identification / screening	533[b]	-	-		100	Beadell et al. (2004)
(1) DW2 × DW4 (2) LeucoF × LeucoR	*cytb*	Identification / screening	818[b]			-	100	Perkins and Schall (2002), Sehgal et al. (2006)
(1) HML × HMR	*cytb*	Screening	367	-			68	Martinez et al. (2009)
(1) Plas-F × 4292Rw	*cytb*	Screening	422		-		70	Martinez et al. (2009)
(1) Palu-F × Palu-R	*cytb*	Screening	391[d]	-	-		64	Martinez et al. (2009)
(1) CytF1 × CytR1 (2) CytFN × CytRN	*cytb*	Phylogeny	1181[b]	-	-	-	100	Schmid et al. (2017)
(1) F x R (2) Finternal × Rinternal	Whole mtDNA	Phylogeny	5451	-	-	-	100	Pacheco et al. (2018b)
(1) 213F × 372R	n-c	Screening	160[e]	-	-		0	Beadell and Fleischer (2005)
(1) 343F × 496R	n-c	Screening[f]	188	-	-	-	0	Fallon et al. (2003b)
(1) AE064 × AE066			1109	-	-	-	100	Pacheco et al. (2018a)
(2) AE980 × AE982	*cytb*	Screening	346	-			0	
(2) AE983 × AE985			580		-		47	

(continued)

Table 4.1 (continued)

Primer combinations[a]	mtDNA Region	Main use	Length[b] (bp)	H	P	L	MalAvi Overlap (%)	Reference
(1) PMF × PMR	n-c		377	-			0	
(1) HMF × HMR	n-c	Screening	525		-		0	
(1) LMF × LMR	co1		212			-[c]	0	

The main use of the protocols is indicated as screening (presence/absence on gels), lineage identification, or phylogenetics (whole *cytb* or whole mtDNA genome). The sequences of the primers can be found in the original publications

[a](1) Denotes primers in first reaction, (2) the primers in a second nested reaction

[b]Excluding the length of primers (i.e., the length of novel sequences obtained)

[c]The primers appear to fail in amplifying many lineages of *Leucocytozoon* having fusiform gametocytes (Lotta et al. 2019)

[d]Treatment of the amplicon with the endonuclease H*py* CH4III cuts *Haemoproteus* sequences in two fragments (363 bp and 27 bp) and *Plasmodium* sequences in three fragments (327 bp, 36 bp, and 27 bp)

[e]Treatment of the amplicon with the endonuclease *Xba*I cuts *Haemoproteus* sequences (121 bp and 39 bp), whereas *Plasmodium* sequences remain intact (160 bp)

[f]Works also for estimating total parasitemia by qPCR (Ishtiaq et al. 2017)

4.2 Barcoding, Species Limits, and Population Structures

A genetic region suitable for barcoding must first of all exist in all of the species attempted to be barcoded; second, it should show sufficient variation so that species can be distinguished. At the beginning of this century when the first MalAvi sequences were generated (Bensch et al. 2000), there was so little knowledge in the field that these assumptions could not be critically tested. A few parasites have failed amplification even when tested by multiple primers (Valkiūnas et al. 2016), in one case possibly indicating that the mitochondrial genome had been deleted (Zehtindjiev et al. 2012) like in *Cryptosporidium*, a distant relative within Apicomplexa parasites (Kuo et al. 2008). With a few possible exceptions, it appears that the MalAvi region is present in the genomes of all avian haemosporidians investigated to date. Encouraging for species discrimination, it was observed that the sequence diversity seemed to be many fold higher than estimates of species diversity based on morphological analyses alone (i.e., the observed genetic variation showed potential for investigating the presence of cryptic species). It also seemed that the MalAvi region captured most of the lineage diversity when comparing the same samples sequenced for the full mtDNA *cytb* coding region; Hellgren et al. (2007a) tested 8 lineages (2–13 samples / lineage) without finding additional lineages with the full *cytb* coding region data set. Not surprisingly, later studies have found cases of the same MalAvi lineage breaking up into more lineages when sequencing the full *cytb* gene (Musa et al. 2018) or the whole mtDNA genome (Pacheco et al. 2018a, b; Huang et al. 2018). However, these cases are comparably few. Hence, for barcoding avian haemosporidians and trying to minimize sequence

length while maximizing lineage discrimination, it seems that the MalAvi region meets the purpose quite well. That more diversity can be uncovered with longer mtDNA sequences is not in itself a problem for barcoding. Note, for example, that adding sequences from nuclear genes up to the complete nuclear genome will always increase the resolution. The purpose of barcoding is to have one sufficiently informative and easily studied region that a research community agrees to sequence across studies, enabling unambiguous identification of parasites for direct comparisons of parasite diversity among host species and geographic regions.

The mitochondrial genome of haemosporidian parasites is minimal in size (6 kb) with a linear instead of a circular organization, which is otherwise the common arrangement in eukaryotes (Hikosaka et al. 2013). In mammal *Plasmodium* parasites, each mitochondrion contains multiple copies of the 6 kb genome, typically arranged in tandem and repeated dozens to up to 150 times (Wilson and Williamson 1997). Direct studies of avian haemosporidian mitochondrial DNA structure have not been carried out, but it is plausible that the situation is similar. An important consequence of this is that DNA samples will always contain many more copies of parasite mtDNA (10–100×) than any nuclear gene of the parasite. Hence, protocols targeting mtDNA fragments will be correspondingly more sensitive than protocols amplifying nuclear genes. Attempts have been done to use primers for 18S rRNA for avian malaria infections screening (Feldman et al. 1995), a commonly used marker for identification of a wide range of organisms, particularly protozoans. This protocol has not been widely used, as it seems to be specific for *Plasmodium relictum*. In contrast to animals that often have hundreds of tandem repeated rRNA copies in the nuclear genome, in haemosporidians, they are single copy genes arranged on different chromosomes and are often highly divergent from each other (Gunderson et al. 1987; Rooney 2004). Hence, it seems unlikely that we will find sensitive and universal primers for amplifying rRNA of haemosporidians.

Within the mitochondrial genome, any, or at least many other regions would probably work equally well for barcoding. One main reason for keeping focus on the MalAvi region is the wealth of information already available for comparisons. It is important to note that screening for prevalence using primers that generate long amplicons will more likely result in false negatives. This is because such reactions are more sensitive to variation in template quality, quantity, and PCR conditions. Hence, a more efficient strategy is to first use protocols for shorter amplicons, such as the primers for the MalAvi region, to identify the infected samples. The barcoding sequence of these samples can then form the basis when selecting samples for generating more complete mtDNA sequences, preferably to be combined with sequences from multiple nuclear genes (Borner et al. 2016) for robust phylogenetic reconstructions.

A lingering question has been whether similar mitochondrial lineages (i.e., sequences that differ by 1–5 bp) represent variation within species or indeed unique species. To date, there is no consensus regarding the rate of molecular change for the mitochondrial genes in the haemosporidian parasites. There is however good evidence that parasite mitochondrial DNA evolves at a slower rate than the mitochondria of hosts, and the parasite's nuclear genes evolve 6–10 times faster than

their mitochondrial DNA (Nilsson et al. 2016). Rate estimates for the *cytb* gene range between 0.1% and 1.3% divergence per million year (Ricklefs and Outlaw 2010; Bensch et al. 2013; Pacheco et al. 2018b). In practice, this would translate to one mutation within the MalAvi fragment (equal to 0.2% sequence divergence) every 154,000 to 2 million years. This has two important implications. First, cases where lineages only differ by a single mutation will include both very newly arisen mutations where the lineages are part of the same recombining population and cases where these have diverged for up to four million years (assuming a divergence rate of 0.1% and that the lineages are analyzed just before the second mutation has occurred). Second, when parasites are sharing the same *cytb* lineage, these might consist of populations that have been isolated for up to 154,000–2 million years. With a higher rate of change in nuclear genes, such populations can be substantially divergent in their nuclear genomes.

It was early observed that lineages that differ by only 1–2 bp sometimes had drastically different host species distributions (e.g., HIICT1 and HIPOL1) (Reullier et al. 2006) or gametocyte morphology (*Haemoproteus minutus* and *Haemoproteus pallidus*; Hellgren et al. 2007a). Moreover, the few studies that have investigated nuclear genes of closely related *cytb* lineages have found that these frequently are associated with distinctly different nuclear alleles: in *Haemoproteus* (Nilsson et al. 2016), *Plasmodium* (Beadell et al. 2006), and *Leucocytozoon* (Galen et al. 2018b). This suggests an absence of successful reproduction between these closely related lineages. Hence, such strict associations between mitochondrial and nuclear variants support that they should represent independently evolving nonrecombining units (e.g., Bensch et al. 2004), a hallmark for good biological species. With that said, it is important to point out that similar lineages also can represent within species variation; for example, the *Plasmodium relictum* lineages SGS1 and GRW11 share MSP1 alleles (Hellgren et al. 2013b), suggesting that they are recombining. Unfortunately, there does not seem to be a general cut-off, or so-called barcoding gap, which can separate within-species variation from between-species variation.

To what extent similar lineages represent one or several species needs therefore to be addressed case by case. Compared to animals, it is surprising that mtDNA distances as low as 0.2% often appears to represent unique species in haemosporidian parasites. In birds, within species divergence is generally <2%, whereas the between-species divergence is >4% (Ward 2009). The lack of a clear barcoding gap for haemosporidians emphasizes the importance of recording the findings of all unique lineages in the primary publications (i.e., not lumping lineages that do differ into the same entity), as their species status may be recognized in the future.

4.3 The Design of Molecular Studies

Before the introduction of molecular techniques, most of the species or community surveys of haemosporidians reported data on the overall prevalence of the three genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, without further attempts

to separate the species within these genera (Pierce 1981; see Chap. 1 for a synthesis of avian haemosporidian research in tropical regions during the twentieth century). This is understandable given that microscopic identification requires high infection levels and high-quality blood smears, which is rarely the case in data sets of hundreds of samples from wild captured birds. Molecular methods now make it possible also for the nonspecialist to identify haemosporidian parasites to a level that enables unambiguous identification of the parasites, not only to genus but also down to lineages (see Chap. 2 for avian haemosporidian life cycles and study methods). However, even if molecular methods have a very strong identification power, they can be implemented very differently and also come with a number of caveats that will be discussed below (see also Chap. 2).

4.3.1 Selecting Buffer for Storing Blood Samples

There are many buffers equally good for obtaining high-quality DNA from avian blood. These include inexpensive alternatives such as ethanol and more expensive commercial buffers. It has been reported that buffers containing sodium dodecyl sulfate (SDS) may reduce the success of amplifying haemosporidian DNA (Freed and Cann 2006) and in general, one should use buffers and sampling procedures that minimize polymerase inhibitors, such as heparin, EDTA, and Giemsa staining (Palinauskas et al. 2010; Owen 2011). Because each buffer will require its specific extraction technique, the choice of buffer for storing the blood should be decided with the plan of how the DNA will be extracted. A good starting point is to find out what buffers and extraction techniques are normally used in the laboratory where the work is to be carried out. When working with a technique that is already well established in a laboratory, troubleshooting will be easier as there will be experienced colleagues who can give advice on how to deal with low-yield or low-purity DNA.

4.3.2 Selecting Primers

There are many published PCR primers that work equally well if correctly optimized (Table 4.1). Importantly, the optimal condition for a specific set of primers may differ between laboratories due to different brands of DNA polymerase, PCR instruments, and other reagents. Just because one set of primers works better than another pair in a particular laboratory, it does not predict global success. Most of the general PCR and sequencing protocols have been developed based on parasites in passerine birds. Hence, there are reasons to believe that lineages from nonpasserines may be undetected by these primers. High lineage richness can result in failure to amplify some parasites, as some of the ~10,000 lineages just by chance may have mutations at crucial primer-binding sites. The task for any project is to develop an

optimized assay for the study: an assay that generates repeatable results and that does not miss infections identified by alternative methods (i.e., microscopy of blood smears). However, many data sets consist of just blood samples (no blood smear for verification). Starting screening such data without verifying that the selected protocol is working well in the laboratory is risky. A recommended strategy is to select about 30–50 samples for testing with two different screening protocols that produce absence/presence data (Fallon et al. 2003b; Beadell and Fleischer 2005; Ishtiaq et al. 2017; Pacheco et al. 2018a; Ciloglu et al. 2019), in parallel with developing the protocol for generating sequence data.

4.3.3 Coinfections

Wild birds are frequently infected with two or more different parasites (Valkiūnas et al. 2006). If both parasites have similar infection intensities and the primers bind equally well to the DNAs of these parasites, they will be amplified together and sequencing electropherograms will show double base-calling at the positions where the parasites have different nucleotides (Fig. 4.2). If the common parasites of the host species at the specific study site are known, one might be able to resolve the coinfections, by finding two lineages that can explain all the positions of the double base-calling. If the mix is from more than two parasites, this approach does not work. The standard way to resolve coinfections in unknown or complex situations is by cloning the PCR product and sequencing multiple clones (Perez-Tris and Bensch 2005). Note that sequences of cloned PCR products contain polymerase errors that normally go undetected when employing direct sequencing of PCR products, as the sequence is the average of millions of sequence reads. To verify the correct sequence from sequenced clones, multiple clones need to be analyzed.

In many instances, the PCR will favor only one of the parasites, either the one with the highest parasitemia or the one with the best match to the primers,

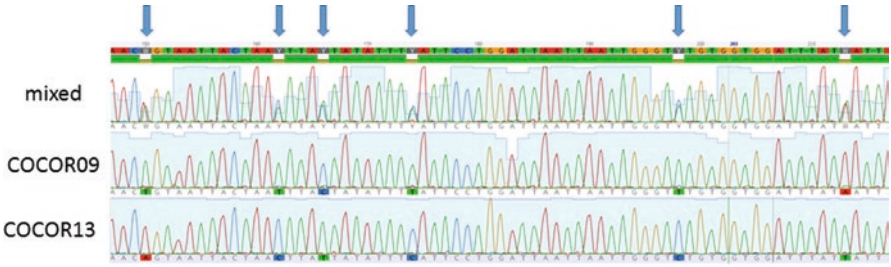

Fig. 4.2 At the top is a sequence of coinfections (six positions of double base-calling: blue arrows) that can be explained by a combination of the *Leucocytozoon* lineages COCOR09 and COCOR13. The samples are from Jackdaws (*Corvus monedula*)

erroneously showing the result of single infections (Bernotienė et al. 2016). The presence of infections that include parasites of different genera can be efficiently identified by examination of blood smears or by protocols with multiplex primers (Ciloglu et al. 2019) or by combining PCR with restriction enzymes (Beadell and Fleischer 2005; Martinez et al. 2009). However, revealing the hidden sequence by sequencing may require design of primers that exclude the masking sequence. Coinfections of lineages from the same genus that go undetected by standard PCR and sequencing protocols will generally be missed unless blood smears show presence of morphologically distinct species or that lineage-specific primers are used.

The consequences of failing to detect and determine the sequences in coinfections depend on the specific questions asked. If the aim is to describe the lineage diversity of a host species, missing some coinfections is probably not so important as all lineages of the species at some point will occur in single infections (assuming the infections are fairly independent). In bird species with generally high prevalence (> 70%), rare lineages will often be in coinfections and thus easy to miss. Hence, the higher the prevalence, the more samples need to be screened in order to find most of the lineages infecting a species. The failure to identify coinfections should generally be more problematic in studies aimed at precisely estimating lineage prevalence, particularly in bird species with high lineage diversity and high prevalence where the lineages will mask each other occurrence. In such cases, lineage-specific qPCR may be a solution (Asghar et al. 2011). However, each lineage needs its own PCR design and optimization, which can be very laborious and comes with fairly high running costs. It is important to remember that even if all efforts are taken, the observed frequency of coinfections will most likely be an underestimate of the true frequency (Valkiūnas et al. 2006).

4.3.4 Contamination

A successful PCR may generate many millions of copies of the targeted DNA fragment, each copy a potential source of contamination in future experiments. This is the reason why it is important to add negative template controls (NTC) to check for the presence of contaminants in the preparation of reagents. However, adding one or a few NTC on a 96 plate may not be sufficient to identify low-level background contamination. If low levels of contaminating PCR products are present in the laboratory, it may show up in only some of the NTCs. For example, in our laboratory in Lund, we ran a project screening 382 samples from birds from the Azores that had overall low rates of infections (Hellgren et al. 2011). We used 1 NTC per row of 8 samples, that is, 12 NTC per plate. All of these were negative as were most of the wild samples from 10 species of birds with the exception of blackbirds (prevalence 57%). Sequencing showed that one European robin (*Erithacus rubecula*), one blackcap (*Sylvia atricapilla*), one Eurasian blackbird (*Turdus merula*), and two house sparrows (*Passer domesticus*) were each infected by the lineage

PARUS1, a common parasite of tits in Europe, and not previously recorded in these species. We examined blood smears from these specimens but could not find gametocytes and analyses of subsequent DNA extractions failed to amplify. So, how did we find the lineage PARUS1 in these samples? The most likely reason was due to spill-over from a previous project in the lab, completed just a few months earlier that included amplifications from 53 blue tits (*Cyanistes caeruleus*) infected with PARUS1 (Stjernman et al. 2008). In this case, we could detect the contamination because the records stood out as surprising (wrong host species) and we had blood smears to check with microscopy, but in many other situations, such contamination may go undetected (including in our lab in Lund). This calls for a general awareness of contamination, not only between samples within a study but also from previous projects carried out in the particular laboratory. To confirm that a surprising finding indeed comes from a parasite infecting the bird and not from a PCR-contaminated product, one can check the sample with primers targeting other parts of the mitochondrial genome and also under the microscope to search for gametocytes.

4.3.5 Mixing Up the Samples

This sounds like a problem so obvious that it is not even worth bringing up. However, when working with hundreds of samples, it can happen easily, and if it goes undetected, it will lead to erroneous host records of parasites. Samples can be mislabeled in the field, during DNA extraction, or when preparing dilutions for the PCR. In studies of single species, this is not fatal beyond the study itself, although mixing up the tubes from two individuals, one of which is infected and one is not, will give incorrect parasite status to two individuals in the data set. This will reduce the power of the statistical analyses aimed at finding phenotypic predictors (e.g., sex, age) of parasite infection status. The consequences of mixing up samples will however be more severe in studies including several species. For example, if the mixed-up samples belong to two different species, each infected with a host-specific lineage of parasites, it would lead to two erroneous host records in the databases that will inflate future calculations of host specificity. Mixed-up samples of host species can easily be detected by amplifying and sequencing a smaller fragment of the bird's mtDNA (e.g., *coxI* or *cytb*) using universal primers (Kocher et al. 1989), but due to the extra labor and costs for sequencing, this is rarely done on complete data sets. However, checking the host species identity of samples that have resulted in "surprising findings" is a good practice to reduce errors of parasite host records. For example, a surprising finding would be a typical host-specific parasite in very different host species. Particularly vulnerable to this kind of problem are studies including relatively few samples from many previously not investigated species (i.e., the common situation for community studies in the tropics).

4.4 How Can We Obtain Data from Nuclear Genes?

Many evolutionary and ecological questions require more genetic information than can be retrieved from mtDNA alone. However, to obtain data on nuclear genes has been far from easy for several reasons. The main reason is the fact that the avian hosts have nucleated erythrocytes and that there is a big difference in genome size between the host and the parasite, as well as a difference in ploidy during the vertebrate stage. Even if all the red blood cells were infected, such an infected sample would mainly contain bird DNA (< 1% would be from the parasite). In nature, the level of parasitemia is typically <1%, resulting in a parasite proportion of <0.01% of the total DNA. Although sequencing costs are decreasing and sequencing platforms steadily become more efficient, it would require a tremendous sequencing effort to obtain a good coverage parasite genome from a naturally infected bird. In fact, there would be a need to sequence around 10 trillion base-pairs in order to obtain a 20× genome of the parasite (Videvall 2019). This bias is reflected in the low yield of parasite reads found when looking for avian haemosporidian sequences in databases of genome-sequenced hosts (Borner and Burmester 2017). Due to these problems, until recently, the only way to amplify nuclear genes was by using primers designed based on genomes from either mammalian malaria parasites and an unpublished fragmented genome of *Plasmodium gallinaceum*. Despite much effort from many laboratories, the success was restricted because designing working primers using alignments of these very divergent genomes was problematic. Between 2000 and 2013, publications included one apicoplast gene (clpc) and a handful of nuclear genes (*DHRF-TS*, *TRAP*, *ASL*, *MAELB*), of which many only could be amplified from a limited number of lineages (Jarvi et al. 2003; Bensch et al. 2004; Martinsen et al. 2008; Santiago-Alarcon et al. 2010; Farias et al. 2012; Martinez et al. 2013). Simultaneously, a lot of unsuccessful effort was put into sequencing the first genome of avian haemosporidians. Since then, several different methods have been developed in order to circumvent the problem of skewed DNA ratios when obtaining nuclear data of the parasites. At present, there are three major strategies of obtaining large number of sequences from nuclear genes: 1) RNA/transcriptome sequencing, 2) Parasite enrichment followed by genome sequencing, and 3) Multigene sequencing using sequenced genomes as the backbone.

4.4.1 RNA/Transcriptome Sequencing

When the parasite has infected a host, the ongoing asexual replication will require the molecular machinery responsible for the basic metabolic processes along with those for the biological processes during host invasion. This means that DNA transcription in the parasites will be fully switched on to produce mRNA, followed by translation into the required proteins. The reason for using RNA sequencing instead of DNA sequencing to obtain nuclear data rests on the notion that the parasite is producing abundant mRNA in the peripheral blood, resulting in a more favorable ratio of parasite-host RNA for sequencing. The first avian haemosporidian RNA

data was from a Crossbill (*Loxia* sp.) experimentally infected with the *P. relictum* lineage SGS1 (Bensch et al. 2014). This first transcriptome was of low coverage and included relatively few and incompletely sequenced genes. Nevertheless, this partial transcriptome was sufficient for developing primers for investigating several nuclear genes in the parasite *P. relictum* (Hellgren et al. 2013a, 2015). As RNA isolation and sequencing have been improved, there are now several well-annotated transcriptomes available, primarily from different *Plasmodium* species (Lauron et al. 2014; Videvall et al. 2017; Weinberg et al. 2018), but also from the genus *Leucocytozoon* (Pauli et al. 2015). Strong advantages of using RNA sequencing are that there is no need to enrich for the parasite in the samples, it does not require any prior information of the genome when constructing the protocol, and offers the possibility of investigating the expression levels of different genes of the parasite. To date, transcriptomes have primarily been obtained from birds that have been experimentally infected as this allows for collecting blood samples when the parasitemia is at its peak (see Fig. 2.2 from Chap. 2). It therefore remains to be tested whether RNA sequencing will work on samples from wild caught birds that typically have low parasitemia. Samples for RNA sequencing must however either be stored directly at −80 °C (freezer or liquid nitrogen) or in suitable RNA buffers that prevent the RNA from degrading.

When obtaining the RNA sequences from an infected sample, the first challenge is to filter out the reads originating from the host. This can be done either by (1) deleting reads that map to a reference genome of the host, (2) filtering reads or contigs based on the composition of G and C where the GC% is much lower in the parasite compared to the host, (3) selecting reads or contigs that map to a related parasite genome or through homology searches using BLAST algorithms or, (4) a combination of these approaches. To date, sequencing transcriptomes seems to be one of the easiest ways to obtain nuclear data of the parasites, as it requires no manipulation of the parasite or prior knowledge of the parasites genome.

4.4.2 Parasite Enrichment Followed by Genome Sequencing

In theory, there are many ways to enrich samples of blood or tissue for parasites, to increase the yield of parasite DNA when aiming for whole-genome sequencing. The first *Haemoproteus* (*H. tartakovskyi*) genome was sequenced from samples of harvested microgametes (Bensch et al. 2016). For this to work, it requires access to live birds having infections that contain a high number of gametocytes. When the blood is withdrawn, the gametocytes respond as if they were in the gut of a vector (i.e., exflagellation is induced), probably triggered by the direct exposure to oxygen and the drop in temperature (see Chap. 2). Due to the size difference between microgametes and host cells, the microgametes can be enriched by simple centrifugation of the samples (Palinauskas et al. 2013). Some host DNA might still be left in the sample due to ruptured red blood cells; however, the proportion of parasite to host DNA can be sufficiently enriched for conducting whole-genome extraction and sequencing. Though this method works for *Haemoproteus* species, it seems more

difficult to induce exflagellation for species of *Plasmodium* and *Leucocytozoon* (Arai et al. 2001; Valkiūnas 2005; Valkiūnas et al. 2013, 2015). Another approach is to use laser capture microdissection microscopy (LCMM) on blood smears to directly separate parasites cells from host cells before sequencing (Lutz et al. 2016). This is a promising method for isolating parasite DNA; however, it is time consuming to collect a sufficient number of parasites needed for sequencing, and requires that the blood is prepared on specific membrane slides. A further challenge is that the microscopy must be done on unstained blood smears as Giemsa staining is a potent PCR inhibitor (Palinauskas et al. 2010).

Another way to harvest parasites was used by Böhme et al. (2018) before sequencing the genome of *P. relictum* (Böhme et al. 2018). In captivity, mosquitoes were fed blood from birds infected with *P. relictum*. After 7 days, oocyst-infested midguts were dissected out from the mosquitoes, DNA was extracted and used as a template for genome sequencing. This method is specifically suitable for *Plasmodium* species as they often produce a large number of oocysts, where each may contain thousands of developing sporozoites (see Chaps. 2 and 6). For enrichment of *Leucocytozoon* spp., one can take advantage of the large difference in shape of infected blood cells compared to uninfected cells. This morphological difference was used to separate infected from uninfected cells by flow-cytometry (Chakarov et al. 2012). As this method requires a size or shape difference of infected and uninfected cells, it is less likely to work for *Plasmodium* and *Haemoproteus*; however, it might be worth testing. In the future, there will most likely be other ways to manipulate or to take advantage of the biology of the parasites life cycle in order to separate the parasites from the host cells.

The following two enrichment methods have not yet been applied successfully for avian haemosporidians but might be worth considering. It has been suggested that host and parasite DNAs can be separated based on the observation that CpG methylated sites are much more abundant in the nuclear genomes of the vertebrate hosts than in the genomes of the host mitochondria and the parasites. Feehery et al. (2013) developed this method for *Plasmodium falciparum* and observed a tenfold increase in the number of parasite reads (Feehery et al. 2013). An alternative approach is called selective whole-genome amplification (SWGA). By selecting short primers and amplifying sequence motifs that are common in the genome of the parasite but rare in the host, the sequence coverage of the parasite genome can be enriched a tenfold (Leichty and Brisson 2014). However, for natural infection levels of avian haemosporidians that are typically <1%, a tenfold enrichment is probably not sufficient to reduce the excessive amount of host DNA.

4.4.3 Nuclear Gene Sequencing Using Available Genomes as Backbone

In the beginning of the molecular era of avian malaria research, much effort was put into using sequenced human or rodent malaria genomes as backbones when designing primers for avian malaria parasites. In some cases, these efforts have been

successful (Bensch et al. 2004; Borner et al. 2016), especially for genes that are evolutionarily conserved. However, many of the ecological and evolutionary questions that we want to answer involve gaining knowledge of genes that, due to their function (i.e., genes involved in host invasion or immune system evasion), evolve at a faster rate, or because such fast-evolving genes can provide us with a higher resolution about population structures or species boundaries. In those cases, primers or protocols need to be developed using genomes/transcriptomes from closely related species or using the combined information from multiple genomes of more distant parasites.

Several studies have used traditional Sanger sequencing when developing sequencing protocols either for single- (Bensch et al. 2004; Hellgren et al. 2013a; Garcia-Longoria et al. 2014; Nilsson et al. 2016) or multiple-gene approaches (Borner et al. 2016; Galen et al. 2018a). For studies requiring data from multiple genes, PCR protocols need optimization for each single gene under investigation, which are, of course, time, money, and template consuming. An alternative method for sequencing multiple genes is by sequence capture, which recently has been tested in haemosporidians with satisfying results (Huang et al. 2018; Barrow et al. 2019). When developing a sequence capture protocol, a researcher utilizes one or multiple genomes as a template for designing probes for the targeted genes. The protocols developed by Barrow et al. (2019) and Huang et al. (2018) targeted 498 and 1000 preselected genes, respectively. In brief, the method exploits the fact that target sequences are bound to biotin-labeled probes that can be captured by streptavidin-coated magnetic beads. Following a step of amplification with sample-specific index primers, many samples can be sequenced in parallel on a next-generation sequencing platform. Since samples can be indexed before capture (Barrow et al. 2019), the cost can be reduced substantially. One advantage with this method is that multiple probes, designed from different species, can be used in parallel, which should increase the success of recovering sequences from species and lineages that are evolutionarily distant from the available reference genomes. In the studies by Barrow et al. (2019) and Huang et al. (2018), approximately half of targeted loci were retrieved from lineages with an mtDNA difference of 5% from the reference genomes. It should however be noted that the success rate of loci recovered decreases drastically when the parasitemia drops below 0.1% (Barrow et al. 2019).

The number of available genomes and transcriptomes will progressively make it easier to develop specific protocols that include a larger phylogenetic range of the parasites. This will in turn enable larger phylogenetic comparative studies (see Chap. 3 for an introduction to systematic and phylogenetic concepts) of parasites with different life-history traits, varying, for example, in host specificity or virulence. This will vastly increase our knowledge of the epidemiology of the parasites, and what might be limiting transmission success and host range. However, in order to take the next step in this line of research, there is a need to expand the genomic research, which to date has primarily been done for parasites transmitted in temperate regions, to also include more data on avian parasites that are transmitted in the tropics.

4.5 Challenges and Opportunities with Molecular Methods

4.5.1 Molecular Methods to Estimate Prevalence

We now know that the three genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* are very species rich. To set up a molecular study with the goal of just determining the prevalence of genera seems therefore a waste of resources, given the relative ease by which lineage identification can be obtained. No ornithologist would be satisfied with a study that describes bird communities by the proportion of individuals belonging to different orders, Passeriformes, Galliformes, etc. Although the age of the genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* is a contentious issue (Bensch et al. 2013), it seems likely that they diverged before the radiation of extant birds (Pacheco et al. 2018b) and thus represent very divergent organisms that deserve as much attention as the species they infect.

The ideal goal of any screening project is to identify all infections in the collected samples (i.e., to find the true parasite prevalence and diversity). In practice, this is impossible because low-level infections will always be missed. Consider a small songbird of 10 grams that has a total blood volume of ~1.0 ml, or about 5 billion erythrocytes (assuming five million erythrocytes/μl). For PCR, we typically add 25 ng of total DNA as a template, which corresponds roughly to 20,000 bird genomes. In order to confirm an infected sample to be positive by PCR, it then follows that it must have at least 250,000 infected erythrocytes! Admittedly, these calculations are rough and since the parasites have several mtDNA genomes per cell, the sensitivity of the PCR might be a lot higher. Dilution experiments have suggested that the detection limit is about 1 parasite / 100,000 erythrocytes for the most commonly used nested PCR protocol (Waldenström et al. 2004). The take-home message is that the bird must be infected by tens of thousands of parasites before we can detect infection by PCR. From these calculations, one would be tempted to add more template DNA to the PCR (e.g., 100 ng rather than 25 ng that theoretically would increase the sensitivity fourfold). We are not aware of any test of this possibility, however, because too much DNA is in itself a PCR inhibitor, it may in the end not increase the sensitivity.

A common experience when retesting samples is that, for some samples, the infection status is inconsistent (e.g., out of a total of five PCRs, only three are positive). Based on the calculations above, this is precisely what we are to expect if samples are close to the detection limit. When taking 25 ng of template repeatedly from the extract, sometimes it contains enough parasites for the PCR to amplify them, but sometimes there are no parasites in the template volume.

Whatever efforts we take, our estimates of prevalence will always be an underestimate of the true prevalence. However, the knowledge of the distribution of natural parasitemia can inform us on the reliability of our estimates. Each protocol has a detection curve relative to the infection intensity of a sample, with highly infected samples being less likely missed than low infection samples (Fig. 4.3). If the distribution of the parasitemia is high for the given sample set, there will be few false

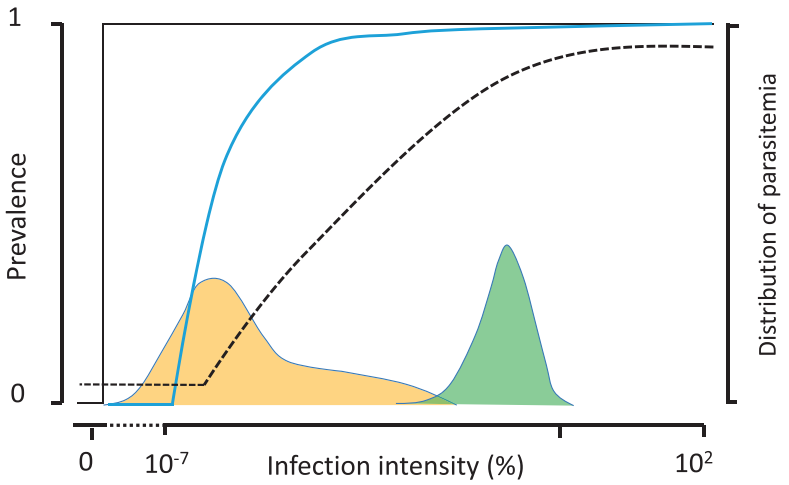

Fig. 4.3 A schematic illustration of the relationship between the successes of identifying true positive infections relative to infection intensity (i.e., parasitemia). The blue line shows the detection curve for a well-optimized protocol, the stippled line for a protocol that misses many infections already at median infection intensities and also results in false positives. The natural distributions of infection intensities of two parasites (orange and green) are illustrated at the bottom of the graph. The optimized protocol will correctly recover the infections of the green parasite, but for the orange parasite, it will result in many false negatives

negatives. However, if the distribution of the parasitemia overlaps with the decline of the detection curve, we can be sure that we have missed many infected samples. Thus, variation in parasitemia may lead to wrong conclusions of variation in prevalence. It is therefore important to consider the temporal aspect of parasitemia when estimating prevalence. Consider a parasite that has a seasonal variation in parasitemia, for example, higher in the wet season than in the dry season. By PCR, we will miss more infections in the dry season samples than in the wet season samples and conclude that prevalence is higher in the wet than in dry season, when in fact it is the parasitemia, but not the prevalence, that varies between the seasons. For any study that aims for carefully estimating prevalence, it is important to collect and examine blood smears or if these are not available, use qPCR to estimate parasitemia.

4.5.2 Aborted Development

To be a competent host, the parasite must be able to reach the blood and develop into mature gametocytes that are able to be transmitted to the vector and subsequently to new vertebrate host individuals. Similarly, to being a competent vector, the parasite must be able to develop into sporozoites that reach the insect's salivary glands (see

Chaps. 2 and 6). However, molecular screening of haemosporidian infections is based on determining the presence or absence of parasite DNA, without differentiating where this DNA came from. Molecular methods, particularly those that involve amplification steps, are very sensitive and may therefore detect DNA from other life stages of the parasites (e.g., sporozoites recently injected by a vector or parasite DNA released into the blood from exoerythrocytic replication points). Sporozoites circulating in the blood are probably only rarely amplified by PCR although these can sometimes been seen on blood smears (Valkiūnas et al. 2009). Based on the calculations above, we can assume that 25 ng of total DNA corresponds to 0.004 μl blood. To be regularly picked up by PCR, sporozoites hence need to reach a density of 250 μl^{-1} (to have one sporozoite genome/25 ng of total DNA). For a ten-gram bird, this would correspond to a total of 250,000 sporozoites in the blood volume and would require bites from hundreds or thousands of infected vectors, which is probably a rare situation in nature. In contrast, it has become increasingly clear that primers for haemosporidians can amplify parasite DNA released into the blood from replication points in other organs (Olias et al. 2011). Importantly, molecular detections of parasites cannot distinguish whether infections are within competent host species or cases of aborted development of the parasites. A striking example comes from the parasite *Haemoproteus witti*, which uses hummingbirds as competent hosts, but is frequently amplified from various species of passerines in which gametocytes do not seem to develop (Moens et al. 2017). It has been shown that abortive development infections can severely harm both hosts (Olias et al. 2011) and vectors (Valkiūnas et al. 2014), thus sometimes presenting a higher selection pressure than competent parasites. Although infections resulting in abortive development might be dead ends for both the parasite and sometimes also for the host, such infections are of both ecological and evolutionary interest as they might represent the first signs of host range expansion; the parasite obviously has reached the capacity to establish and replicate to some degree in the novel host(s) (one of the first stages of the aggressive symbiosis hypothesis; Ryan 2009).

The traditional way to verify that a host or a vector is competent is by confirming presence of gametocytes in analyses of blood smears or sporozoites in the salivary glands of the vector. For human and rodent malaria (*P. falciparum* and *P. chabaudi*), there are already molecular methods that utilize genes that are specifically expressed in the gametocytes that can be used to detect if transmissible stages exist in the vertebrate host (Wargo et al. 2006; Babiker and Schneider 2008). It would be possible to develop similar protocols for detection of sporozoites due to expression differences of genes involved in different development stages in the vector (Roth et al. 2018). For avian haemosporidians, there are already several RNA-sequencing studies that can be used to look for life-stage-specific genes (Videvall et al. 2017; Weinberg et al. 2018) and soon, there will be transcriptome data for life stages in the vectors. However, a particular challenge for identifying life-stage-specific genes in avian haemosporidians is that the data comes from samples with un-synchronized life cycles (i.e., the samples include parasites that are in different life stages). To overcome this problem, there is a need to be able to culture the parasites in vitro, as is done in human malaria research. For the vectors, this problem might be less

important as it is possible to dissect out the parasites belonging to the different life-cycle stages before sequencing, (i.e., separating the blood meal with zygote formation, oocysts, and sporozoites into different sequencing batches). Once we have this information, it might be possible to develop protocols that identify stage-specific RNA profiles of the parasite, thus allowing us to identify whether the parasite has completed its life cycle in that specific host.

4.5.3 Population Structure

Today, we are only at the beginning of understanding the genetic structure of parasites that have identical *cytb* lineages. This question needs to be addressed with a large number of nuclear genes across the genome in order to evaluate levels of linkage disequilibrium and gene flow between potential populations. Many *cytb* lineages have been recorded across vast geographical areas as well as in taxonomically divergent hosts (Bensch et al. 2009). Do these cases represent large genetically unstructured parasite populations or do these consist of a multiple isolated population with no or very little gene flow occurring between them? The lineage SGS1, found in 126 species in 39 countries (June 2019), appears to have active transmission both in temperate regions in Eurasia as well as in tropical parts of Africa (Beadell et al. 2006; Hellgren et al. 2007b, 2015; Marzal et al. 2011). However, when investigating data from a fast-evolving nuclear gene (MSP1), the lineage SGS1 was found to consist of several populations, each with its own MSP1 haplotypes with different variants transmitted in tropical and temperate regions (Garcia-Longoria et al. 2015; Hellgren et al. 2015). Another example is the lineage SISKIN1 (*Haemoproteus tartakovskyi*) that is a common parasite of siskins (*Spinus spinus*) and crossbills in northern Eurasia, but also of house finches (*Haemorhous mexicanus*) in Mexico. Huang et al. (2019) analyzed ~1000 genes of SISKIN1 isolates from Europe and Mexico using the sequence capture technique. The complete mtDNA genomes were found to be very similar although not identical (6 differences corresponding to 0.1% divergence), whereas the average nuclear gene divergence was 20-fold higher (2.85%) between the European and Mexican isolates. This shows that minimal divergences in mtDNA in some cases are associated with highly divergent genomes. In order for future avian haemosporidian researchers to understand the epidemiology/epizootiology of the parasites, host range evolution, and processes of speciation, it will be important to identify the genetic structure of populations across transmission areas as well as across phylogenetically divergent hosts.

4.5.4 Molecular Phylogenies

To be able to study the direction of evolution (e.g., do generalists evolve from specialists or vice versa?), how selection is acting on certain genes or to be able to account for phylogenetic constraints when studying any life-history trait of the

parasite, we need robust phylogenies (see Chap. 3 for a thorough introduction to systematic and phylogenetic concepts and methods). For phylogenetic analyses, the MalAvi fragment is too short for obtaining well-supported topologies. Ellis and Bensch (2018) constructed ML trees for all the lineages that presently were in MalAvi (n = 2451) and determined that only 20% of the nodes had bootstrap support values >70%. In order to obtain better-supported phylogenies, it would clearly be advantageous to amplify and sequence the whole *cytb* or even the whole mtDNA genome (Pacheco et al. 2018b), or – even better – to use multiple gene-sets across the whole genome of the parasites.

We will approach the ultimate goal of a robust phylogeny of all avian haemosporidians much faster if future studies will direct efforts toward generating data for the same set of nuclear genes. Borner et al. (2016) developed primers for 21 nuclear genes that can also be found in the published genomes of avian haemosporidians (Bensch et al. 2016; Böhme et al. 2018). These consist of a promising set of genes for future studies. As mentioned above, it is hard work to obtain data for nuclear genes by traditional PCR and sequencing. Such genes will also be biased toward those that evolve slowly, which on the one hand will be helpful for resolving deeper nodes in the phylogeny, but may not provide enough variation for resolving phylogenies of closely related species at the tips of the tree. As more genomes and transcriptomes become available for avian haemosporidians, they will facilitate researchers in developing specific protocols and molecular markers for "their" group of parasites, whether these efforts will be done by PCR and sequencing, sequence capture, or other methods based on reference genomes.

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Chapter 5
Introduction to the Taxonomy and General Biology of Diptera (Insecta) Involved in the Transmission of Avian Haemosporida

Sergio Ibáñez-Bernal, Karina D. Rivera-García, and Carlos A. Abella-Medrano

Abstract Diptera is one of the most diverse orders of insects with more than 159,000 valid species described worldwide, which are arranged in approximately 158 families. A general overview of insect species of the order Diptera with hematophagous habits is presented. This includes some important characteristics to recognize those families with blood-sucking habits and a simple taxonomic key for their identification. Of the 13 blood-feeding families included, four are known to be involved as vectors of avian haemosporidia and are treated in more detail. As such, an overview of the morphology, biology, importance, taxonomy, and diversity of Culicidae, Simuliidae, Ceratopogonidae, and Hippoboscidae is presented. Basic literature for their study is also provided. The novice is reminded that many technical taxonomical terms will be used, so make sure you follow closely the figures legends and drawings throughout the text; we also include a glossary of terms to help you traverse the journey (remember patience and dedication pay off!).

Keywords Ceratopogonidae · Culicidae · Hematophagous flies · Hippoboscidae · Simuliidae

S. Ibáñez-Bernal (✉) · K. D. Rivera-García
Red Ambiente y Sustentabilidad, Instituto de Ecología, A.C. (INECOL), Xalapa, Veracruz, Mexico
e-mail: sergio.ibanez@inecol.mx

C. A. Abella-Medrano
Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico

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5.1 Introduction

Diptera includes the true flies and is one of the most diverse and successful orders of insects around the world; it has more than 159,000 valid species described worldwide, which are arranged in ~158 families (Pape et al. 2011). This species richness correlates with a wide diversity of habits and habitats; flies can be found in almost any environmental conditions with the exception of the highest elevations (with permanent snow) and the Poles. The bulk of species has been recorded in tropical areas, corresponding to all biogeographical tropical or at least subtropical zones of the world (Konstantinov et al. 2009; García Molinos et al. 2017). Zoogeographic Regions vary in their described diversity, with 32,500 species in the Neotropics (Borkent et al. 2018); 20,400 species in the Afrotropical Region (Kirk-Spriggs 2017); 45,198 in the Palaearctic and 22,545 in the Oriental Region (Grootaert 2009); 21,454 in the Nearctic Region (Thompson 2009); and an estimated 6,400 species in the Australian Region (Yeats et al. 2009). However, it is important to note that the different biogeographic regions have been explored with different intensity; there are still many unexplored areas and some families have not been studied in detail.

The order Diptera includes the Endopterygota (see Glossary) insects, which are characterized as adults by having the head highly mobile, usually with large compound eyes, with or without ocelli, antenna from long multiarticulate to short with the last segment compact with flagellomeres forming a stylus or an arista, mouthparts variously modified but haustellate, adapted to suck liquid food. The thorax has the first segment or prothorax that is very small and fused with the second segment or mesothorax, which is the largest portion of the thorax that bears the two functional wings, and the third segment or metathorax that is small, with a pair of halters, which are the second pair of wings modified as gyroscopic organs used during flight; each thoracic segment has a pair of legs, which in general are adapted to lay down or perch rather than walk or run (Fig. 5.1). Diptera pupae may be adecticous, obtect, and free in the nematocerous families (Figs. 5.7c, 5.10d, and 5.12b,c) and Orthorrhapha Diptera, or exarate, enclosed in puparium (Fig. 5.14f), which is the last larva exuviae, as occurs in Cyclorrhapha. The larva varies considerably in form and is always apodous (Fig. 5.12a), but in some groups, pseudopods may be present on various segments (Fig. 5.10a), with sclerotized head capsule (Figs. 5.7a and 5.10a–c) or with the head not differentiated. Normally, Diptera are oviparous and lay eggs, but there are cases of ovoviviparity or viviparity, laying larvae that may pupate immediately (Fig. 5.14f).

In general, Diptera adults are saprophagous, but there are some predators, parasitoids, parasitic (including hematophagous), and nectarivorous species. Adults are generally good fliers and usually can be found near the habitats in which the immatures develop. Immatures may be aquatic, semiaquatic, or occupy dry environments, but usually, they use moist or semiaquatic microhabitats (e.g., epiphytes). Some feed on plants, act as predators or parasites of other animals including vertebrates, or can feed on feces or other organic decomposing matter. This group of insects is only relatively well known for those few families that have adult hematophagous

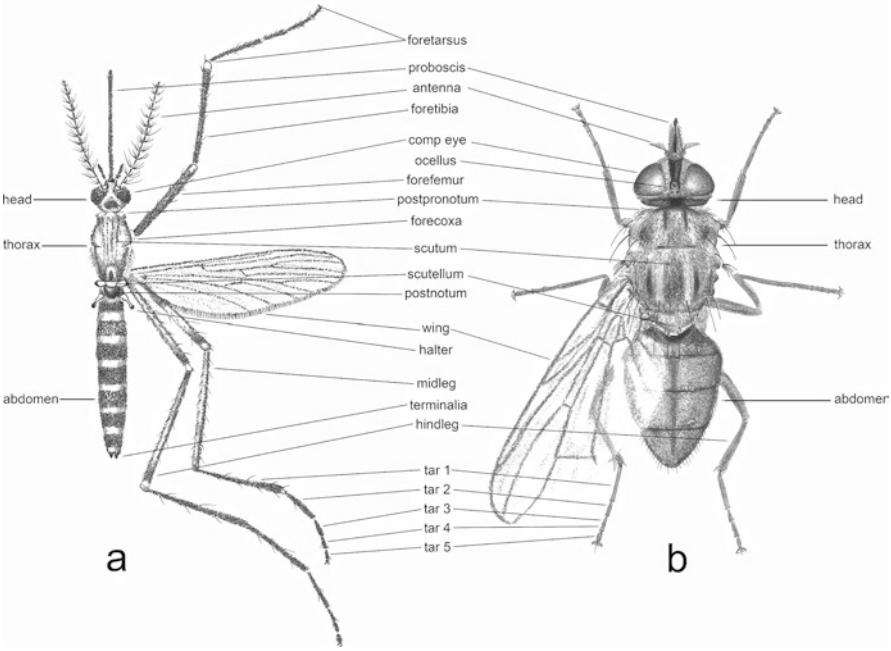

Fig. 5.1 General morphology of adult Diptera. (**a**) Culicidae (mosquito). (**b**) Muscidae (horn fly). Abbrv. comp eye compound eye, tar tarsomere

species, and which are important in the transmission of parasites producing disease in vertebrates (i.e., pathogens). Diptera may be involved in the transmission of virus, bacteria, rickettsia, protozoa, and helminths, that produce severe diseases in humans and animals, having high economic impacts.

5.1.1 Diptera as Parasites

Some Diptera species are parasites of other invertebrates and of vertebrates. Parasitism in Diptera can be of different types, and some classifications have been proposed taking into consideration different attributes. Diptera can be classified as protelean or imaginal parasites, which correspond to the immature and adult stages, respectively (Askew 1971). They could be considered as obligate or facultative, considering how much they depend on hosts to survive. Another classification of parasitism is based on the diversity of hosts they use, as monoxenous (i.e., one host species), stenoxenous (i.e., some host species that belong to the same phylogenetic group), and eurixenous (i.e., can use indiscriminately any available hosts) (Dawes 1976). Another useful classification of parasitism in insects is based on how much time of their life cycle they spend as parasites. Continuous parasites if they require

all their life to be intimately related with the host, transitory parasites in the case where the parasite only has parasitic habits during one developmental stage, and intermittent parasites corresponding to those parasites that are free living but turn to hosts only to feed (James and Harwood 1969).

Regardless of the parasitic classification used, the most relevant Diptera in regard to pathogen transmission are the ones belonging to the hematophagous type. Hematophagy is here defined as the trophic relationship in which the parasite (i.e., the adult fly) feeds on vertebrate blood (i.e., the host). It is important to mention that most of the hematophagous nematocerous Diptera also feed on nectar or other sugar sources to fuel their flight. It is also important to emphasize that hematophagous flies are few species in comparison with the rest of Diptera, considering that there are 159,051 known species of Diptera (Borkent et al. 2018; Pape and Evenhuis 2018), about 9.6–10% are hematophagous. Hematophagy in Diptera has evolved independently, at least three times in lower Diptera (formerly suborder Nematocera) in Psychodomorpha and Culicomorpha, two times in the lower Brachycera but restricted to the Tabanomorpha (Athericidae and Tabanidae) (Wiegmann et al. 2000; Grimaldi and Engel 2005; Courtney et al. 2009), and at least two times in the higher Diptera or Calyptratae Cyclorrhapha in superfamilies Hippoboscoidea and in Muscoidea and Oestroidea (Fig. 5.2). The oldest fossil records of hematophagous Diptera are known from the Early Cretaceous in Psychodidae-Phlebotominae (Azar

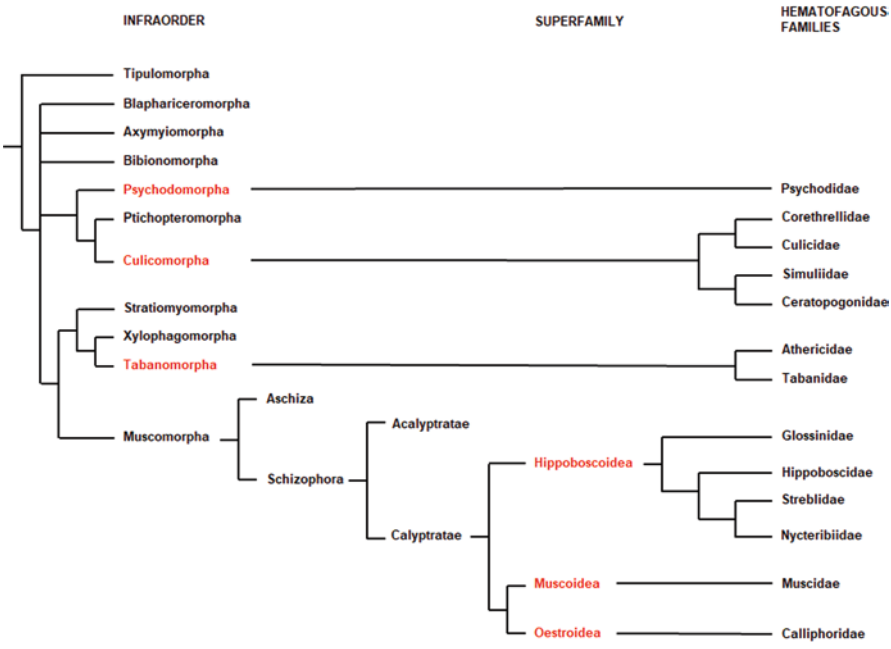

Fig. 5.2 Simplified cladogram of superfamilies of Diptera, highlighted in red the groups containing species with hematophagous habits. (Modified from McAlpine 1989; Woodley et al. 2009)

et al. 1999; Stebner et al. 2015), Ceratopogonidae (Borkent 1997, 2000), Simuliidae (Kalugina 1991), Corethrellidae (Borkent 2008), and at least from the Mid Cretaceous in the case of Culicidae (Poinar et al. 2000; Borkent and Grimaldi 2016), Tabanomorpha from the Early Cretaceous, and Cyclorraphous from the Cenozoic period (Lukashevich and Mostovski 2003).

5.1.2 Hematophagous Families

Of the approximately 150 families of Diptera, only 13 include hematophagous species. In the nematocerous families we find the Psychodidae, Simuliidae, Ceratopogonidae, Corethrellidae, and Culicidae; in the lower Brachycera families we have the Athericidae and Tabanidae, and in the Calyptratae Cyclorrhapha families there are the Muscidae, Calliphoridae, Glossinidae, Nycteribiidae, Streblidae, and Hippoboscidae (Fig. 5.2). Culicidae, Ceratopogonidae, Simuliidae, and Hippoboscidae are treated in detail in Sect. 5.4 as they include species that transmit Haemosporida in birds (Santiago-Alarcon et al. 2012; see also Chap. 6 for a recent synthesis of studies on vector transmission of avian Haemosporida).

The family Psychodidae is classified into six extant subfamilies, of which two subfamilies have females with blood-sucking habits, Sycoracinae where females feed on frogs (Bravo and Salazar-Valenzuela 2009) and were once found infected with a filarial worm (Desportes 1942), and Phlebotominae with females that suck blood from reptiles, birds, but predominantly mammals (Young and Duncan 1994). Phlebotominae are known as vectors of *Leishmania* to vertebrates including humans (Killick-Kendrick 1990), but also other trypanosomatids such as genus *Trypanosoma* infecting bats, lizards, toads, and nonflying mammals (e.g., *Didelphis* spp.; Anderson and Ayala 1968; Herrer 1942; Christensen and Herrer 1975; Williams 1976; Williams and de Vasconcellos Coelho 1978), *Endotrypanum* and *Brimont* spp. in sloths (Franco and Grimaldi 1999), *Bartonella bacilliformis* producing bartonellosis, Carrion's disease or verruga Peruana (Noguchi et al. 1928; Cáceres et al. 1997), and arbovirus of the genus *Phlebovirus*, *Orvivirus* and *Vesiculovirus* (Maroli et al. 2012).

The family Corethrellidae is the sister group of Culicidae + Chaoboridae, all belonging to the only included genus *Corethrella*, with 109 species worldwide (Borkent 2008, 2014; Yu et al. 2013; Amaral and Pinho 2015; Caldart et al. 2016; Kvifte and Bernal 2018). The members of this group are known as frog-biting midges, females suck blood exclusively from calling male frogs. They transmit *Trypanosoma* to their hosts (Borkent 2008; Bernal and Miguel Pinto 2016).

The family Athericidae includes the genus *Suragina*, which are known as blood feeders on frogs, birds, and mammals (Woodley 2009), but there is no evidence of pathogen transmission by these flies yet. There are ten genera and about 100 species described for the family around the world, of which about 47 species correspond to the genus *Suragina* (Rafael and Henriques 1990; Stuckenberg 2000; Pape and Evenhuis 2018).

The family Tabanidae comprises the commonly named deer flies and horse flies. Currently, there are about 4,406 named species worldwide (Borkent et al. 2018; Pape and Evenhuis 2018), representing four subfamilies. Females of few species may be nonhematophagous, but most of them feed on the blood of mammals and few species on the blood of reptiles and birds (Middlekauff and Lane 1980; Fairchild 1986). Their bites are painful, and there are species recognized as mechanical vectors of some pathogens, including *Bacillus anthracis* producing anthrax, *Francisella tularensis* that causes tularaemia, and have been implicated as possible vectors of *Borrelia burgdorferi*. Some species of the genus *Chrysops* are the biological cyclical vectors of the nematode *Loa loa* in the forested areas of Central and West Africa (Chainey 1993).

The family Muscidae includes the well-known houseflies, stable flies, and horn fly (Savage and Vockeroth 2010). It is a diverse family of flies with about 5,200 species described across the world (Borkent et al. 2018; Pape and Evenhuis 2018), and can be found in nearly all types of environments. Most of the species feed as adults on decomposing organic matter, being saprophagous, on plant or animal exudates as sugars, honey, sweat, tears, etc., but there are predaceous species too. Larvae can be found in a variety of habitats, breeding in decomposing vegetable matter, in the soil, insect and bird nests, burrows, water, carrion, and so on. There are some common annoying species inside human dwellings, stalls, and barns, and these may constitute severe pests transmitting mechanically some pathogens to animals. Greenberg (1973) synthesized pathogens related to some families of flies, including Muscidae, causing diseases in humans and other animals. Few species especially of the genus *Philornis* are hematophagous during the larva stage and are found as subdermal parasites of birds. As adults, hematophagous muscids are *Musca crassirostris* (subfamily Muscinae), and about 12 species of the genera *Haematobosca*, *Stomoxys*, and *Haematobia* (subfamily Stomoxyinae) (Zumpt 1973). From the basic morphology of surface-sucking type of mouthparts, few species have adapted their proboscis to scrape off the skin to gorge the blood of mammals. They may transmit some pathogens under certain circumstances, but there is dubious evidence that hematophagous muscids may be involved as mechanical vectors of pathogens (Crosskey 1993b).

The family Calliphoridae is usually known as blowflies and includes about 1,522 species worldwide (Borkent et al. 2018; Pape and Evenhuis 2018). In the larval stage, some are decomposers of animal matter, and few in dead vegetable tissues, but some species of the genus *Auchmeromyia* feed on mammals' blood, and species of the genus *Protocalliphora* suck blood from birds and are found in their nests (Vargas and Wood 2010). As adults, they feed on carrion and feces, but can consume nectar; species of *Cochliomyia*, primarily *Cochliomyia hominivorax*, are parasites on wounds and feed available blood, and when present in high numbers may kill the host (Laake 1936).

The family Glossinidae includes the commonly known tsetse flies distributed across the sub-Saharan Africa. All species belong to the genus *Glossina*, and the adults are blood feeders on reptiles, birds, and mammals. They are hippoboscoids flies having a viviparous reproduction. Females develop a single larva in their uterus to their maturity, larviposit on the ground, with the larva quickly transforming into

a pupa. Depending on the classification, there are between 23 or 31 species that vector *Trypanosoma brucei*, causing nagana in mammals and sleeping-sickness in humans (Jordan 1993).

The family Nycteribiidae contains species commonly known as spider bat flies. Nycteribiids are hippoboscoid flies closely related to Streblidae to an extent that this family and Streblidae have been placed by some authors within the family Hippoboscidae (Griffiths 1972). Currently, there are about 274 described species worldwide in 11 genera, with *Basilia* being the richest genus with 116 (Graciolli 2010; Graciolli and Dick 2018). They are viviparous and the adults are obligate hematophagous parasites of bats. It is important to mention that members of this family have been incriminated as vectors of haemosporidians infecting bats (Garnham 1973; Marinkelle 1995).

The family Streblidae or bat flies comprise about 239 species worldwide (Borkent et al. 2018; Pape and Evenhuis 2018). As indicated by their common name, streblid adults are obligate hematophagous ectoparasites feeding exclusively on bats, similar to those in the family Nycteribiidae. As other hippoboscoids flies, they are viviparous and have morphologic and physiological adaptations for parasitic life. Five subfamilies are currently recognized, of which Brachytarsininae and Ascodipterinae are exclusive of the Old Word, and Nycterophiliinae, Trichobiinae, and Streblinae are from the New World (Dick and Miller 2010). There is evidence of haemosporidian infections in bats and bat flies since the Tertiary (Poinar 2011), and currently also infections by other pathogens, such as *Bartonella* (Reeves et al. 2005; Dick and Dittmar 2014; Obame-Nkoghe et al. 2016).

5.2 General Taxonomic Characteristics of Adult Diptera, with Special Reference to Hematophagous Families

There are many publications in which the general morphology of Diptera is presented in more detail. The following paragraphs summarize important aspects to introduce morphology and taxonomy of the hematophagous families, but if more information is needed, we recommend McAlpine (1981a), Teskey (1981), Cumming and Wood (2009), Merz and Haenni (2000), Sinclair (2000), Kotrba (2000), and Kirk-Spriggs (2017).

The body is divided into three tagmata (sing. tagma) or functional units: head, thorax, and abdomen, from which specialized appendages originate (Fig. 5.1).

5.2.1 Head

The head is apparently formed by six segments in two groups: the preorals (acron, antennal, and intercalary segment) and the postorals (mandibular, maxillary and labial segments). It is a body unit strongly sclerotized, which protects the brain and

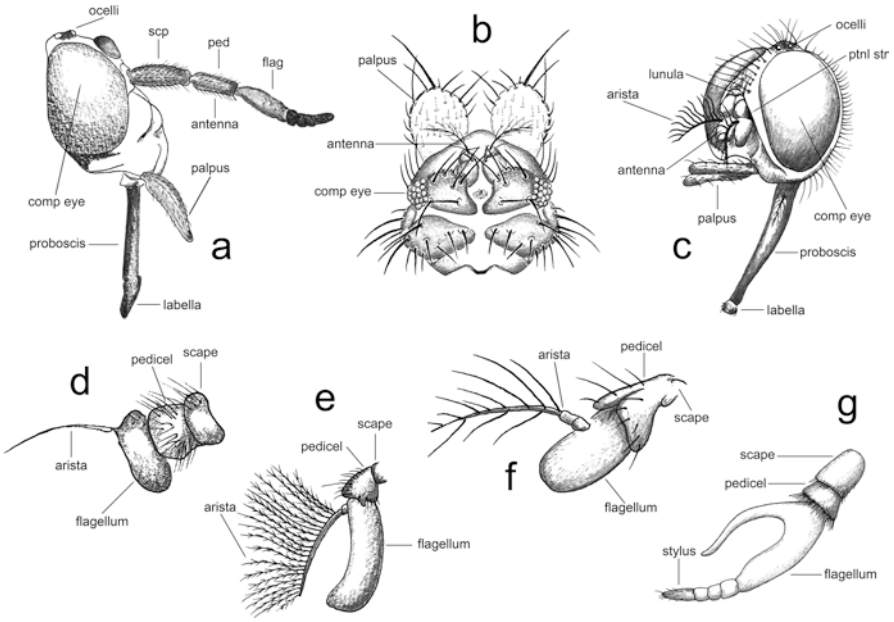

Fig. 5.3 Head. (**a**) Tabanidae, lateral view; (**b**) Streblidae, dorsal view; (**c**) Muscidae, frontolateral view. Antenna types of different families: (**d**) Athericidae; (**e**) Glossinidae; (**f**) Muscidae; (**g**) Tabanidae. Abbrv. comp eye compound eye, flag flagellum, ped pedicel, ptnl str ptilinial suture, scp scape

the subesophageal ganglia of the nervous system, in which special sensory organs and also the mouthparts are situated. In Diptera, it is usually somewhat spherical with high capacity of movement with the thorax, and it commonly has large compound eyes, sometimes ocelli, and a pair of antennae (Figs. 5.1 and 5.3a–c). The mouthparts usually are directed downward (hypognathous condition, e.g., Simuliidae, Tabanidae, Muscidae) (Fig. 5.3a, c) or may be directed forward (prognathous condition, e.g., Hippoboscidae, Nycteribiidae, Streblidae) (Figs. 5.3b and 5.14c).

Principal appendages in head are the antennae (Fig. 5.1). Each antenna is composed of three segments, the basal or scape that can be very short, as in Culicidae and Simuliidae (Fig. 5.9c), the second or pedicel, that is large and globular in Culicomorpha (Fig. 5.6a), and the third or flagellum, which may be long and multiarticulated (each subsegment is a flagellomere) as in nematocerous Diptera (Fig. 5.9c) or short with a tendency to the reduction of the number of subsegments of the stylate (e.g., Tabanidae) (Fig. 5.3a, g) or aristate type (as in Muscomorpha) (Fig. 5.3d–f). Head bristles and setae are also important in taxonomy of Diptera, especially in higher Diptera.

The mouthparts are composed basically of a labrum-epipharynx, a pair of mandibles, a pair of maxillae, and a hypopharynx, which in the hematophagous families

are styliform and are useful to penetrate the skin and suck blood, all of them covered and protected by the labium (Fig. 5.6a). All these elements constitute the proboscis. Originating from each maxilla, there is a maxillary palpus with five (Figs. 5.9b and 5.11b) or fewer segments depending on the group (Fig. 5.3a, c); the labial palpi of other insects are transformed in Diptera to form a pair of small cushions at the end of the proboscis, named labella, which helps to lick superficial fluids (Fig. 5.3a), or in the case of some hematophagous species to suck blood from the host's skin surface (Fig. 5.3c). Reduction of mouthparts in male Diptera makes the process of blood sucking impossible, as is the case of the males of nematocerous and lower brachyceran Diptera. In the higher Diptera, mouthparts are more specialized and composed only of the labrum-epipharynx and hypopharynx, which form the food channel, both are protected by the labium, which is capable of retraction; in some species, the labella are rigid and sclerotized with spines for scratching the hosts' skin (Fig. 5.3c). Two mechanisms for taking blood can be recognized in Diptera, solenophagy or capillary feeding directly from blood capillaries of vertebrate hosts as found only in Culicidae, and telmophagy or pool feeding where the fly breaks the skin and capillaries bleed, forming a tiny blood pool on the skin's surface (e.g., Ceratopogonidae, Simuliidae). As a consequence of differences in proboscis structure, different families of Diptera have different competencies as pathogen vectors, which also explain the differences between host clinical reactions to fly bites (Bouchet and Lavaud 1999).

5.2.2 Thorax

As in all insects, the thorax is composed of three segments: the prothorax, the mesothorax, and the metathorax, each bearing a pair of legs (Fig. 5.4a). In Diptera, the prothorax is strongly reduced (Fig. 5.4a, blue area), the mesothorax, which bears the pair of functional wings, constitutes the major portion of this tagma (Fig. 5.4a, orange area), and the metathorax, which also is reduced but to a lesser degree, bears a pair of halters that are modified hind wings (Fig. 5.4a, green area). In dorsal view, the prothorax is usually represented by the postpronotum, the mesothorax by the scutum (that may be divided into a prescutum and a scutum *sensu stricto*), the scutellum and the mesopostnotum, whereas the metathorax is represented by the metanotum that is reduced and difficult to see. In lateral view, the prothorax is composed of the propleuron, may be rather indistinctly divided into a proepisternum and a proepimeron, with the anterior spiracle opening between the propleuron and the mesopleuron; the mesothorax laterally is called mesopleuron and is divided by the pleural suture separating the anterior mesepisternum from the posterior mesepimeron; subsequently, the mesepisternum is divided transversely into a upper part of mesanepisternum and a lower portion or meskatepisternum, whereas the mesepimeron is divided transversely by a suture into an upper mesanepimeron and a lower meskatepimeron; finally the lateral portion of the metathorax, known as metapleuron, is separated by a suture into an anterior metepisternum and a posterior

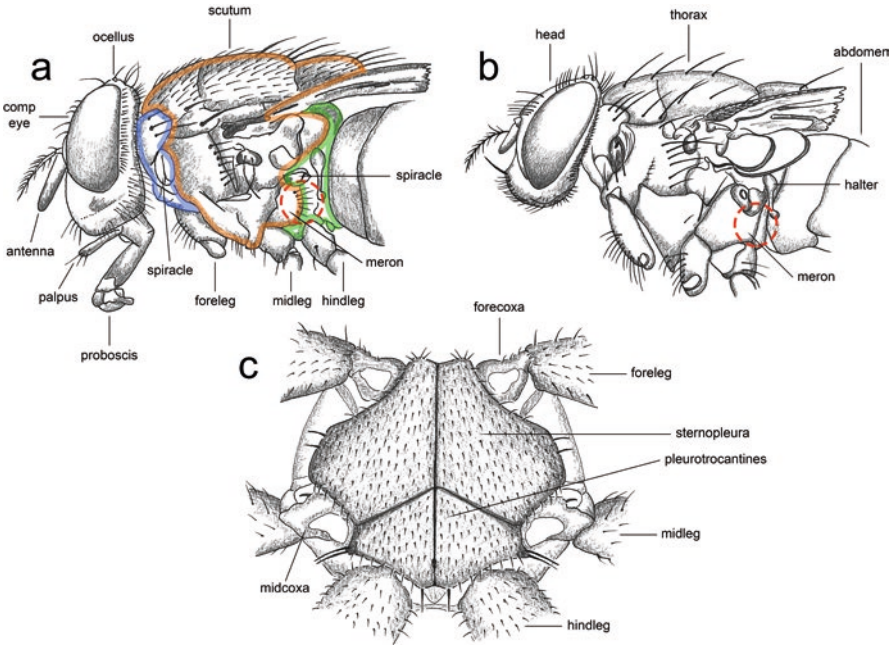

Fig. 5.4 Thorax. (**a**) Calliphoridae, lateral view; (**b**) Muscidae, lateral view; (**c**) Streblidae, ventral view. Blue contour: prothorax; orange contour: mesothorax; green contour: metathorax. Abbrv. comp eye compound eye

metepimeron; the metepisternum is divided into a dorsal metanepisternum and a ventral metakatepisternum; anterior and below the metakatepisternum is meron dorsal to the hind coxa (Fig. 5.6b). Ventrally, the thoracic segments in the large majority of Diptera have the sclerites reduced, as the legs originate ventrally very close to each other; an exception to this condition are the Hippoboscoidea (Hippoboscidae, Nycteribiidae, and Streblidae), which have the ventral thoracic sclerites more developed, with the legs originating laterally far from each other, a condition related to the parasitic position on the host surface (Fig. 5.4c). Ventrally, prothorax is represented by the prosternum, the mesothorax by the mesosternum, and the metathorax by the metasternum.

5.2.3 Legs

There are three pairs of legs, one pair for each segment of the thorax (Figs. 5.1 and 5.4a). They usually are adapted for landing or perching, with a limited capacity to walk in most taxa. They are divided into the following segments from base to apex: coxae, trochanter, femur, tibia, and tarsus; each tarsus is generally divided into five

tarsomeres (Figs. 5.1 and 5.9a, d). At the end of the fifth tarsomere, there is an acropod, composed of an unguitractor plate that bears a pair of claws, and sometimes, there is at the center a pad named arolium, which produces a sac-like structure (pulviliform) or setiform empodium (Fig. 5.14e). From the arolium, a pair of flap-like processes or pulvilli arise below each tarsal claw. All these pads are useful for holding to surfaces even smooth ones. The form, color, and size of each segment of the legs are variable among species, and the legs also have bristles and hairs with size and position that help in the recognition of genera and species (Figs. 5.9a, d and 5.11a).

5.2.4 Wings

In Diptera only, the mesothoracic wings are functional for flying (Fig. 5.1), but these are reduced in a number of taxa. The second pair on the metathorax that are present in other insects (e.g., Lepidoptera) is modified as halters, which are little club-like organs that serve as gyroscopes and stresses on the halters inform the fly how it is flying (Fig. 5.1a). The mesothoracic or functional wings are very variable in shape, number, and disposition of veins, and for that reason, wings are very useful in the recognition of the families, genera, and even species. The structure of the wings is complex, but for identification, the size, shape, and the longitudinal veins and cross veins, as well as the cells they form (i.e., spaces delimited by veins), are important. In general, the longitudinal veins are, from the anterior to the axillary posterior margin: Costa (C) that can be short or extend along the complete wing margin, Subcosta (Sc), Radial vein (R) that may branch into no more than five veins (R_1, R_2, R_3, R_4, and R_5) but can join together forming complex veins (R_{2+3} and R_{4+5}), Media (M) with no more than three veins (M_1, M_2, and M_3), Cubital (Cu) with two anterior branches (CuA_1, CuA_2), and one posterior (CuP), and Anal with no more than two branches (A_1 and A_2). Cross veins are those transversally directed that connect two longitudinal veins, and usually the subcostal-radial (sc-r), radial-medial (r-m), medial (m-m), medial-cubital (m-cu) may be present. Common and important closed cells in Diptera wings are basal radial (br), basal-medial (b-m), discal (d), and sometimes the posterior cubital (cup) can be closed, and the open cells are named according to the vein, which is anterior to the cell (Figs. 5.5, 5.6c, 5.9a, 5.11d, and 5.14d).

5.2.5 Abdomen

As in all insects, the primary number of abdominal segments are 11, but the posterior segments are modified to conform the terminalia (genital and postgenital regions) (Figs. 5.6d and 5.9e, f). The primitive number of spiracles is eight pairs, but it is most common to find seven; in some groups, the number of spiracles can be

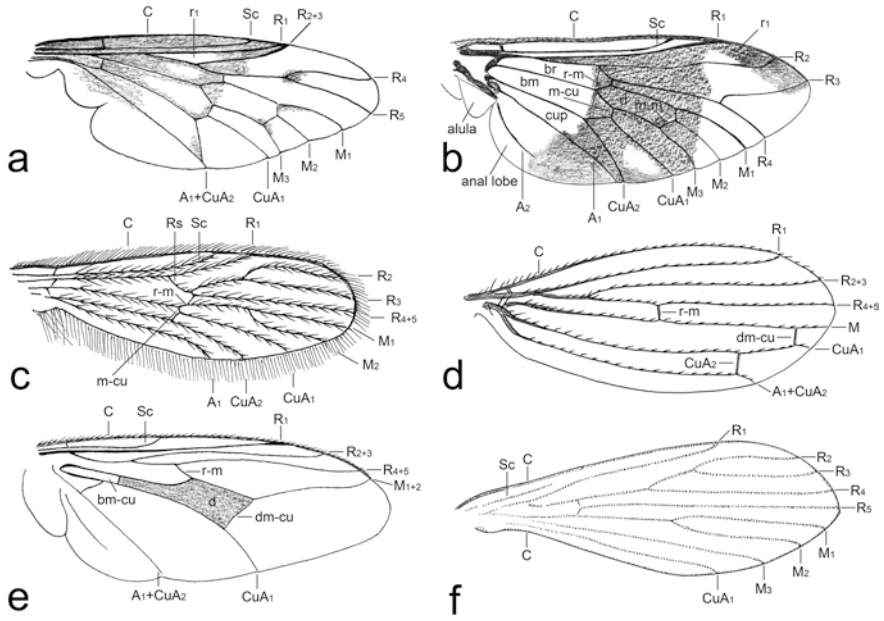

Fig. 5.5 Wings. (**a**) Athericidae; (**b**) Tabanidae; (**c**) Corethreliidae; (**d**) Streblidae; (**e**) Glossinidae; (**f**) Psychodidae (Phlebotominae). Abbrv. A anal, bm basal-medial, br basal radial, C costa, CuA anterior cubital, cup posterior cubital, d discal, M media, m-cu medial-cubital, m-m medial, R radial, r-m radial-medial, Rs radial sector, r_1 cell r_1, Sc subcosta

reduced. The dorsal sclerotized plates are terga (sing. tergum), and the ventral plates are sterna (sing. sternum). Laterally, they are connected by a pleural membrane to which the spiracles are intimately associated. Tergum I almost always is reduced and closely associated with tergum II, to an extent that they may form a syntergum I + II as it happens in Muscomorpha. Sternum I is more or less reduced and sometimes associated to sternum II. Segments I-V or I-VI are not strongly modified and constitute the preabdomen. The terminal segments from the sixth or seventh to the tip constitute the terminalia or postabdomen, which are greatly modified for reproduction (Fig. 5.6d). Terminalia usually provide the characteristics that are necessary to recognize species. In male Diptera, the principal structures are the IX segment (epandrium + hypandrium), gonopods (gonocoxite + gonostylus), the intromittent organ or aedeagus, and cerci (Figs. 5.6d and 5.9f). In female Diptera structures that usually are important for taxa recognition are the sternum VIII, cerci, internally the spermathecae (Fig. 5.9e) and sternum IX.

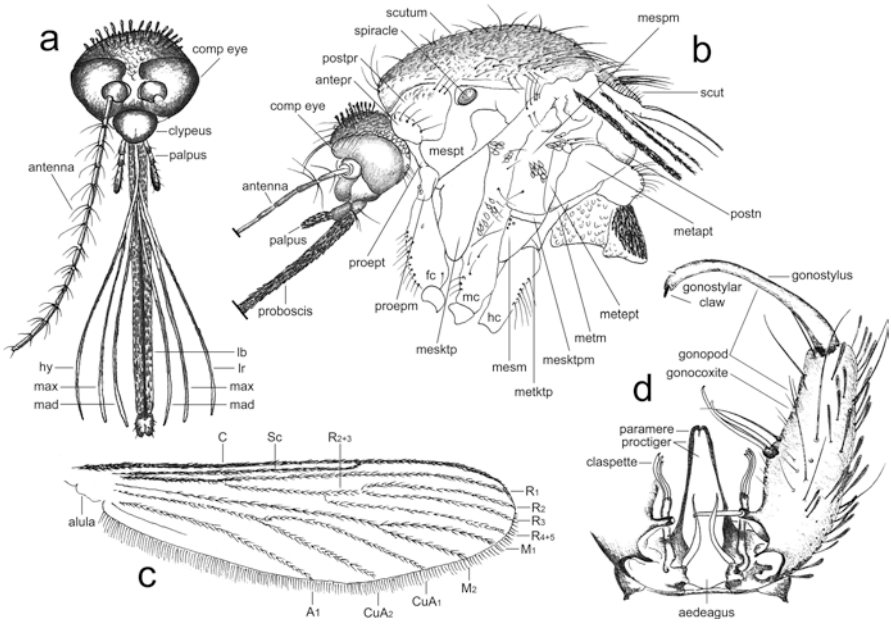

Fig. 5.6 Culicidae (Adult). (**a**) Head, frontal view; (**b**) head and thorax, lateral view; (**c**) wing; (**d**) male terminalia. Abbrv. A anal, antepr antepronotum, C costa, comp eye compound eye, CuA anterior cubital, fc forecoxa, hc hindcoxa, hy hipopharynx, lb labium, lr labrum-epipharynx, mad mandible, max maxilla, M media, mc midcoxa, mesktp meskatepisternum, mesktpm meskatepimeron, mesm mesomeron, mespm mesepimeron, mespt mesanepisternum, metapt metapisternum, metept metepisternum, metm metepimeron, metktp metakatepisternum, postpr postpronotum, postn mesopostnotum, proepm proepimeron, proept proepisternum, R radial, Sc subcostal, scut scutellum

5.3 Key to Hematophagous Families of Diptera

The following keys are simplified with the intention to recognize adults of Diptera families with obligate hematophagous species. For a more detailed and precise identification, we recommend the keys provided by McAlpine (1981b), Papp and Schumann (2000), Buck et al. (2009), and Kirk-Spriggs (2017).

1. Legs with coxae very close to each other near the ventral midline (Fig. 5.1). Intermittent blood feeders. . . . . 4.
   - Legs with coxae widely spaced, originate ventrolaterally (Figs. 5.4c and 5.14a). Ectoparasites of vertebrates. . . . . 2.
2. Head vertically oriented, partially protected by a thorax hollow of the anterior margin of scutum. Legs with tarsomere 1 at least as long as the rest of tarso-

meres. Exclusive ectoparasites of bats. . . . . Family Nycteribiidae.

- Head horizontally directed, freely articulated to thorax (Figs. 5.3b and 5.14c). Legs with tarsomere 1 short, similar in length to tarsomere 2 (Fig. 5.14a). Ectoparasites of bats, birds, and mammals. . . . . 3.

3. Compound eyes large, at least 0.75 the head height, each one is oval and with more than 100 facets (Fig. 5.14a). Ectoparasites of birds and mammals, except bats. . . . . Family Hippoboscidae.

- Compound eyes small, not larger than 0.50 the height of head, each one rounded and formed by no more than 40 facets (Fig. 5.3b). Ectoparasites of bats. . . . . Family Streblidae.

4. Antennal flagellum with four or more articulate flagellomeres (Figs. 5.6a, 5.9c, and 5.11c). Palpus with three to five segments (Figs. 5.6a, 5.9b, and 5.11b). Head without ocelli (Figs. 5.6a, 5.9b, and 5.11b). . . . . 5.

- Antennal flagellum with only one compound flagellomere with other flagellomeres forming a dorsal or apical arista (Fig. 5.3d–f) or a consolidated apical stylus (Fig. 5.3g). Palpus with no more than two segments (Fig. 5.3a, c). Head with or without ocelli. . . . . 9.

5. Wing with C continuing all margin to reach the posterior axilla (Fig. 5.5f). . . . . 6.

- Wing with C ending before M_1, vein commonly coincident with the wing apex (Fig. 5.5a–e). . . . . 8.

6. Wing with Sc complete, ending in C near the middle of wing or distally to it; two veins, Sc and R_1 reaching C anteriorly to R_{2+3}; vein M with two branches. Wing veins covered by scales (Figs. 5.5c and 5.6c). . . . . 7.

- Wing with Sc incomplete or ending in C or in R_1 before the middle of the wing; one vein (R_1) reaching C anteriorly to the first radial branching; M with three branches (Fig. 5.5f). Body and wing veins pilose or with scales. Head with a relatively short proboscis, not longer than two times the length of head. . . . . Family Psychodidae.

7. Proboscis long and slender, at least twice the length of head (Figs. 5.1a and 5.6a). Head, legs, and other body regions clothed with scales (Fig. 5.1a). . . . . .

. . . . . Family Culicidae.

- Proboscis short, less than twice the length of head, both lacking scales. Wing with R_1 ending in wing margin nearer to apex of Sc than to apex of R_2 (Fig. 5.5c). Midfemur thicker than hindfemur. . . . . Family Corethrellidae.

8. Antenna moniliform with flagellomeres about as long as wide, short, antenna length about as long as the head, and without long setae in both sexes (Fig. 5.9c). Wing broad, with expanded anal lobe, anterior veins (Sc, R_1, R_{2+3}, R_{4+5}) thick, posterior veins weak (Fig. 5.9a). . . . . Family Simuliidae.

- Antenna filiform with flagellomeres longer than wide, antenna much longer than head, and each flagellomere with long setae that are much longer and abundant in males than in females (Fig. 5.11c). Wing with anal lobe not greatly expanded, with no more than two branches of R reaching the wing margin, but if vein R_{2+3} present, it forms a closed cell (r_{2+3}) (Fig. 5.11d). . . . . Family Ceratopogonidae.

9. Head with ptilinal suture surrounding antennae, and with lunula above antennal insertions (Fig. 5.3c). Wing vein CuA_2 usually short nearly always reaching A_1 near the wing base (Fig. 5.5e). . . . . 12.

- Head without ptilinal suture and lunula (Fig. 5.3a). Wing vein CuA_2 usually long, nearly always reaching the wing margin or vein A_1 near wing margin (Fig. 5.5a, b). . . . . 10.

10. Tarsi empodia pulviliform, that is, with three similar pads below the tarsal claws..... 11.

- Tarsi empodia setiform or absent, that is, with only two pads below the tarsal claws (as in Fig. 5.14e). . . . . Some nonhematophagous families.

11. Antennal flagellum with a nonarticulated arista (Fig. 5.3d). Wing cell r_1 closed by R_1 and R_{2+3} that are confluent with C (Fig. 5.5a). . . . . Family Athericidae.

- Antennal flagellum forming an annulated stylus (Fig. 5.3g). Wing with cell r_1 open, as R_1, R_2, and R_3 reach C separately (Fig. 5.5b). . . . . Family Tabanidae.

12. Antennae arista with branched setae (Fig. 5.3e). Wings folded completely one on top of the other; wing cell d with a characteristic hatchet shape resembling a meat cleaver (Fig. 5.5e). Proboscis long and thin attached to the bottom of the

head and pointing forward. . . . . Family Glossinidae.

- Antennae arista without branched setae (Fig. 5.3f). Proboscis not so long and not pointing forward (Fig. 5.4a). Wing discal cell d not hatch shaped; wings folded over body more separated between each other. . . . . 13.

13. Meron without row of bristles (Fig. 5.4b). Wing with distal half of Sc usually curved towards C; A_1 + CuA_2 long, fading out beyond midpoint between its base and wing margin; lower calyptra (see Glossary) convex at hind margin. Hindtibia/hindleg usually with setae in anterodorsal and posterodorsal position. Apex of scutellum bare ventrally. . . . . Family Muscidae.

- Meron with row of at least three bristles (Fig. 5.4a). Other characteristics variable. . . . . Family Calliphoridae.

5.4 Families Involved in the Transmission of Avian Haemosporida

5.4.1 Family Culicidae

Members of the family Culicidae are commonly known as mosquitoes, true mosquitoes, zancudos, moyote, moustique, etc., according to the region and language. It is an important and well-represented group of Diptera that mostly include species with hematophagous females. Species of this family are found in all geographic regions, except Antarctica and mountain peaks with permafrost. Immature stages are strictly aquatic, occupying nearly all water reservoirs from salt or polluted to potable fresh water. This group includes the most important insect species associated to major epidemic diseases (e.g., malaria, dengue fever, yellow fever, zika).

5.4.1.1 Morphology

The following descriptions of mosquitoe's life stages are based primarily on the works of Carpenter and LaCasse (1955), Belkin (1962), Harbach and Knight (1980), and Stone (1981).

Adult (Figs. 5.1a and 5.6) Mosquitoes are small insects, between 3.0 and 9.0 mm long, characterized by body completely or partially covered with squamae or scales that provide particular color patterns, wings with veins also covered with scales, with long thin proboscis and legs (Fig. 5.1a).

Head and its appendages The head is small and spherical, with reniform compound eyes formed by ommatidia (eye functional unit) whose facets have equal size, similar in both sexes. Ocelli absent. Proboscis longer than thorax (Fig. 5.1a), with a set of six fine stylets (labrum-epipharynx, two mandibles, two maxillae, and hypopharynx) protected by the labium (Fig. 5.6a); females are adapted for piercing the vertebrate skin, and in males which only feed on nectar, maxillae and mandibles are reduced. Maxillary palpi differ in length according to genera and species; however, in most species of subfamily Culicinae, females have short palpi (Fig. 5.6a) and males long palpi similar in length to proboscis, whereas in subfamily Anophelinae, female's palpi are as long as the proboscis, and in males they are longer than proboscis with the apical segments widened. Antennae are long and filiform with a very short scape, a globular large pedicel sometimes named torus, and flagellum is composed of 13 subunits or flagellomeres, which usually have abundant long setae or verticils in males as compared with females (Figs. 5.1a and 5.6a) (Knight and Stone 1977; McAlpine 1981a; Darsie and Ward 2005).

Thorax and its appendages Scutum covering near all dorsal surface, with short arched or trilobed scutellum presenting transversal row or three groups of setae; antepronotum and postpronotum well differentiated but small, meskatepisternum well developed with scales and setae that are useful in taxonomic identification (Fig. 5.6b). Wings long and thin covered with scales and with a characteristic pattern of uniform veins. The entire posterior margin of the wing from the alula to the tip bears a close-set row of long slender fringe scales; the first unbranched and marginal anterior vein is C, typically extending around the apex of wing; Sc is located closely behind the C; R forks into an anterior branch R_1 and a posterior branch, or radial sector Rs, which branches again into R_{2+3} and R_{4+5}; Vein R_{2+3} divides once more into R_2 and R_3, while R_{4+5} remains unbranched; M bifurcates into M_1 and M_2, CuA divides into two veins; finally, there is one vein A_1 (Fig. 5.6c) (Harbach and Knight 1980). Legs with tarsus composed of five tarsomeres (Fig. 5.1a), the apical tarsomere with two simple or toothed unguis or tarsal claws at apex, and an empodium between them (Knight and Stone 1977; McAlpine 1981a; Darsie and Ward 2005).

Abdomen including terminalia Composed of ten segments, segments I–VIII not modified, segments V–VIII progressively smaller; segments IX and X constitute the terminalia and are modified for reproduction; in males, the last segments turn 180° after the adult emergence. The structure in females is not of primary interest for taxonomic identification, but in males it is very important for the species recognition (Fig. 5.6d). Important structures of the male terminalia include the abdominal segment VIII, the tergal lobes, and the form of tergum IX; also, there are important structural differences of proctiger, aedeagus, gonocoxite, gonostylus, and claspettes (Fig. 5.6d) (Harbach 2013; Becker et al. 2010). In some species, abdominal segment VIII may be sexually differentiated from the preceding ones in one or more detail and would in such cases be treated as a part of the terminalia. The gonocoxites and the gonostyli, together with a pair of parameres, grip the female during copulation,

and guide the median intromittent organ, the aedeagus (Fig. 5.6d) (Harbach 2013; Becker et al. 2010).

Coloration patterns of scales on nearly all the body are used for species identification, but there are some species that are impossible to recognize as adults and need to be identified by larval or male terminalia characteristics, for which individual rearing procedures are highly recommended (see below).

Pupa (Fig. 5.7c) Head and thorax fused forming a prominent cephalothorax, which anterolaterally have a pair of respiratory trumpets associated with the prothoracic spiracles. The abdomen is slender with eight segments and terminates in a pair of flattened paddles. The respiratory trumpet has a tubular part, the meatus, and an open part, the pinna. In the Anophelinae, the respiratory trumpets are short, truncate apically, and have a large oblique opening that terminates in a split. The respiratory trumpets in Culicinae are variable, but they are usually elongated or broadly conical and unsplit. In general, chaetotaxy (see Glossary) are extremely useful to recognize species. The shape, position, or absence of setae; the length and structure of paddles and paddle fringe; and the form and length of respiratory trumpets are characters useful for species recognition (Harbach 2013).

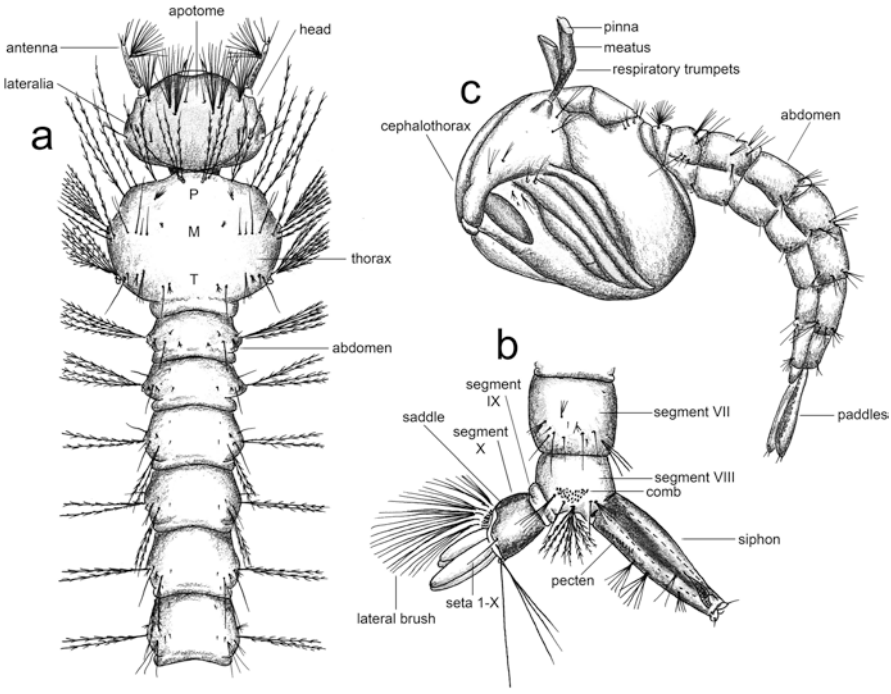

Fig. 5.7 Culicidae (immature stages). (**a**) Larva: head, thorax and first six abdominal segments, dorsal view; (**b**) larva: posterior segments of larval abdomen; (**c**) pupa, lateral view. Abbrv. M mesothorax, P prothorax, T metathorax

Larva (Fig. 5.7a, b) Most mosquito larvae obtain oxygen from the atmosphere by rising to the surface of the water and obtaining air with a siphon or a spiracle plate associated to the abdominal segment VIII; other species obtain oxygen from the air vessels of aquatic plants by means of a specialized siphon. The larva goes through four instars; the tegument in general is smooth and presents different sclerotized structures. There are three well-differentiated body regions, the head, the thorax that is wider than the other two, and a slender abdomen, each possessing variable structures used for taxonomic identification (Clements 1963).

Head is flattened dorsoventrally and is formed by three large sclerites, a pair of lateroventral sclerites or lateralia, and a dorsal sclerite named dorsal apotome divided by the U-shaped frontal ecdysial line. Mouthparts are represented by the usual structures, but in this group, the labrum has lateral lobes that carry the lateral palatal brushes (Harbach and Knight 1980). Antennae vary in length, shape, color, and may present spicules and one evident seta on the shaft and other apical setae. Head chaetotaxy are extremely useful for species identification, as they vary in size, type, and number of branches (Clements 1963).

The pro-, meso-, and metathorax are distinguished only by groups of setae, particularly the pleural setae. All thoracic and abdominal segments have no more than 15 pairs of setae each. It is important to point out that these setae vary in shape, size, and number of branches, depending on the species, and for this reason, it is useful for species identification (Clements 1963).

Other important taxonomic structures are found in the abdominal segments VIII and X; segment IX is reduced. In Anophelinae, segment VIII bears the spiracular plate posterodorsally with pecten formed by long and short teeth laterally, and setae. In Culicinae larvae, abdominal segment VIII may present one or more sclerotized plates or a comb of scales on each side and bears the posterodorsal siphon, that usually has a bilateral pecten composed of a row of scales that extends lateroventraly from its base, and setae in specific number and location according to species (Carpenter and LaCasse 1955; Clements 1963). The abdominal segment X or anal segment bears a sclerotized dorsal saddle, which may completely encircle the segment, a lateral brush on either side near the posterior margin of the saddle, a dorsal brush composed of upper, and lower setae, and seta 1–X arising from the dorsoapical angle on either side (Clements 1963).

Egg Mosquito eggs are elongated and bilaterally symmetrical and bounded by a thick shell, which is pierced at the anterior pole by the micropyle. Mosquito eggs are thus white when laid but gradually become dark brown or black (Clements 1963). It is possible to identify species at egg stage in some genera.

5.4.1.2 Biology

About 75% of mosquito species inhabit the tropical and subtropical areas of the world. Warm and humid climate favors the rapid development of immature ones and the survival of the adult, while the diversity of available habitats allows the

evolution of a greater number of species (Clements 1992). Mosquitoes may live in practically any aquatic habitat, from those that are temporary or permanent, with a high or few nutrients, and from very small to large aquatic habitats, from brackish or polluted to clean fresh water. Some species have the capacity to support desiccation and may survive in egg diapause some months (Becker et al. 2010).

Male mosquitoes are not sexually mature at emergence as they have to rotate their hypopygium (see Glossary) through 180° before they are ready to mate, which takes about one day and usually they emerge before females (Becker et al. 2010). Mating in most mosquitoes takes place when females enter swarms of flying males over a marker object at low light intensities during the evening and morning (Roth 1948). Females are male-receptive only once (Teesdale 1955; Clements 1963; Becker et al. 2010).

Male and females feed upon nectar or fruit exudate, and in hematophagous species only females ingest blood. Females of some species do not require blood to produce eggs (autogenous), other species may deposit one egg-batch without ingesting blood, but others require a blood meal from the start (anautogenous) (Carpenter and LaCasse 1955). Female blood preferences are diverse, but they may be generalists (zoophilous), may present preference for birds (ornithophilous) or for mammals (mammophilous) (Santiago-Alarcon et al. 2012). Female mosquitoes lay between 50 and 500 eggs, 2–4 days after the blood meal. Some mosquito females deposit their eggs onto the water surface either singly (*Anopheles*) or in batches (e.g., *Culex*, *Uranotaenia*, *Coquillettidia*, *Orthopodomyia*), or stick them on substrate surfaces that will be or are in contact with water (e.g., *Aedes*). Timing of larval hatching normally coincides with the presence of ideal developmental conditions such as high temperatures and rainfall, being prerequisites for successful development in temporary water bodies (Becker et al. 2010).

Duration of the larval period depends upon the species and the water temperature but can take only a few days. The larvae of the *Coquillettidia* and *Mansonia* live submerged. Based on their feeding behavior, they may be classified into filter or suspension feeders, browsers, or predators. Pupal stages usually last about two days; however, this period may be reduced or extended at higher or lower temperatures, respectively. Although the pupae do not eat, they are very mobile and can move through the water column with a flip of their abdomen. In *Mansonia* and *Coquillettidia*, the trumpets are modified for penetrating plant tissues (Clements 1963; Becker et al. 2010).

With respect to the transmission of Hemosporidian blood parasites, there are many species of mosquitoes that can be potential vectors of avian malaria. *Culex restuans* was infected with avian malaria and it is considered a competent vector of *Plasmodium* spp. (Abella-Medrano et al. 2018). *Culex quinquefasciatus* and *Culex pipiens* are other avian *Plasmodium* vectors, besides being a species with ornithophilous and mammophilous preferences (Žiegytė et al. 2014). In a study conducted by Inci et al. (2012) *Cx. pipiens*, *Culex theileri*, *Aedes vexans*, and *Culiseta annulata* were positive to *Plasmodium* spp. African species such as *Coquillettidia aurites*, *Coquillettidia pseudoconopas*, and *Coquillettidia metallica* have tested positive for

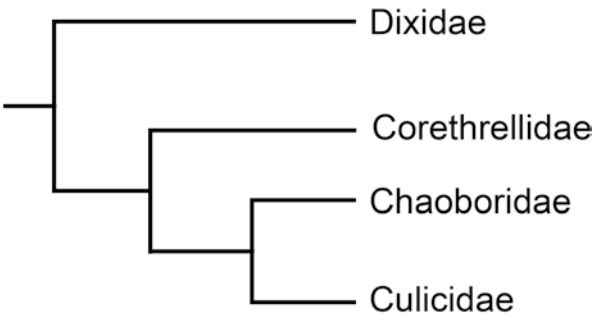

Fig. 5.8 Strict consensus tree of superfamily Culicoidea. (From Borkent 2012)

avian *Plasmodium* (Njabo et al. 2009) (see Chap. 6 for a thorough review and synthesis on Diptera vectors of avian haemosporidians).

5.4.1.3 Taxonomy and Fauna

Family Culicidae is closely related to Chaoboridae that is considered as the sister group, both related to Corethrellidae and all of them with Dixidae are considered under superfamily Culicoidea in the infraorder Culicomorpha (Figs. 5.2 and 5.8) (Borkent 2012). Currently, there are 3,563 species in the world classified into 41 genera (Harbach 2013; Wilkerson et al. 2015; Gaffigan et al. 2019). Two subfamilies are recognized: Culicinae and Anophelinae, both include species with hematophagous females that feed on vertebrates (Harbach 2013). Subfamily Anophelinae is represented only by tribe Anophelini (three genera), whereas subfamily Culicinae includes 11 tribes: Aedeomyiini (one genus), Aedini (10 genera), Culicini (four genera), Culisetini (one genus), Ficalbiini (two genera), Hodgesiini (one genus), Mansoniini (two genera), Orthopodomyiini (one genus), Sabethini (14 genera), Toxorhynchitini (one genus), Uranotaeniini (one genus) (Gaffigan et al. 2019). About 178 species are known in the Nearctic region, 1,069 species in the Neotropical region, about 492 species in Palaearctic region, about 795 species in the Afrotropical region, 1,061 species in the Oriental region, and about 764 species in the Australian region (Rueda 2008).

Useful keys for identification of mosquitoes genera and species are found in the following references: for the Nearctic region, the works by Wood et al. (1979), Stone (1981), and Darsie and Ward (2005); for the Neotropical region, the works by Lane (1953a, b), Belkin (1962), Belkin et al. (1966), Darsie (1985), and Chaverri (2009); for the Palearctic region, the works of Tanaka et al. (1979), Severini et al. (2009), and Becker et al. (2010); for the Afrotropical region, the works by Service (1990) and Coetzee (2017); for the Oriental region, the works of Chow (1949), Mattingly (1971), Darsie and Pradhan (1990), Rattanarithikul and Panthusiri (1994), Azari-Hamidian and Harbach (2009); and finally, for the Australian region, the works of Lee et al. (1989) and Webb et al. (2016).

5.4.2 *Family Simuliidae*

Members of the family Simuliidae are commonly known as black flies, name given due to their usually dark coloration. It is a relatively small group of Diptera that mostly include species with hematophagous females. Species of this family are distributed across all geographic regions, except Antarctica. Their immature stages are closely related to lotic environments and are strictly aquatic, specially flowing rivers and streams. Immature black flies have some morphological adaptations that make possible to inhabit these types of environments.

5.4.2.1 Morphology

The following description is based on Peterson (1981), Crosskey (1990), Crosskey (1993a), and Adler et al. (2004).

Adult (Fig. 5.9) Size about 1.2–6.0 mm, body and legs stout, broad wing, proboscis short, palpi long, and scutum strongly convex (Fig. 5.9a). Head hypognathous as

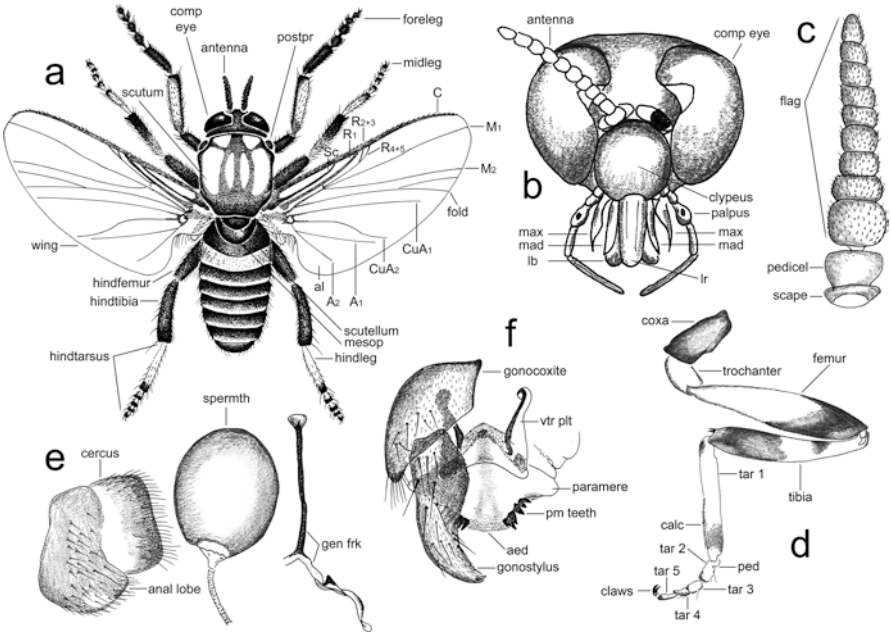

Fig. 5.9 Simuliidae (Adult). (**a**) Habitus; (**b**) head, frontal view; (**c**) antenna; (**d**) hindleg; (**e**) female terminalia; (**f**) male terminalia. Abbrv. A anal, aed aedeagus, al anal lobe, C costa, calc calcipala, comp eye compound eye, CuA anterior cubital, flag flagellum, gen frk genital fork, lb labium, lr labrum-epipharynx, M media, mad mandible, max maxilla, mesop mesopostnotum, pm teeth parameral teeth, ped pedisulcus, postpr postronotum, R radial, Sc subcosta, spermth spermatheca, tar tarsomere, vtr plt ventral plate

mouthparts are directed downward and the thorax is strongly convex dorsally. Most males are black in color, usually females are paler, and some species are blackish brown, dark gray, reddish brown to yellow or orange.

Head and its appendages Head is broad, but shape differs between females and males because of eye features. Members of this family present sex dimorphism, which is evident in the shape of eyes and the number and types of ommatidia (see Glossary). In females, the eyes are separated by a frons (head dichoptic), kidney-shaped, and each are formed by about 900–1,200 ommatidia (Fig. 5.9b). In males, the frons is greatly reduced; for this reason, the eyes are linked together in the front of the head (head holoptic) and present a greater number of ommatidia than in females, about 1,300–1,700 (O'Grady and McIver 1987). Usually, males have a *fovea ocularis*, presenting the upper portion with large ommatidia, and the lower portion with small ommatidia. Ocelli are absent.

The shape of antenna is one of the most important diagnostic characteristics in Simuliidae, with seven, eight, or nine short and wide flagellomeres, except the apical that is cone shaped; however, in females, the flagellomeres are usually more compact than in males (Fig. 5.9c).

Proboscis is adapted for cutting skin and sucking blood of vertebrates in females (Fig. 5.9b) and for the suction of sugary liquids in adults of both sexes; proboscis may differ in length according to species but it is short and stout compared with other blood-sucking Diptera, as Culicidae. Labrum is articulated basally with the clypeus and presents a pair of two or three sclerotized teeth apically (absent in males and nonbiting females); it forms an internal alimentary canal with the participation of mandibles and maxillae. Mandibles are behind the labrum and are blade shaped with serrated external margin in biting females. Posterolaterally to mandibles are the maxillae, represented by laciniae and maxillary palpi. Maxillary palpi are five segments, of which the third segment presents a sensory vesicle or pit in each palpus. Laciniae in blood-sucking females are armed with a row of recurved teeth. Internally, the cibarium (see Glossary) in females may present teeth or spines; their number, size, and arrangements vary according to species. At the time of feeding, the labium retracts and the labrum teeth and hypopharyngeal spines stretch the skin; at the same time, the mandibles, the hypopharynx, and labrum penetrate and open the skin, with the maxillary laciniae anchoring the mouthparts to the host (Fig. 5.9b).

Thorax and its appendages Thorax in Simuliidae is short and slightly curved or dorsally convex, strongly arched in males. In the prothorax, the postpronotum are well developed; the scutum is the most conspicuous area of the mesothorax, with coloring patterns that are taxonomically important and it is covered with pubescence, microtrichia, setae, and sometimes with scales; scutellum is setose and the mesopostnotum is subtriangular and usually bare (Fig. 5.9a).

Wings are broad and short, never patterned, with a strongly developed anal lobe; in the frontal area of the wing, there are three conspicuous veins: C, Sc, and R, the

rest of veins are thinner (Fig. 5.9a). The microtrichia in veins are of relevant taxonomic importance; they are present in all veins except in M and sometimes in Sc and R.

The legs are short and stout compared with others nematocerous Diptera; they usually are unicolorous, but in some cases, are banded; the basitarsus (see Glossary) is important taxonomically in the hindleg, is always long, and can exceed the length of the rest of tarsomeres and has at its apex a process called calcipala or inner apical surface (Fig. 5.9d). Tarsomere 2 presents a wrinkle in the basal portion at the posterior margin called pedisulcus; tarsomere 3–5 are short, and tarsomere 5 has two claws with taxonomic information according to the shape or size (Fig. 5.9d).

Abdomen including terminalia Abdomen consists of nine well-defined segments, with functional spiracles on segments III–VII. The abdominal shape differs between sexes and biting or nonbiting females. The terga in males and in some nonhematophagous females are well developed covering practically all the dorsal part of the abdomen. However, in blood-sucking females, the terga III–V are smaller than the terga VI–VIII; the first tergum in males and females are short and modified as a conspicuous ring with long setae. The sterna also differ between sexes; in males, they are well developed and defined, but in females, it depends on their feeding habits. The abdomen is covered with setae or hairs, and the color is usually like the scutum.

Female terminalia begins in the segment VIII, which is modified forming the hypogynial valves, which may be short lobes or long processes. Sternum IX is modified to form the internal genital fork (Fig. 5.9e), which is a strong, slender structure that usually is attached to tergum IX; the shape of the genital fork is of taxonomic importance. The terminal part of the abdomen is composed of the segment X, in which the anal lobes and the cerci are projected; their form and size are important for species recognition (Fig. 5.9e). Spermatheca is the internal most conspicuous portion of the reproductive system; it is a sclerotized and usually pigmented structure that, except for a few species, always is single (Fig. 5.9e). Male terminalia is not rotated. Gonopods (see Glossary) act as claspers to hold the female during the copulation; the gonostyli are more variable than the gonocoxites; gonostylus has spinules in the apical or subapical area, and the number is characteristic of the different taxa. The aedeagus (Fig. 5.9f) is a complex structure situated between the gonopods formed by two or three sclerites, being the ventral plate the most conspicuous, variable in shape and useful for the species recognition (Fig. 5.9f).

Pupa (Fig. 5.10d, e) Size about 2–7 mm (usually 3–5 mm); the size depends entirely on the species and nutrition of the larval stage. Pupae are obtect with appendages soldered to the body. The silk cocoon is a conspicuous and common structure in the family; the shape, size, texture, and color is considerably different between species and in a few, it may be absent; the opening of the cocoon is commonly a circular or semicircular opening directly toward the substrate or above it, or small just leaving the respiratory organs free. The general structure of the pupae of all black flies is homogeneous, but the respiratory organ, surface sculpture, armature, and the shape of the cocoon are all characteristics with greater differences

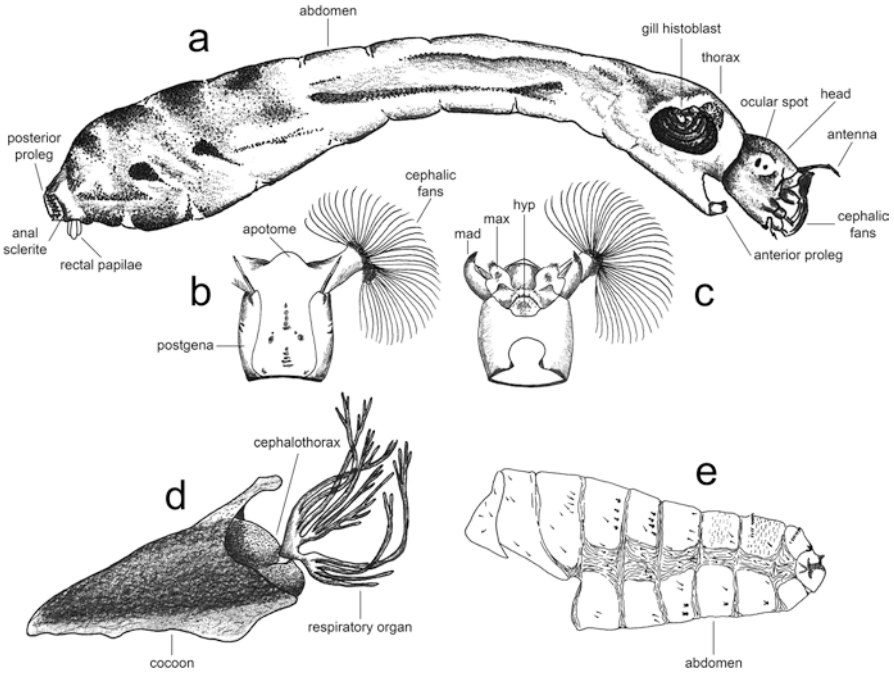

Fig. 5.10 Simuliidae (immature stages). (**a**) Larva, lateral view; (**b**) larva: head, dorsal view; (**c**) larva: head, ventral view; (**d**) pupa with cocoon; (**e**) abdomen, lateral view. Abbrv. hyp hypostoma, mad mandible, max maxilla

between taxa. The respiratory organ on thoracic portion of cephalothorax has at least three branches, abdomen without functional spiracles; apex of abdomen blunt or with a pair of nonarticulated projections (Borkent 2012). As in adults, the shape of the eyes, which can be clearly seen in a mature or well-developed pupa, can easily distinguish the pupae of both sexes.

Only the dorsal part of the cephalothorax has some kind of ornamentation or reticulated patterns, the rest of the tagma is bare, except for small trichomes that usually vary between four and seven pairs depending on the species, but may be absent, or may cover completely its surface. The structure of respiratory organs, also known as spiracular gills, is one of the most important taxonomic characteristics of simuliids pupae. They are cuticular projections on the dorsolateral portion of cephalothorax, and near to the base of these structures is the basal fenestra that opens for the larva to pupa ecdysis (see Glossary).

Abdomen has 5–9 segments, usually with nine well-defined segments. Pleural membrane is located between the terga and sterna on the sides of the abdomen; it may present sclerotized plates or pleurites, mainly in segments IV and V. The hook ground plan is called onchotaxy; in the terga III, IV and sterna V–VII, there are about 28 recurved hooks (about 16 dorsally and 12 ventrally) along their posterior margins; these hooks can be single, divided, or conformed by various combs. In

addition, sometimes, there is a spine comb present in the abdominal terga V–IX forming a complete or incomplete row in the anterior margins. In segment IX, usually, there is a pair of terminal spines or hooks.

Larva (Fig. 5.10a–c) Size about 3.4–15.0 mm, apneustic (see Glossary), prognathous (i.e., directed forward) and worm-like. Head capsule sclerotized, slightly elongated, subcylindrical, with three ocular pigmentary spots on each side of the head. It is composed of two sclerites, with pigmentation patterns usually spot-like distributed in groups (anteromedial, posteromedial, anterolateral, posterolateral) on the frontoclypeal apotome region, which is U-shaped bounded by ecdysial line; postgenae along the lateral area and part of the ventral, and ventrally by the hypostoma. Hypostoma is a sclerotized trapezoidal structure, with anteriorly toothed (usually nine teeth) and serrated on the lateral margins of those teeth, which differs between species.

The labrum, a pair of cephalic fans, a pair of mandibles, a pair of maxillae and labio-hypopharynx compose mouthparts. The cephalic fans are composed of primary (on the apex of the stem), secondary (inside and below primary fan), and median fans (on the median side of the stem), and additionally with a scale-fan. Labrum is between the stems of cephalic fans, in anteroventral position of the head; it is covered with setae, usually arranged in brushes. Mandibles are strongly sclerotized, with three to five apical teeth, and rows of smaller teeth basally, and with bristles arranged in rows usually present. Maxillae are below the mandibles, are rounded, and with five brushes and a maxillary palp. Labio-hypopharynx is provided with setae and sensilla. Antennae are located above the base of the cephalic stem; they are tubular, long and thin or short and stout depending on the habitat. Pigmentation patterns are of relevant taxonomic importance.

Thorax is the widest part of the body, except in some cases for the last abdominal segments. Prothorax has two conspicuous structures in mature larva, the larger gill histoblast and another five histoblasts that correspond to the legs, wing and halter of the adults (six histoblasts in total at each side); the anterior proleg is located ventrally on prothorax and usually has lateral sclerites in the distal part with small hooks arranged in a ring-like (anterior circlet).

Abdomen is composed of nine abdominal segments, of which usually V–VIII expand and differ from the rest, with nine nonfunctional spiracles. In the abdominal segment IX, the posterior proleg is much larger than prothoracic proleg and consists of the same structures (in this case, posterior circlet), except for a few small tubercles. An anal sclerite is located dorsoapically at the end of abdomen with different shapes depending on the taxa (but usually X shaped), but may be absent in some genera or in the initial larval instars. Anterior to anal sclerite, there are rectal papillae, structures that can be retracted into the rectum, and are composed of three finger-like lobes, each usually divided in three more small lobes.

Eggs Size about 0.18–0.46 mm, asymmetrically oval shape or triangular. Color white, darkening with age and embryonic development. The morphology of eggs of Simuliidae is uniform and provides little information to distinguish species.

5.4.2.2 Biology

In most black fly species, the females are completely adapted to cut and suck blood of vertebrates intermittently, mainly from birds and mammals, but they also include nectar in their diet. The genera *Austrosimulium*, *Cnephia*, *Prosimulium*, and *Simulium* include species with hematophagous females; those belonging to the *Simulium* genus have the most important medical implications. In mammals, flies of the genus *Simulium* transmit parasitic nematodes of the family Onchocercidae (*Onchocerca*, *Dirofilaria* and *Mansonella*); *Onchocerca volvulus* is one of the most important as it is the causative agent of human onchocerciasis (Crosskey 1990; Crosskey 1993a).

The genus *Simulium* is one of the most important genera implicated in the transmission of some species of avian protozoan parasites of the genus *Leucocytozoon* that causes leucocytozoonosis; also transmit filarial worms (Crosskey 1993a). In North America *Simulium rugglesi* and *Simulium innocens*, among others, are important vectors of *Leucocytozoon simondi* that affects about 30 species of birds, especially ducks and geese (Fallis et al. 1956; Herman et al. 1975). In the United States, *Prosimulium* is also implicated in the transmission (Crosskey 1990). Species of the genus *Austrosimulium* participate in the transmission of *Leucocytozoon tawaki* in New Zealand (Fallis et al. 1976); *Leucocytozoon sakharoffi* is transmitted by *Simulium* species, in particular *Simulium ruficorne* group in Asia, Australia, and Europe; *Leucocytozoon berestneffi* affects species of the family Corvidae, and it is transmitted by *Simulium aureum* group and sometimes by *Prosimulium decemarticulatum* in Canada (Khan and Fallis 1971). *Simulium aureum* group and other species of the *Simulium vernum* group are important vectors in the Holarctic region of *Leucocytozoon bonasae*, *Leucocytozoon debreuili*, and *Leucocytozoon fringillinarum* (Fallis and Bennett 1958; Khan and Fallis 1970; see Santiago-Alarcon et al. 2012 and Chaps. 1, 2, and 6 for more information regarding avian haemosporidian vectors and transmission ecology).

Males and some females of this family feed exclusively on nectar, which is how they acquire their carbohydrates, because their mouthparts are not adapted to bite and to drawing blood. In addition to nectar, females feed on blood to produce viable descendants. Females of approximately 2.4% of the world total species (about 37 species) are nonblood sucking and are distributed mainly in the northern Holarctic regions, there are a few in the southern hemisphere including all species of *Gymnopais* and *Parasimulium*, and some of *Cnephia*, *Prosimulium*, *Twinnia*, and *Simulium* (Crosskey 1990). There are also reports of simuliid species of the genus *Simulium* biting and feeding on insect hemolymph (Hagen 1883; Pryer 1887; Theobald 1892).

All immature stages of Simuliidae develop in all types of aquatic lotic environments, and their degree of specialization is such that each species is usually associated with particular conditions of their microenvironment (e.g., on leaves of aquatic or submerged plants, on rocks, waterfalls, etc.). In Simuliidae, the number of larval instars is variable, usually from four to nine, but up to 11 larval stages have been reported in some species (Craig 1975; Colbo 1989) and all larval development can

take from one to six months; filtering is the most usual method of feeding and the last larval instar is the one building the cocoon as a sign of the beginning of pupation. The pupal stage lasts about four to seven days. Larval or pupal stages of several species use other organisms such as crabs to move (phoresis) (Crosskey 1993a).

5.4.2.3 Taxonomy and Fauna

Simuliidae is within the infraorder Culicomorpha (Fig. 5.2), according to Woodley et al. (2009), belongs to the superfamily Chironomoidea. Recently, Borkent (2012) included Simuliidae in the superfamily Simuloidea. Chironomidae has been proposed as the sister group of the other Culicomorpha; Simuliidae is the sister group of Thaumaleidae, and they together are the sister group of Ceratopogonidae (Fig. 5.13).

There are 2,351 described species worldwide; 2,335 extant and 16 fossil, classified in a total of 32 genera (nine with fossil species, of which six have exclusively fossil species) (Adler and Crosskey 2018) grouped in two subfamilies: Parasimuliinae (one genus) and Simuliinae divided into tribe Prosimuliini (eight genera) and Simuliini (23 genera); this classification (two subfamilies and Simuliinae in two tribes) was proposed by Crosskey (1988).

In the Neotropical region there are about 382 species (Borkent et al. 2018), in the Nearctic region there are approximately 265 species, in the Afrotropical region approximately 216 species, in the Australian region about 205 species, in the Palaearctic region about 707 species, and in the Oriental region approximately 524 species. The genus *Crozetia* (two spp.) is subantarctic. It is important to mention that *Simulium* is the largest genus of Simuliidae with about 1,919 species described around the world (Adler and Crosskey 2018). Of the 32 genera, only *Austrosimulium*, *Cnephia*, *Prosimulium*, and *Simulium* have species related to the transmission of pathogens.

For useful keys for genera identification of black flies for the Nearctic region refer to the work by Peterson (1981), for the Neotropical region the work by Adler and Currie (2009), for the Palearctic region the work by Jedlička and Stloukalová (1997), for the Afrotropical region the work by de Moor (2017), for Malaysia the work by Takaoka (2004), and for the Australian region the work by Dumbleton (1963).

5.4.3 Family Ceratopogonidae

Members of the family Ceratopogonidae include a large group of nematocerous Diptera that are commonly known as biting midges, chaquistes, purrujas, and other names depending on the geographic area and language. Species of this family may be very abundant in aquatic or semiaquatic environments across the world. Ceratopogonidae includes some species with hematophagous females that are better documented than those from other trophic habits. Females of some species of the

genera *Forcipomyia* and *Atrichopogon* are ectoparasites of insects and ingest hemolymph from them, some others may be predators, but males and females of these and all other genera feed on nectar. Adults of other genera are melophagous or predators of other small insects, and only species of the genera *Culicoides*, *Leptoconops*, *Austroconops*, and *Forcipomyia* (*Lasiohelea*) include species with hematophagous females, where species of the genus *Culicoides* are vectors of avian Haemosporida (Borkent 2004a, 2004b, 2009).

5.4.3.1 Morphology

The following descriptions of the life stages of biting midges are based primarily on the works of Downes and Wirth (1981), Borkent and Spinelli (2007), and Borkent et al. (2009).

Adult (Fig. 5.11) In general, ceratopogonids are small Diptera, with stout bodies about 1–4 mm of length, antennae that are usually much longer than head, with 13 flagellomeres bearing whorls of long setae that are longer in males than in females, and in most females with distal 3–5 flagellomeres differing morphologically from

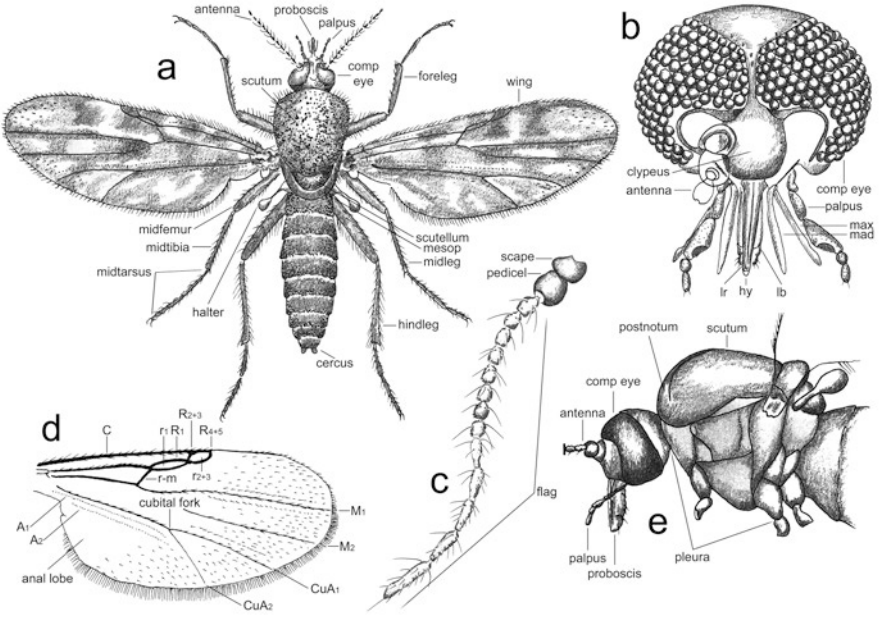

Fig. 5.11 Ceratopogonidae (Adult). (**a**) Habitus; (**b**) head; (**c**) antenna; (**d**) wing; (**e**) head and thorax, lateral view. Abbrv. A anal, C costa, comp eye compound eye, CuA anterior cubital, flg flagellum, hy hipopharynx, lb labium, lr labrum-epipharynx, M media, mad mandible, max maxilla, postnotum (=postpronotum), R radial, r_1 cell r_1, r_{2+3} cell r_{2+3}, r-m radial-medial

proximal flagellomeres (Fig. 5.11c). Wing with C ending at most beyond the last branch of R vein and before the wing apex, nearly always with two branches of M vein, and always no more than two branches of R reaching wing margin; R_{2+3}, if present, is disposed as a cross-vein and enclosing a cell (r_1) (Fig. 5.11d).

Head and its appendages With reniform eyes composed of uniform sized facets, approximated at middle above antennal insertions; without ocelli; mouthparts about as long as head, of female usually with blade-like mandibles with medial teeth. Palpus usually with five segments, the first two short and third with a vesicle/pit or sensory organ, segments 4 and 5 occasionally fused (Fig. 5.11b). Some important taxonomic characters are present on antennae and palpi; flagellomeres may have specialized sensilla in a variable combination among different flagellomeres. The relative size of palpi segments, in particular segment 3, and the form and size of the sensory pit are often very useful for identification of hematophagous species (Fig. 5.11b).

Thorax and its appendages Scutum with pits near anterior margin, and sometimes with specific color patterns that helps in the recognition of species. Pleura is useful for taxonomic identification in various genera (Fig. 5.11e).

Wing with apex rounded; anal lobe usually well developed, very large in a few genera; calypter (see Glossary) usually without fringe; wing membrane usually with microtrichia and sometimes also with macrotrichia (see Glossary). The genus *Culicoides* (the most species-rich genus of the family) usually has a specific pattern of dark or light spots or shadings, which are extremely useful for species recognition (Fig. 5.11a, d). Wing venation is characteristic with R vein complex very close to the anterior wing margin and at some distance before the wing apex near the middle of the wing, with R_1 and Rs short; vein Rs branching into R_{2+3} and R_{4+5}; R_{2+3} joining R_1 to form a closed cell (r_1), and R_{4+5} joining costa to form another cell (r_{2+3}) that rarely may be confluent or are obliterated; veins posterior to R weaker, vein r-m usually present, M_1 and M_2 commonly branching beyond level of r-m; vein m-cu absent; cubital fork evident (Fig. 5.11d).

Legs usually not long; they may have coloration patterns that are useful for species recognition. Tarsi with empodia variable, from well developed to vestigial, according to supraspecific taxa. In predatory species, their fore and other legs may be armed with stout ventral spines that help them to capture and hold prey.

Abdomen including terminalia Pregenital abdominal segments II–VII each with a pair of spiracles, but those on tergum I may be small or possibly fused with tergum II. Male terminalia with tergum IX usually large; gonopods formed by articulated gonocoxite and gonostylus, aedeagus basally articulated to base of gonocoxite, triangular shaped in dorsoventral view and with sclerotized lateral arms or ventral plate and paired or fused parameres that are very useful in taxonomy (see Fig. 5.6d to recognize structures). Female sternum VIII with a bilobate posterior margin; tergum and sternum IX small, the latter usually divided at midline around the spermathecal duct opening; one, two, or rarely three functional spermatheca. Cerci usually short (Fig. 5.11a), greatly elongated in females of *Leptoconops*.

Pupa (Fig. 5.12b, c) Typically conical in shape, with spinose abdomen that ends in a pair of apicolateral processes, not inside a silk cocoon. Antenna extending posteriorly, ending posterior to the posterior margin of head; cephalothorax with a pair of respiratory organs, that may be short and knob-like or long funnel-shaped, clavate or tubular, having at least some apical pores; palpus directed posteriorly, posteromedially, or anteromedially; hindleg curled under wing sheath; abdomen straight, not curled under thorax; apex of abdomen blunt or with a pair of nonarticulated projections or apicolateral process (Wirth and Stone 1956; Borkent and Spinelli 2007; Borkent 2012).

Larva (Fig. 5.12a) The larvae are morphologically very different, as are the habitats and habits of species in this family. In general, all have sclerotized head capsule, with a well-developed pharyngeal complex, and no open spiracles (Borkent and Spinelli 2007). Most species are aquatic or have a strong affinity with water either fresh or salty, or at least develop in microhabitats with high humidity. In most species, aquatic larvae are elongated and apodous (see Glossary), living as predators or scavengers freely in water or in association with algae in lakes or river margins or may be found in sand or mud; other species inhabit water accumulated in leaf axis or tree holes; there are species that occupy the interior of succulent plants. Other larvae may be terrestrial, and commonly they are stouter and setose, have pseudo-

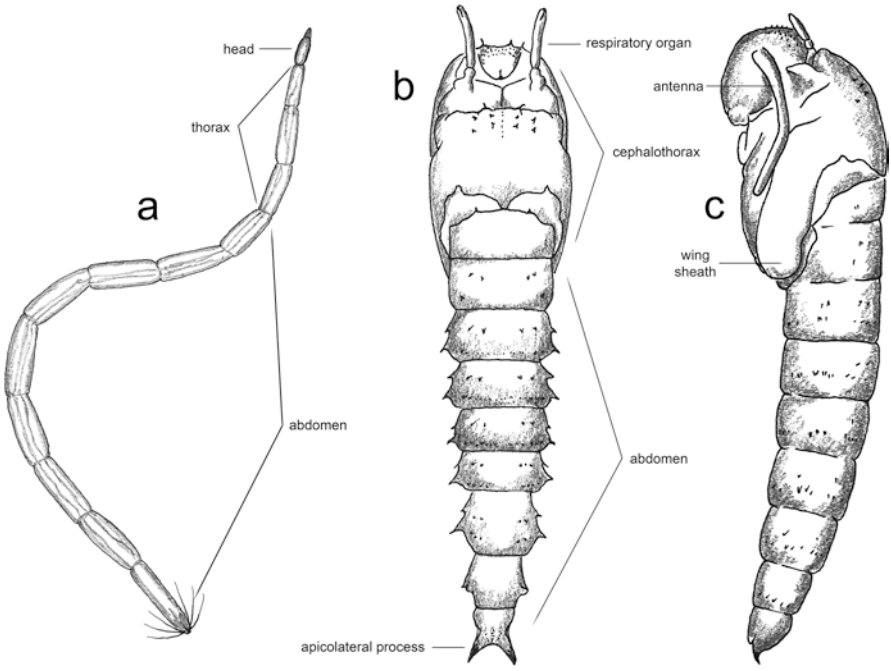

Fig. 5.12 Ceratopogonidae (Immature stages). (**a**) Larva, dorsal view; (**b**) pupa, dorsal view; (**c**) pupa, lateral view

pods (see Glossary), and live in decaying vegetable matter or litter (Wirth and Stone 1956).

Eggs Without distinctive characters.

5.4.3.2 Biology

Females of some species have atrophied mouthparts and do not feed as adults. All males and females are nectar feeders. Adult females of some species suck hemolymph from other insects, but females of the genera *Culicoides*, *Leptoconops*, *Austroconops*, and *Forcipomyia* (*Lasiohelea*) suck blood from reptiles, birds, and mammals (including humans) (Borkent 2004; Borkent et al. 2009). As in other families, blood-sucking ceratopogonid females need blood for egg maturation, but there are species in which the first oogenesis (see Glossary) does not need blood.

Referring to the hematophagous species, some are very abundant and their bites can generate allergic reactions on hosts, making them a seasonal pest. Some *Culicoides* species have been incriminated as intermediate hosts of the filarial worm *Mansonella perstans* in Africa, New Guinea, and Guyana. Other *Culicoides* are involved in the transmission of *Mansonella streptocerca*, *Mansonella ozzardi*, *Onchocerca reticulata*, and *Onchocerca gibsoni*. Also, species of this genus vector a variety of protozoa and viruses, causing diseases in domestic and wild animals. For instance, *Hepatocystis* spp. and *Hepatocystis kochi* in Old World monkeys and other animals, and *Haemoproteu*s in birds (Garnham 1966). Species of Leucocytozoidae such as *Leucocytozoon caulleryi* and other *Leucocytozoon* spp. may also be transmitted by species of this genus (see Chaps. 2 and 6). *Culicoides* spp. are well-known vectors of viruses such as the blue tongue, western equine encephalitis, and Venezuelan equine encephalitis. *Forcipomyia* (*Lasiohelea*) *taiwana* was found carrying the Japanese B encephalitis virus (James and Harwood 1969). Most of the Ceratopogonidae insects involved in avian haemosporidian transmission belong to the genus *Culicoides*, which belongs to the tribe Culicoidini of the subfamily Ceratopogoninae; also it is possible that species of the genera *Leptoconops* and *Austroconops* of the subfamily Leptoconopinae, and species of the genus *Forcipomyia* (*Lasiohelea*) of the subfamily Forcipomyiinae, may be involved in haemosporidian life cycles (Borkent 2016; see Chap. 6 for a thorough review of current research on vectors of avian haemosporidians).

Immature stages of *Culicoides* spp. in general occupy some types of habitats from leaf litter to mud at margins of ponds and lakes, but can occupy crab holes, tissues of succulent plants such as cacti (Wirth and Hubert 1960), those of *Forcipomyia* (*Lasiohelea*) breed in moss, sand, under bark, whereas *Leptoconops* are found in sand on beaches and in deserts (Boorman 1993). Aquatic species may develop in salt marshes, mangrove mud, tree holes, bracket fungus, or plants that retain water (telmatophytes) such as bromelids and pitcher plants (Wirth and Blanton 1959).

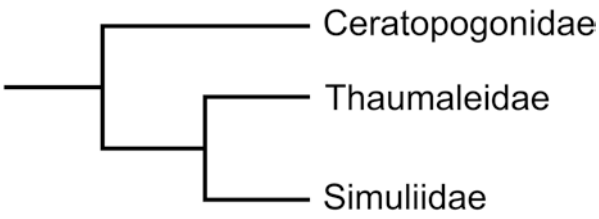

Fig. 5.13 Strict consensus tree of superfamily Simuloidea. (From Borkent 2012)

5.4.3.3 Taxonomy and Fauna

Ceratopogonidae is closely related to Simuliidae and Thaumaleidae, both of which are its sister group, and are classified with Chironomidae in the superfamily Chironomoidea, which along with the Culicoidea form the infraorder Culicomorpha (Figs. 5.2 and 5.13) (Borkent 2012). Currently, Ceratopogonidae includes about 6,267 described valid extant and extinct species in 133 genera around the world (Borkent et al. 2018). A total of two fossil and four recent subfamilies are currently recognized: Lebanoculicoidinae (one fossil genus), Leptoconopinae (six genera), Atriculicoidinae (one fossil genus), Forcipomyiinae (two genera), Dasyheleinae (one genus), Ceratopogoninae divided into eight tribes: Culicoidini (with three genera), Ceratopogonini (67 genera), Hebetulini (one genus), Heteromyiini (seven genera), Johannsenomyiini (17 genera), Palpomyiini (six genera), Sphaeromiini (11 genera), Stenoxenini (two genera), and four unplaced extant and four fossil genera (Borkent 2016). Of the total, an estimation of species in the Neotropics is 1,132 species (Borkent et al. 2018), in the Palaearctic region about 1,041 species (Remm 1988), in the Nearctic region about 600 species (Borkent and Grogan 2009), in the Afrotropical region 622 species, in the Oriental region about 521 species, and in the Australian region about 761 species (Wagner et al. 2008). It is important to mention that *Culicoides* is the largest genus of Ceratopogonidae with more than 1,000 species described around the world (Connelly 2013), which can be consulted in the World catalog that regularly is updated by A. Borkent (see Borkent 2016).

Useful keys for genera identification of biting midges are the following: for the Nearctic region the work by Downes and Wirth (1981), for the Neotropical region the work by Borkent and Spinelli (2007) and Borkent et al. (2009), for the Palearctic region the work by Boorman (1997), for the Afrotropical region the work by Borkent (2017), for the Oriental and Australian regions the work by Borkent (2004).

There have been some attempts to segregate *Culicoides* species into subgenera and species groups, but as commented by Borkent et al. (2009), subgeneric classification is in great need of analysis and restructuration. For *Culicoides* identification the works of Wirth et al. (1985) and Wirth et al. (1988), for the New World, Boorman (1997) for the Palaearctic region (summarizing some works about *Culicoides* species by geographic regions) and Meiswinkel et al. (2004) present advances in the knowledge of species complexes, Labuschagne et al. (2015) for Afrotropical

Culicoides helps to begin and obtain some useful references by region, Dyce et al. (2007) for the Australian region, and Wirth and Hubert (1989) for Asia; all constitute excellent works for recognition of most *Culicoides* species.

5.4.4 Family Hippoboscidae

Members of family Hippoboscidae are commonly known as louse flies. They exhibit some extraordinary adaptations for parasitic life, being the most important the intrauterine complete development of the larva: reason why this group, along with Glossinidae, Streblidae, and Nycteribiidae have been traditionally grouped as Pupipara, literally meaning that females deposit pupae in a puparium (Fig. 5.14f), not eggs or larvae as happens in other groups of Diptera.

5.4.4.1 Morphology

The following description is based on Bequaert (1953), Maa and Peterson (1987), and Wood (2010).

Adult (Fig. 5.14a–e) Size between 1.5 and 12 mm, strongly depressed (dorsoventrally flattened), with legs originating laterally from the thorax, widely separated between each pair by sternal plates, and abdomen membranous (Fig. 5.4c). Hippoboscids are highly specialized flies that exhibit some morphological adaptations for living within feathers or fur of their hosts, in particular leg structure and placement. Larvae develop completely inside the female body, pupae are free living, and adults are ectoparasites of birds and mammals.

Head and its appendages Dorsal surface flattened, mouth parts directed forward (prognathous type), in general rounded posteriorly and capable of free movement, except when postpronotum is large flanking the sides of head (Fig. 5.14c); dorsal surface with conspicuous crescent-shaped ptilinal suture from which the ptilinum inflates at the moment when the adult emerges to break the puparium (Fig. 5.14b); above or posterior to the ptilinal suture is the flattened front between the compound eyes, and the vertex at the same plane, with an ocellar triangular plate that may present three ocelli or none according with the group (Fig. 5.14b); frons large with a median frontal vitta, and with lateral fronto-orbital plates; below or anterior to the ptilinal suture and near the anterior margin of head are the antennae and the mouthparts (Fig. 5.14b). Eyes large, always separated (dichoptic), horizontally oval, and at least 0.75 as high as head, with more than 100 small facets. Inner vertical bristles long, outer vertical bristles absent, orbital bristles variable. Antennae originate from a single face cavity or from separate antennal fossae (Fig. 5.14b); each antenna is composed of a very small scape, and a large flattened pedicel that has a varied form according to the species and presents a hole ventrally containing the reduced flagellum, of which only the arista is appreciated, namely, arista bipectinate or spatulate.

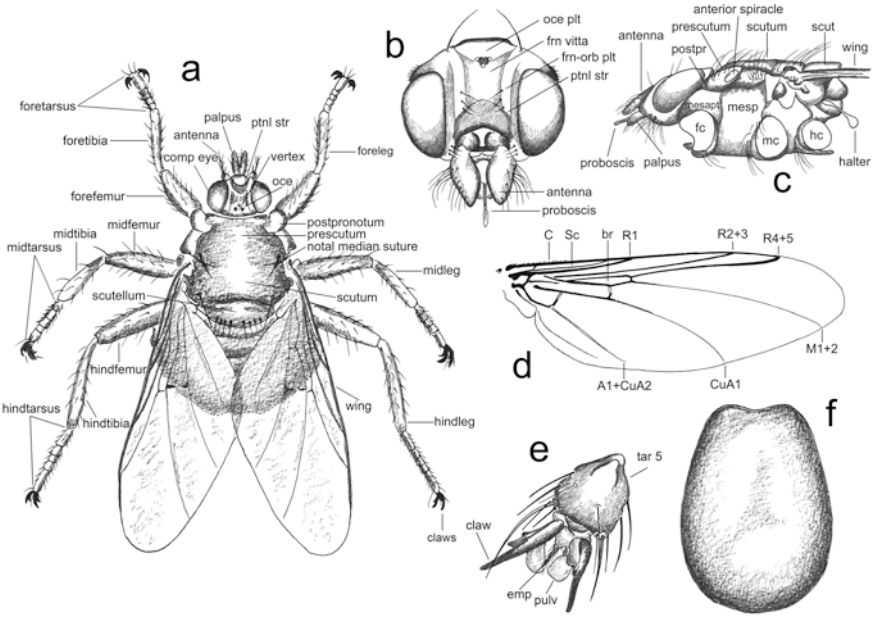

Fig. 5.14 Hippoboscidae. (**a**) Habitus; (**b**) head, frontal view; (**c**) head and thorax, lateral view; (**d**) wing; (**e**) apex of tarsus; (**f**) puparium. Abbrv. A anal, br basal radial, C costa, CuA cubital anterior, comp eye compound eye, emp empodium, fc forecoxa, frn vitta frontal vitta, frn-orb plt fronto-orbital plate, hc hindcoxa, M media, mc midcoxa, mesp mesepimeron, mesapt mesanepisternum, oce ocellus, oce plt ocellar plate, postpr postpronotum, ptnl str ptilinial suture, pulv pulvillum, R radial, Sc subcosta, scut scutellum, tar tarsomere

Mouthparts are adapted for piercing and sucking in both sexes; proboscis or haustellum is long, narrow but strongly sclerotized, capable of retraction when not in use (Fig. 5.14b), flanked and protected by a pair of one-segmented maxillary palpi; haustellum consists of the modified retractable labium, which encloses the labrum-epipharynx and the hypopharynx, which forms the food and salivary ducts, respectively; at apex, there are a pair of small but strong labella with a double crown of prestomal teeth that are responsible for cutting the skin to feed.

Thorax and its appendages Thorax depressed; sternum large with coxae widely separated (Fig. 5.4c). Dorsally, the prothorax is evident by the postpronotum that is variable in form, that is separated from the scutum by a suture; anterior spiracle more or less dorsally placed, sometimes enclosed by the postpronotum; mesonotum large with a complete or incomplete transverse suture that divides the plate in an anterior prescutum and a posterior scutum, sometimes both with a longitudinal notal median suture, with different degree of development according to the group; scutellum often large; mesopostnotum usually evident laterally but can be fused with other nearby sclerites (see Glossary); there are some bristles on the sclerites (Fig. 5.14a, c). Laterally, mesothorax is represented by the mesanepisternum and mesepimeron, the last one with dorsal position. In ventral view, the thorax presents

large sclerites as compared with other Diptera; the prosternum is represented by a pair of separated or medially fused lobes between forecoxae, and mesosternum and metasternum is larger and medially divided by a suture (Fig. 5.14c). Legs with swollen femora, flatted tibiae and short tarsi, being the last tarsomere large, with a pair of tarsal claws strongly curved and toothed, and pulvilli well developed (Fig.5.14e). Wings variable in size and shape, atrophied in a few hippoboscids, with Sc, and R veins well developed up to the wing's margin, basal portion of M and Cu strong, but with their apical portion weak and divergent (Fig. 5.14d). Halteres present in winged species, absent in apterous species.

Abdomen Tergum I represented by a pair of laterotergites, followed by five reduced terga or median tergal plates of the preabdomen dorsally. Only the sternum I is visible, the rest obsolete. Terminal segments poorly differentiated and relatively unimportant for taxonomic purposes. Female and male terminalia simple, male terminalia retracted. Terminalia characteristics are reviewed by Schlein and Theodor (1971).

5.4.4.2 Biology

All hippoboscids are ectoparasites of birds and mammals, feeding on its blood. Birds are the most frequent hosts (e.g., Ibáñez-Bernal et al. 2016), but some species parasitize some ungulates and few other mammals; there are species that are parasitic on domestic animals that are considered of economic importance. Species with fully developed wings can easily fly and change hosts (e.g., Levin and Parker 2013), but those with atrophied wings remain most of their life on the same host. Females reach maturity within few days and in general accept only one male during its lifetime, but there are examples of multiple matings. Only one individual develops at the same time; the embryo (inside the egg) and larva develop in the uterus, they are nourished by secretions produced by specialized glands (adenotrophic viviparity). When larva is fully developed, it exits from its mother and leaves the host to rapidly pupate in the soil. Hippoboscids are univoltine, that is, only one generation occurs each year; but, in some species, up to eight offspring can be produced by each female.

Subfamily Ornithomyinae includes mainly parasites of birds and a few of mammals, and Hippoboscinae species are parasitic on mainly mammals and occasionally on birds of the Old World.

Louse flies are vectors of avian pathogens such as species of the genera *Trypanosoma* and *Haemoproteus* (Aragão 1908; Adie 1915; Baker 1967; Santiago-Alarcon et al. 2012; Valkiūnas et al. 2010).

5.4.4.3 Taxonomy and Fauna

According to McAlpine (1989), Hippoboscidae is grouped in the Infraorder Muscomorpha, section Schizophora, subsection Calyptratae, and superfamily Hippoboscoidea; currently, Glossinidae is at the base of a clade containing

Hippoboscidae, Streblidae, and Nycteribiidae, the last two are monophyletic sister groups having Hippoboscidae as sister family (Fig. 5.2). Griffiths (1972) lumped the families Nycteribiidae and Streblidae with the Hippoboscidae, this idea is not supported by other specialists, because Streblidae seems to be paraphyletic (Petersen et al. 2007).

There are 213 described species worldwide, classified in 21 genera grouped in three subfamilies: Ornithomyinae (16 genera), Lipopteninae (three genera), and Hippoboscinae (two genera) (Maa 1969a; Dick 2006; Wood 2010). An estimation of species indicates that there are 32 species in the Neotropical region (Borkent et al. 2018), more than 35 species in the Palearctic region (Hutson 1984), 31 species in the Nearctic region (Maa and Peterson 1987), more than 32 species in the Oriental region (Maa 1977; Mogi et al. 2002), 46 species in the Afrotropical region, and about 43 species in the Australian region (Maa 1969b; Hutson and Oldroyd 1980). Keys for the identification of Hippoboscidae in the Americas can be found in Maa and Peterson (1987) for Nearctic genera, and in Wood (2010) for the Neotropical genera. Hutson (1984) can be consulted for the Palaearctic and Bequaert (1954) in general for the family around the world.

Glossary

Adecticous Pupa with immobile mandibles (Torre-Bueno 1989).

Aedeagus In male Diptera, the intromittent organ (Torre-Bueno 1989).

Alula In adult Diptera, the restricted membranous basal portion of posterior wing margin, distal to upper calypter and basal to anal lobe (Torre-Bueno 1989).

Apneustic Respiration through the tegument as the tracheal system does not present open functional orifices or spiracles for gas exchange, occurring in some aquatic Diptera larvae (Torre-Bueno 1989).

Apodous Larvae without true articulate legs (Torre-Bueno 1989).

Basitarsus The proximal or basal tarsomere of an insect leg (see tarsomere) (Torre-Bueno 1989).

Bristle A stiff, usually robust seta (Torre-Bueno 1989).

Calypter (sing.), calypteres (plr.) In Diptera wing, the two basal lobes formed from the posterobasal portion of axillary membrane, proximal to alula. Lower and upper calypteres are recognized (Torre-Bueno 1989).

Cercus (sing.), cerci (plr.) Paired appendages originated from abdominal segment XI (Snodgrass 1935).

Chaetotaxy The study of the arrangement and nomenclature of setae or bristles of any part of the body to describe their patterns in taxonomy (Torre-Bueno 1989).

Cibarium The preoral cavity posterior to the base of the hypopharynx situated more or less at the level of the clypeus (Torre-Bueno 1989).

Claspette (s) In male mosquito genitalia, the pair of structures derived from gonocoxites or parameres that often present hooks, claws, or specialized setae (Torre-Bueno 1989).

Compound eye Specialized optic organ of the head in which each functional unit (ommatidium) has two lens and, for that reason, is capable of registering impressions of form. A compound eye may be formed by one to several hundreds of units, externally observed by the cornea or external lens, also called facets (Snodgrass 1935).

Cyclorrhapha An unranked taxonomic category within the infraorder Muscomorpha that refers to the circular aperture through which the adult escapes the puparium.

Ecdysial line Suture or cleavage weakness line along which cuticle splits when the insect molts (Torre-Bueno 1989).

Ecdysis The process of forming the cuticle (Snodgrass 1935).

Endopterygota Term equivalent to Holometabola, referring to those insects, which pass through a complete metamorphosis, larva is very different from adult, passing by an intermediate stage of pupa for dramatic transformation. Specifically, the term Endopterygota refers to the internal development of wings during larval stage (Torre-Bueno 1989).

Epandrium (sing.), epandria (plr.) The ninth abdominal tergum in male Diptera and other insects (Torre-Bueno 1989).

Exarate A type of pupa in which the legs and wings are free from the body and the abdomen is movable (Torre-Bueno 1989).

Exuviae (sing., and plr.) The class of exocuticle and epicuticle of the immature insect tegument posterior to ecdysis (modified from Torre-Bueno 1989).

Gonocoxite(s) The coxites of gonopods (Torre-Bueno 1989). The basal segment of the appendages of the abdominal segment IX, assisting copulation.

Gonopod(s) Appendages of the abdominal segment IX (Torre-Bueno 1989).

Gonostylus (sing.), gonostyli (plr.) Apical segment of the gonopod or stylus, usually modified to form the clasping organ for reproduction (Torre-Bueno 1989).

Gonotrophic cycle The average number of days that gravid nematoceran and lower Brachycera Diptera females take to oviposit after taking a blood meal.

Haustellate Mouthparts modify for sucking (Torre-Bueno 1989).

Histoblast Imaginal disc (Torre-Bueno 1989). Any of the undifferentiated cells forming a mass in the body of an insect larva that develop later into an adult organ or structure (Torre-Bueno 1989).

Hypandrium In male insects, the subgenital plate, which in Diptera corresponds to abdominal sternum IX (Torre-Bueno 1989).

Hypopygium (sing.), hypogygia (plr.) In male Diptera, the abdominal segment IX, or in ample sense all terminalia or genital and postgenital segments (Torre-Bueno 1989).

Lacinia (sing.), laciniae (plr.) A blade; the inner lobe of the maxilla, articulated to the stipes, that in Diptera is present as a flat lancet-like piercing structure (Torre-Bueno 1989).

Macrotrichia (plr.) Trichoid sensilla (Torre-Bueno 1989).

Micropyle One of the openings in the chorion of an insect egg through which spermatozoa enter for fertilization (Torre-Bueno 1989).

Microthrichia Spicules or small hair-like structures (Torre-Bueno 1989).

Obtect A pupa in which the wings and other body appendages are appressed to the body and most of the abdominal segments are immovable (Torre-Bueno 1989).

Ocellus (sing.), ocelli (plr.) A photoreceptive sensorial organ that presents only one lens, and for that reason incapable of registering impressions of form (Snodgrass 1935).

Ommatidium (sing.), ommatidia (plr.) An individual functional unit of a compound eye (Snodgrass 1935).

Oogenesis Egg maturation (Torre-Bueno 1989).

Oviparous Reproducing by eggs laid by the female (Torre-Bueno 1989).

Ovoviviparous Producing living young by the hatching of the ovum while still within the mother (Torre-Bueno 1989).

Paramere In male Diptera, a pair of unsegmented paraphallic processes situated between the posterolateral base of aedeagus and the dorsomedial base of gonocoxite (Torre-Bueno 1989).

Parasitoid An organism that lives in close association with its host and at the host's expense, and which invariably kills it (e.g., some wasp species injecting their eggs inside caterpillars).

Pecten Any comb like structure (Torre-Bueno 1989).

Proctiger The reduced abdominal segment X bearing the anus (Torre-Bueno 1989).

Proleg Any process that serves the purpose of leg, but that is not a true leg (Torre-Bueno 1989).

Pseudopod A proleg.

Ptilinum In Diptera Schizophora, a reversible sac capable of being thrust out of a fissure just above the bases of the antennae, thereby splitting the puparium cover and permitting emergence of the adult (Torre-Bueno 1989).

Scale A flat modified seta (Torre-Bueno 1989).

Sclerite Any plate of the body wall bounded by membrane or sutures (Torre-Bueno 1989).

Sclerotized Hardened through sclerotization (Torre-Bueno 1989).

Sensillum (sing.), sensilla (plr.) A simple sense organ (Torre-Bueno 1989).

Seta (sing.), setae (plr.) A sclerotized hair-like projection of cuticula formed by a single trichogen cell and surrounded at the base by a small cuticular ring (Torre-Bueno 1989).

Spermatheca (sing.), spermathecae (plr.) In females, a receptacle of sperm after coitus.

Spiracle External opening of the tracheal system (Torre-Bueno 1989).

Squama Any scale-like structure. In Diptera wing, the proximal posterior lobe.

Styliform Terminating in a long slender point.

Sulcus (sing.), sulci (plr.) A groove.

Terminalia In male Diptera, the genital and postgenital segments.

Trophic Pertaining to food or nourishment.

Trichoid Like a hair.

Trichome Modified hair-like structures.

Unguitractor plate The ventral sclerite of pretarsus articulating to claws distally (Torre-Bueno 1989).

Vector An organism carrying a microorganism pathogenic for members of another species (Torre-Bueno 1989).
Vermiform Worm-shaped.
Viviparity Retention of the embryo within the female at least until it has almost reached blastokinesis (Torre-Bueno 1989).
Viviparous Displaying viviparity.

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Chapter 6
Diptera Vectors of Avian Haemosporidians: With Emphasis on Tropical Regions

Francisco C. Ferreira, Diego Santiago-Alarcon, and Érika M. Braga

Abstract Haemosporidian parasites are a globally distributed group of protists that infects many species of vertebrates (amphibians, reptiles, birds, and mammals) and are transmitted by bloodsucking dipteran insects (Insecta: Diptera). Although significant progress has been made toward understanding the details of the life cycle of these parasites, our current knowledge about patterns of development of different haemosporidian species in vectors is insufficient. Experimental and methodological studies with vectors and patterns of transmission of wildlife haemosporidian parasites are uncommon. Little is known about the effects of avian haemosporidian infections on vectors, and detailed experimental and field studies on vector competence are critical. These represent significant barriers to better understanding the epizootiology of avian haemosporidians and the evolutionary biology of this diverse group of parasites. This chapter introduces the Diptera families that are commonly associated with the transmission of avian haemosporidian parasites and describes the sexual and sporogonic processes that ultimately produce sporozoites, the infective stage to vertebrate hosts. A brief historical perspective of haemosporidian vector studies is also outlined, as well as an overview of methodological and experimental procedures conducted with different Diptera species during the past 8 years. Furthermore, the chapter explores how the use of molecular methods has contributed to the understanding of vector ecology and biodiversity, highlighting tropical vectors. Finally, we propose directions for further research aiming to improve our knowledge about vector–haemosporidian parasite interactions in the tropics.

F. C. Ferreira (✉)
Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Center for Conservation Genomics, Smithsonian Conservation Biology Institute, Washington, DC, USA

D. Santiago-Alarcon
Red de Biología y Conservación de Vertebrados, Instituto de Ecología, Xalapa, Mexico

É. M. Braga (✉)
Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

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6.1 Introduction

Avian haemosporidian parasites have an obligate heteroxenous cycle with asexual stages in birds, and fertilization of gametes (sexual phase) and asexual development of sporozoites (sporogony) in bloodsucking dipterans (Garnham 1966; Valkiūnas 2005; Santiago-Alarcon et al. 2012a; see Chap. 5 for an in-depth introduction to tropical Diptera). It is generally accepted that mosquito (Culicidae) species from different genera transmit parasites of the genus *Plasmodium*, although some lizard Plasmodiidae are vectored by *Lutzomyia* sandflies. *Haemoproteus* (*Parahaemoproteus*) are known to be transmitted by biting midges, mostly of the genus *Culicoides* (Ceratopogonidae), while *H*. (*Haemoproteus*) are transmitted by louse flies (Hippoboscidae). *Leucocytozoon* (*Leucocytozoon*) species are transmitted by black flies (Simuliidae), but *L*. (*Akiba*) *caulleryi* (the only species of this subgenus infecting birds) is transmitted by ceratopogonid flies (Santiago-Alarcon et al. 2012a). There is some evidence that the only species of the genus *Fallisia* infecting birds is transmitted by culicine mosquitoes (Gabaldon et al. 1985).

Most of the research on haemosporidian vectors is concentrated in the Culicidae family. *Anopheles* mosquitoes are the primary vectors of *Plasmodium* parasites that cause human malaria, and consequently biases research priorities especially in biomedicine and public health epidemiology toward a few species within this mosquito genus (Enayati and Hemingway 2010). Fortunately, research on insect species involved in avian haemosporidian life cycles has increased during the past two decades (Fig. 6.1). These studies included experimental investigation of sexual and sporogonic development of parasites (Kazlauskienė et al. 2013; Bukauskaitė et al. 2015; Gutiérrez-López et al. 2016; Žiegytė et al. 2016) and analyses on the diversity and composition of Diptera insect assemblages in the tropics (Abella-Medrano et al. 2015, 2018; Ferreira et al. 2016; Schmid et al. 2017; Mantilla et al. 2018), which form a basis to understand the ecological dynamics of haemosporidian parasites.

6.2 Developmental Stages of Avian Haemosporidians in Their Vectors

The successful transmission of malaria and related haemosporidian parasites among birds requires a series of complex developmental transformations inside dipteran vectors. Parasite development in their vectors is relatively conserved among the three most known genera (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), but it is different during the asexual phases in their vertebrate host. Figure 6.2 depicts the

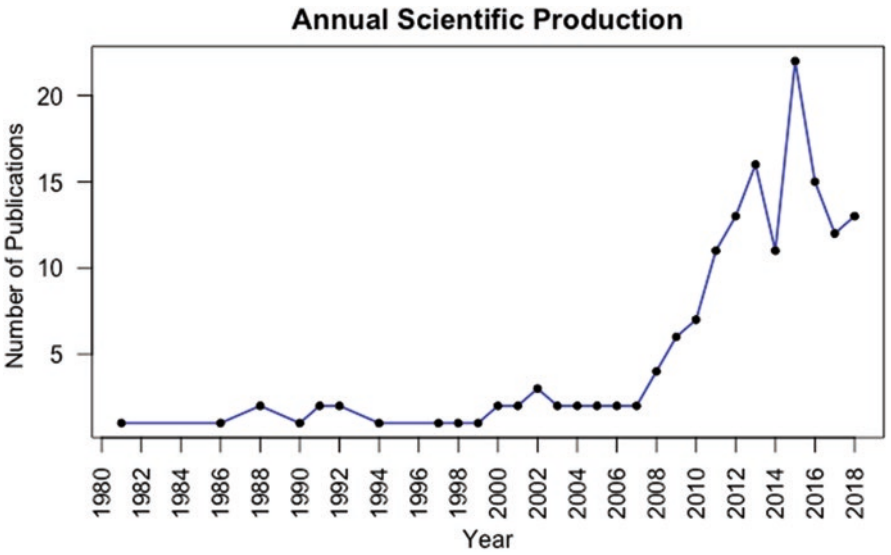

Fig. 6.1 Number of studies on avian haemosporidian Diptera vectors across tropical regions. Results derive from a bibliometric analysis spanning the years 1980–2018 from the Web of Science Core Collection (www.webofknowledge.com) using the R package bibliometrix v. 2.0.0 (Aria and Cuccurullo 2017). We used the following Boolean search keywords: TS = ((Haemosporid* OR avian Plasmodium OR Haemoproteus OR Leucocytozoon) AND (Culicidae OR Ceratopogonidae OR Culicoides OR Simuliidae OR Hippoboscidae OR vector OR Diptera*) AND (Tropic* OR Neotropic* OR Afrotropic* OR Ethiopia* OR IndoMalay* OR Oriental OR Austral* OR Oceania*))

sexual reproduction and sporogony during a haemosporidian life cycle in the dipteran vectors (see Chap. 2 for a thorough introduction to haemosporidian's life cycle in their bird hosts). Insects ingest parasites of various blood stages from infected birds, but only mature gametocytes will form gametes in the insect's midgut by a process known as gametogenesis. Macrogametocytes will develop into one rounded macrogamete; a single microgametocyte undergoes exflagellation to form eight motile microgametes. The microgamete formation seems to be triggered by changes in temperature as well as in oxygen and carbon dioxide concentrations after blood meal intake. This process can start in a few seconds in *Leucocytozoon* parasites or in several minutes in *Plasmodium* and *Haemoproteus* (Valkiūnas 2005). A macrogametocyte and a microgametocyte fuse to form a zygote, which turns into a motile ookinete in 1–24 hours after an infected blood meal is ingested (Kazlauskienė et al. 2013; Žiegytė et al. 2014; Bukauskaitė et al. 2018). This latter form penetrates through the peritrophic membrane formed by the insect around the blood meal and through the midgut epithelia. After escaping the midgut, the ookinete settles under the midgut basal lamina and develops into an oocyst by creating a wall built from host material. Sporogony occurs in the oocyst, producing several infective

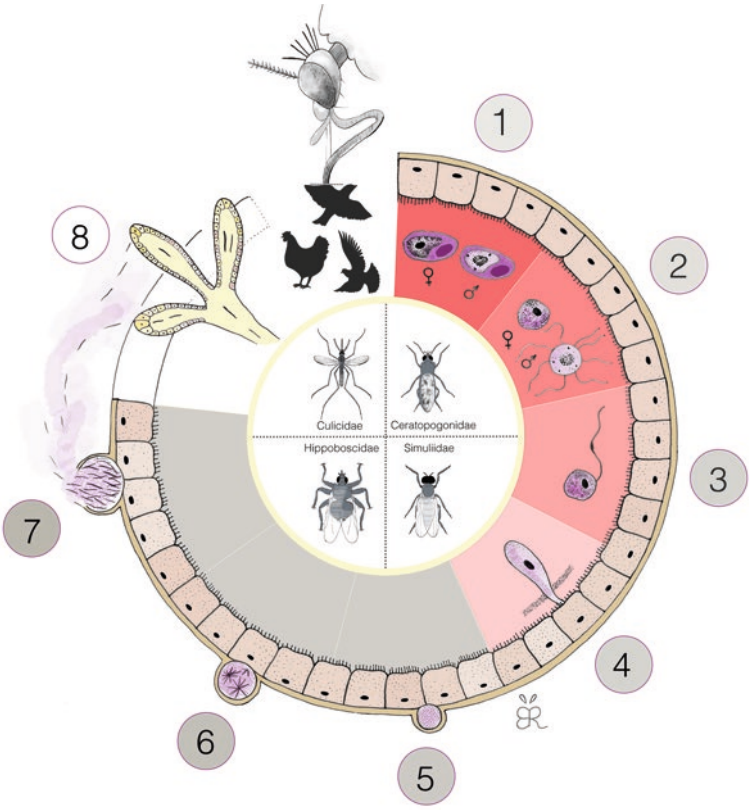

Fig. 6.2 Diagrammatic representation of the sporogonic life cycle of haemosporidian parasites. After feeding on infected birds, mature gametocytes (1) initiate gametogenesis, which usually occurs in the midgut of the vectors. A macrogametocyte produces one rounded macrogamete, while a microgametocyte undergoes exflagellation, as a result of which eight motile thread-like microgametes are formed (2). Fertilization occurs extracellularly (3). The zygote is transformed into an ookinete that penetrates through the peritrophic membrane and through the epithelial layer of the midgut (4). The ookinete rounds up under the basal lamina and develops into an oocyst, which is surrounded by a capsule built from the material of the host (5, 6). After maturation, oocysts rupture (7), the sporozoites move into the hemocoel and then penetrate the salivary glands of the vector (8), where sporozoites become infective to birds. Adapted from Valkiūnas 2005

sporozoites. In *Plasmodium relictum*, for example, oocysts are visible from 5–24 days after feeding, generating sporozoites during this period (LaPointe et al. 2005; Kazlauskienė et al. 2013; Valkiūnas et al. 2015). Sporozoites are gradually released into the insect hemocoel and circulate around the vector body via hemolymph flow. Some *P. relictum* sporozoites reach and penetrate the salivary gland, where they can be first detected between 6 and 14 days after feeding, and remain

infective for weeks (Kazlauskienė et al. 2013; Valkiūnas et al. 2015). To complete the cycle, infectious vectors inject sporozoites together with salivary gland secretions (saliva) into the vertebrate host during subsequent blood meals (Fig. 6.2; see also Fig. 2.1 from Chap. 2). Only female Culicids, Ceratopogonids, and Simuliids ingest blood, while both female and male Hippoboscids are hematophagous.

The cycle inside vectors is temperature dependent and can be completed between 7 and 10 days for *Plasmodium* and *Haemoproteus* (*Parahaemoproteus*) at 24 °C and 20 °C, respectively. Development cycles can be longer than 10 days for *Haemoproteus* in Hippoboscidae flies and can be completed in 3–5 days by *Leucocytozoon* in *Simulium* black flies (Valkiūnas 2005). It is important to take into account that sporogonic development cycle of some parasite species can be longer than the average for the genus. For example, sporozoites were observed in the salivary glands of mosquitoes experimentally infected with *Plasmodium elongatum* only between 18 and 22 days post exposure to infected birds (Palinauskas et al. 2016).

Oocysts size and the numbers of produced sporozoites vary among haemosporidian parasites. Parasites of the genus *Plasmodium* usually form the biggest oocysts, with diameters usually equal to or greater than 40 μm. *Haemoproteus* (*Haemoproteus*) form oocysts bigger than 20 μm, with *Haemoproteus columbae,* the most studied species from this subgenus, forming oocysts exceeding 40 μm in diameter. Oocysts from both *Plasmodium* and *H.* (*Haemoproteus*) possess multiple germinative centers and can produce hundreds of sporozoites. Oocysts of *Haemoproteus* (*Parahaemoproteus*) and *Leucocytozoon* are of diameters between 5 and 20 μm and usually produce less than 100 sporozoites derived from a single germinative center. The length of sporozoites in haemosporidians ranges from 8 μm in some *Leucocytozoon* species, up to 14 μm in *Plasmodium*, and between 9 and 13 μm in *Haemoproteus* (Kazlauskienė et al. 2013; Žiegytė et al. 2014; Valkiūnas et al. 2015).

Morphometrics and ultrastructure are important components to differentiate parasite life stages (ookinetes, mature oocysts, and sporozoites) of each parasite group, and microscopic visualization of these forms is essential to determine vector competence. Vector competence determination (the intrinsic ability of dipteran species to support the replication and transmission of a given haemosporidian – Cohuet et al. 2010) is central for a better understanding of avian haemosporidian ecology and is assessed by experimental infections using infected birds with a parasitemia of at least 1% (e.g., Palinauskas et al. 2008). However, most natural infections have parasitemias lower than 1%; therefore, Carlson et al. (2016) developed a method for short-term storage (48 h) of infected avian blood with low *Plasmodium* parasitemias (e.g., <0.05%) that successfully completed its cycle in competent mosquito species. This procedure allows the use of low parasitemia samples for experimental infections and opens opportunities to work with a large array of wild haemosporidians to uncover vector–parasite associations that would otherwise go unnoticed. However, caution must be exercised when working with low parasitemia blood samples, as undetected coinfections can confound vector competence assays (Carlson et al. 2018).

6.3 Brief Review of the First Studies on Diptera Vectors of Avian Haemosporidian Parasites

The first discovery of a mosquito species involved in the transmission of avian *Plasmodium* was made by the British physician Ronald Ross in 1897 (reviewed in Cox 2010). Ross established the life cycle of an avian malaria parasite, *Plasmodium relictum*, in *Culex* mosquitoes, probably *Culex fatigans*. Ross demonstrated that the sexual phase of the parasite takes place in the mosquito's midgut proving transmission by insect bite. Ronald Ross was awarded the Nobel Prize in Medicine for this discovery in 1902. Koch (1899) was one of the first to confirm Ross's results by infecting domestic canaries with malaria parasites through mosquito bites. The use of bird hematozoa became the model in malaria research following Ronald Ross's discovery but was later replaced with the murine model after several rodent *Plasmodium* species were discovered, including *Plasmodium yoelii* and *Plasmodium berghei* (Cox 2010).

Mosquitoes of the genus *Aedes* were demonstrated to be experimentally susceptible to bird malaria (*Plasmodium gallinaceum*) by Huff (1927, 1932). Subsequently, there was a surge of studies conducting experimental infections mostly on mosquitoes of the genera *Culex* and *Aedes*, using mainly canaries and chickens to identify *Plasmodium* competent vectors (Ruge 1901; Huff 1927, 1932; Herman 1937; Laird 1941; Micks 1949). By the 1960s, it was already known that at least nine different mosquito species from five genera were capable of transmitting *Plasmodium* parasites among human, nonhuman primates, rodents (*Anopheles*), and birds (*Anopheles*, *Culex*, *Aedes*, *Culiseta*, *Coquillettidia*), and that some vector species were capable of feeding on different and phylogenetically diverse vertebrate hosts (Cox 2010; Santiago-Alarcon et al. 2012a). In addition, previous studies suggested that some mosquito species were able to transmit many *Plasmodium* species (e.g., *An. quadrimaculatus* vectoring monkey and avian malaria), whereas other mosquito vectors were more specialized (Coggeshall 1940). Intriguingly, in 1970, the lizard parasite *Plasmodium mexicanum* was confirmed to be transmitted by two sand fly species (*Lutzomyia vexatrix* and *Lutzomyia stewarti* – Ayala & Lee 1970) despite its close phylogenetic relationship to avian *Plasmodium* parasites that are transmitted by mosquitoes (Pacheco et al. 2018).

Pseudolynchia canariensis, a fly species of the Hippoboscidae family, was the first to be identified as a vector of *Haemoproteus columbae* (Sergent and Sergent 1906; Aragão, 1908; Adie 1915), a common parasite of pigeons and doves (Santiago-Alarcon et al. 2010). The quail *Lophortyx californica* is another bird species used in previous studies on the transmission of *Haemoproteus* parasites, in which hippoboscid flies were tested as putative vectors (Tarshis 1955). However, field experiments were inconclusive since cages with a mesh size that did not exclude *Culicoides* vectors were used. Indeed, *H. lophortyx* can be experimentally transmitted by *Culicoides* spp. as demonstrated later, placing this parasite in the *H.* (*Parahaemoproteus*) subgenus (Mullens et al. 2006).

During the early years of the past century, the vectors of *Leucocytozoon* parasites were not conclusively determined, and suspected vectors erroneously included lice and flies of family Muscidae (Skidmore 1931). The impact of an outbreak of *Leucocytozoon smithi* on turkey farms in Nebraska, USA, during the 1930s provided an opportunity to identify the vector, ultimately incriminating Simulid black flies (*Simulium meridionale*, previously referred to as *S. occidentale*) (Skidmore 1931). Other studies conducted during later outbreaks conclusively demonstrated that *Simulium jenningsi* and *Simulium venustum* were also vectors of *L. smithi* in turkeys and *Leucocytozoon anatis* in ducks (Johnson et al., 1938). Blackflies had been well studied because of their involvement in human onchocerciasis (Blacklock 1926) and due to the economic losses generated in the poultry industry (Barnett 1977).

Ceratopogonidae was the last Diptera family to be determined as vector of avian haemosporidians despite its medical and veterinary relevance (Mellor et al. 2000; Borkent 2005). The first studies on vector competence for the parasite *Haemoproteus nettionis* analyzed insect species from the Simuliidae, Hippoboscidae, Culicidae, and Ceratopogonidae, implicating ceratopogonid flies from the genus *Culicoides* as competent vectors (Fallis and Bennett 1961a, b). Interestingly, *Leucocytozoon* (*Akiba*) *caulleryi* is transmitted by *Culicoides arakawae* in Japan (Akiba 1960; Morii et al. 1965), which is an exception given that Simulid flies transmit *Leucocytozoon* parasites. Recent molecular analyses place *L.* (*Akiba*) *caulleryi* within the genus *Haemoproteus* (Pacheco et al. 2018), but morphologically, it is clearly a *Leucocytozoon* parasite (Hernández-Lara et al. 2018). Such result suggests that vectors play an active and important role in the phylogenetic history of the parasites they transmit (see also Chap. 2).

6.4 Vector Studies Worldwide with Emphasis on Tropical Areas

In this section, we collate data from studies on Diptera research published since 2012 (Table 6.1) because vector research on avian haemosporidians from the period 1890s to 2011 has already been synthesized (Santiago-Alarcon et al. 2012a). Some aspects on avian haemosporidian vectors are still understudied in the tropics, and consequently, we used studies conducted in temperate areas to illustrate and discuss some topics in this chapter. Among the nine studies in the tropics since 2012, five studies sampled and analyzed mosquitoes for the presence of haemosporidians, two evaluated Simuliidae, and two sampled louse flies. Surprisingly, no study evaluated biting midges.

It is worth mentioning that the Hawaiian Islands is by far the most studied tropical area in relation to haemosporidians due to the dramatic effects caused by avian malaria in the local avifauna. Accidental introductions of *Plasmodium relictum* and the vector *Culex quinquefasciatus* to these islands directly contributed to the extinction of several endemic bird species, and it is still a strong force limiting the

Table 6.1 Studies on Diptera vectors and avian haemosporidians from the period 2012-2019

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex erraticus	pGRW6-*P. elongatum*	–/–	NA	USA	*Ardea alba*	NA	+	NA	Egizi et al. (2018)
	pTUMIG03	–/–	NA	USA	*Butorides virescens*	NA	+	NA	
	pNOVEL	–/–	NA	USA	*Butorides virescens, Ardea alba, Phalacrocorax auritus*	NA	+	NA	
	–	–/–	NA	USA	*Ardea alba; Butorides virescens; Phalacrocorax auratus; Gallus gallus domesticus; Gavia immer; Gallinula chloropus; Ardea herodias; Nycticorax nycticorax; Branta canadensis; Egretta thula; Homo sapiens; Odocoileus virginianus; Lontra canadensis; Pseudacris crucifer*	NA	–	NA	
Culex pipiens (includes a mixture of *Cx. pipiens* and *Cx. restuans*)	pCHI02PL; pCHI04PL; pCHI05PL pCHI07PL; pCHI09PL; *P. elongatum* (AY733088)[c]; *P. cathemerium* (AY377128)[c].	–/–	NA	USA	*Agelaius phoeniceus; Cardinalis cardinalis; Carpodacus mexicanus; Dumetella carolinensis; Melospiza melodia; Molothrus ater; Passer domesticus; Quiscalus quiscula; Sturnus vulgaris; Turdus migratorius;*	+	NA	NA	Medeiros et al. (2013)

Culex pipiens	Unspecified *Plasmodium* and *Leucocytooon* lineages	–/–	NA	USA	*Quiscalus quiscula*	NA	+	NA	Mehus and Vaughan (2013)
Culex pipiens	pP01; pMMK-2009a; pJA7_J25; pM-2011	–/–	NA	USA	NA	+	NA	NA	Fryxell et al. (2014)
Culex pipiens	pCHI01PL; pCHI04PL; pCHI02PL	–/–	NA	USA	*Turdus migratorius*	NA	+	NA	Boothe et al. (2015)
	hCHI23PA; hCHI20PA	–/–	NA	USA	*Turdus migratorius; Passer domesticus*	NA	+	NA	
	–	–/–	NA	USA	*Cardinalis cardinalis; Carpodacus mexicanus*	NA	+	NA	
Culex pipiens (includes a mixture of *Cx. pipiens* and *Cx. restuans*)	pCHI02PL; pCHI04PL; pCHI07PL; pCHI03PL; pCHI06PL	–/–	NA	USA	*Turdus migratorius; Passer domesticus; Cardinalis cardinalis; Haemorhous mexicanus*[b]	+	NA	NA	Medeiros et al. (2016)
Culex pipiens	pHOWR_CA_ELW_10P; pHOWR_CA_ELW_2P; han. punc_CA_JSC_1H;	NA/–	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
Culex pipiens (includes hybrids of *Cx. pipiens* and *Cx. quinquefasciatus*)	p HOFI_CA_JSC_1P-*P. cathemerium*	+/NA	Sporozoite	USA	NA	NA	NA	+	Carlson et al. (2016)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex pipiens	pSPHUjJ; pRinshi-1;	–/–	NA	Spain	*Passer domesticus; Streptopelia decaocto; Turdus merula;* other birds; *Homo sapiens;* other mammals and reptiles	NA	+	NA	Muñoz et al. (2012); Ferraguti et al. (2013a)
Culex pipiens	pSGS1-*P. relictum*; pGRW11-*P. relictum;* pP5-*P. relictum*; pTURDUS1-*P. circumflexum*; pSW2-*P. polare*; pSYAT05-*P. vaughani*	–/–	NA	Switzerland	*Parus major*[b]	NA	NA	+	Glaizot et al. (2012)
Culex pipiens	pKayseri2; pKYS3; pKYS4; pKYS5; pKYS6; pKYS7; pKYS8; pKYS9; pKYS11	–/–	NA	Turkey	NA	NA	+	+	Inci et al. (2012)
Culex pipiens	pSGS1	–/–	NA	Romania	NA	NA	–	+	Ionică et al. (2017)

Culex pipiens	pPADOM02; pCXQUI01; pCXPIP09; pCXPIP10; pGALLUS01; pGALLUS02; pGRW4-*P. relictum*	−/−	NA	Japan	NA	+	NA	NA	Kim and Tsuda (2012)
	pGRW4	−/−	NA	Japan	*Acrocephalus orientalis; Emberiza cioides; Carduelis sinica; Ixobrychus sinensis; Lanius bucephalus; Passer montanus; Phasianus versicolor; Sturnus cineraceus; Homo sapiens; Lepus brachyurus*	NA	+	NA	
Culex pipiens	pSGS1-*P. relictum* pGRW11-*P. relictum;* pGRW4-*P. relictum;* CXPIP09	−/+	Sporozoites	Japan	NA	NA	+	+	Kim and Tsuda (2015)
Culex pipiens	pGRW4-*P. relictum*	+/NA	Sporozoites	Lithuania	NA	NA	+	+	Valkiūnas et al. (2015)
Culex pipiens	pERIRUB01- *H. elongatum*	+/−	Sporozoites	Russia	NA	NA	NA	NA	Palinauskas et al. (2016)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex pipiens (includes the forms: *pipiens, molestus,* and hybrids)	pSGS1; pSYAT05; pGRW6; pDELURB4; pDONANA03; pLINN1	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)
Culex pipiens	pSYAT05; pSGS1; pAFTRU05; pGRW11; pPADOM01; pPADOM02-*P. relictum*; pCOLL1	–/–	NA	Switzerland	NA	NA	NA	+	Lalubin et al. (2013)
Culex pipiens	pSYAT05; pSGS1-*P. relictum*; pCOLL1; pYACHO1	–/–	NA	France	NA	NA	–	+	Larcombe and Gauthier-Clerc (2015)

Culex pipiens	pSGS1; pDELURB4; pDELURB5; pPADOM01; pGRW06; pSYAT05; pCOLL1; pCXPIPS1; pCXPIPS2; pGRW11; pLINN1; hCSPIPS3; hGAGLA03	−/−	NA	France	NA	+	NA	NA	Zélé et al. (2014)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex pipiens	pAFTRU5; pLINN1; pDelurb4; pSGS1; pGRW11; pSYAT05; hTURDUS2; hCIRCUM05; hltCxpip01; hPadom3	–/–	NA	Italy	*Turdus merula; Passer domesticus; Gallus gallus; Streptopelia decaocto; Columba livia; Passer montanus; Athene noctua; Meleagris gallopavo; Columba palumbus; Pica pica; Anas platyrhynchos; Sturnus vulgaris; Accipiter nisus; Cairina moschata; Carduelis carduelis; Gallinula chloropus; Jynx torquilla; Numida meleagris; Nycticorax nycticorax; Oriolus oriolus; Parus major; Phasianus colchinus; Sylvia atricapilla; Homo sapiens; Felis silvestris; Canis familiaris; Equus caballus; Sus scrofa; Bos taurus; Erinaceus europaeus;Podarcis muralis; Lacerta* spp.	NA	+	NA	Martínez-de la Puente et al. (2015b)

Culex pipiens (includes the forms: *pipiens, molestus,* and hybrids)	pSYAT05; pSGS1	–/–	NA	Spain	NA	NA	NA	+	Martínez-de la Puente et al. (2016)
	hPADOM05	–/–	NA	Spain	*Alectoris rufa; Ana crecca; Anthus pratensis; Ardeola ralloides; Asio otus; Bubulcus ibis; Burhinus oedicnemus; Carduelis chloris; Columba livia; Galerida cristata; Gallus gallus; Hippolais polyglotta; Ixobrychus minutus; Larus ridibundus; Nycticorax nycticorax; Passer domesticus; Streptopelia decaocto; Sturnus* sp.*; Sylvia melanocephala; Turdus merula; Bos taurus; Canis familiaris; Equus caballus; Felis silvestris; Homo sapiens; Lepus granatensis; Oryctolagus cuniculus; Rattus rattus; Sus scrofa*	+	NA	NA	
Culex pipiens	hAAS-1	–/–	NA	Kuwait	NA	+	NA	NA	Reeves (2018)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex pipiens	hCp1; hCp2; pCp1; pMosquito132	–/–	NA	Madagascar	*Foudia omissa; Xanthomixis cinereiceps; Nesillas typica; Hypsipetes madagascariensis; Copsychus albospecularis; Zosterops maderaspatanus; Monticola sharpei; Ploceus nelicourvi; Foudia madagascariensis; Xanthomixis zosterops; Saxicola torquatus; Tylas eduardi; Philepitta castanea; Pseudobias wardi*	NA	+	+	Schmid et al. (2017)
Culex pipiens	pSGS1	–/–	NA	Portugal	*Sturnus unicolor*	+	–	–	Ventim et al. (2012)
Culex pipiens (complex)	pSYAT5; hTURDUS2	–/–	NA	Czech Republic	NA	NA	NA	+	Synek et al. (2013)
Culex quinquefasciatus	*Plasmodium juxtanucleare*	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)
Culex quinquefasciatus	pGRW4-*P. relictum*	+/NA	Sporozoites	Lithuania	NA	NA	NA	+	Valkiūnas et al. (2015)

Culex quinquefasciatus	pERIRUB01- *H. elongatum*	+/–	Sporozoites	Russia	NA	NA	NA	NA	Palinauskas et al. (2016)
Culex quinquefasciatus	pHOFI_CA_ELW_8P-*P. cathemerium*	+/NA	Sporozoites	USA	NA	NA	NA	+	Carlson et al. (2018)
Culex quinquefasciatus-Culex pipiens hybrid	pSPTO_CA_ELW_6P-*P. cathemerium*	+/NA	Sporozoites	USA	NA	NA	NA	+	Carlson et al. (2018)
Culex restuans	pBAEBICO2	–/–	NA	Mexico	*Sturnira hondurensis; Bos taurus; Homo sapiens*	NA	+	+	Abella-Medrano et al. (2018)
	pTROAED21	–/–	NA	Mexico	*Sturnira hondurensis; Bos taurus*	NA	+	–	
	pZOCAP12	–/–	NA	Mexico	*Homo sapiens*	NA	+	+	
Culex restuans	pP01; pMMK-2009a	–/–	NA	USA	NA	+	NA	NA	Fryxell et al. (2014)
Culex restuans	pCx.stig_CA_JSC_15P; pSPTO_CA_ELW_6P- *P. cathemerium*	NA/+	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
	p*SOSP*_CA3P-*P. homopolare*; han. punc_CA_JSC_1H	NA/–	NA	USA	NA	NA	NA	+	
Culex sitiens	pTacy7; pCXBIT01	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex torrentium	pSGS1; pSYAT05; pGRW6; pDELURB4; pDONANA03; pLINN1	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)
Culex vishnui	pTacy7	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)
	Plasmodium juxtanucleare	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)
Culex modestus	pSPHUjJ; pRinshi-1; pRinshi-11; pDONANA02; pDONANA03; pDONANA04; hPADOM05	–/–	NA	Spain	*Anas platyrhynchos; Anser anser; Gallus gallus;* mammals (no humans or horses) and reptiles	NA	+	NA	Muñoz et al. (2012); Ferraguti et al. (2013a)
	pDONANA03	–/–	NA	Romania	NA	+	NA	NA	Ionică et al. (2017)
	pDONANA03	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)

Culex perexiguus	pDelurb5; pSPHUjJ; pRinshi-1; pRinshi-11; pYacho-1; pDonana01; pDonana05, pDonana07; pDonana10; hPADOM05; hSYBOR1	–/–	NA	Spain	*Anas platyrhynchos; Anser anser; Gallus gallus; Passer domesticus; Streptopelia decaocto; Turdus merula; Equus caballus*	NA	+	NA	Muñoz et al. (2012); Ferraguti et al. (2013a)
Culex theileri	pSPHUjJ; pDelurb5; pRinshi-1; pRinshi-11; hSYBOR1	–/–	NA	Spain	*Anser anser; Gallus gallus; Passer domesticus;Equus caballus; Homo sapiens;* other mammals	NA	+	NA	Muñoz et al. (2012); Ferraguti et al. (2013a)
Culex theileri	pKYS10	–/–	NA	Turkey	NA	NA	+	+	Inci et al. (2012)
Culex theileri	pSYAT05	–/–	NA	Portugal	–	+	–	–	Ventim et al. (2012)
Culex tritaeniorhynchus	pCXTRI01	–/–	NA	Japan	*Lepus brachyurus*	+	–	NA	Kim and Tsuda (2012)
Culex tritaeniorhynchus	–	–/–	NA	Japan	*Homo sapiens; Mus* sp.	–	NA	NA	Tanigawa et al. (2013)
Culex inatomii	pCXQUI01; pGALLUS01; pCXPIP10; pCXINA01; pPADOM02; pSYBOR02; pCXPIP09	–/–	NA	Japan	*Acrocephalus orientalis; Homo sapiens; Micromys minutus; Mauremys reevesii*	+	+	–	Kim and Tsuda (2012)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex inatomii	pCXQUI01; pCXINA01; pCXINA02	NA/+	Sporozoites	Japan	NA	NA	NA	+	Kim and Tsuda (2015)
Culex bitaeniorhynchus	pPADOM02; pCXBIT01	–/–	NA	Japan	*Ardea cinerea; Anas platyrhynchos; Emberiza cioides; Homo sapiens*	+	+	–	Kim and Tsuda (2012)
Culex ocossa	pPAN2; pPAN3; pPAN7	–/–	NA	Panama	*Turdus grayi*[b]	+	NA	NA	Loaiza and Miller (2013)
Culex stigmatosoma	pHOWR_CA_ ELW_10P; pHOFI_CA_ ELW_8; pHOWR_CA_ ELW_2P; pCx. stig_CA_ JSC_17P; pCx. stig_CA_ JSC_16P; Cx.stig_CA_ JSC_15P; pSPTO_CA_ ELW_6P-*P. cathemerium*; pSPTO_CA_ ELW_4P; AMRO_CA_ ELW_11P	NA/+	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
	hAn. punc_CA_ JSC_1H	NA/–	NA	USA	NA	NA	NA	+	

Culex stigmatosoma	p HOFI_CA_JSC_1P-*P. cathemerium*	+/NA	Sporozoite	USA	NA	NA	NA	+	Carlson et al. (2016)
Culex stigmatosoma	p SPTO_CA_ELW_6P-*P. cathemerium*; pHOFI_CA_ELW_8P-*P. cathemerium*	+/NA	Sporozoite	USA	NA	NA	NA	+	Carlson et al. (2018)
Culex tarsalis	Unspecified *Plasmodium* and *Leucocytooon* lineages	–/–	NA	USA	*Bos taurus;* Cervidae; *Canis familiaris; Oryctolagus cuniculus; Homo sapiens; Turdus migratorius; Passer domesticus; Bombycilla cedrorum; Spinus tristis; Spizella passerine; Petrochelidon pyrrhonota; Quiscalus quiscula; Butorides virescens*	NA	+	NA	Mehus and Vaughan (2013)

(continued)

Table 6.1 (continued)

Vector species	Parasite lineage/ species	Proven experimentally/ proven natural vector	Most advanced developmental stage observed in vector	Region	Blood meal host detected by PCR	PCR method			References
						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culex tarsalis	pHOWR_CA_ ELW_10P; pHOFI_CA_ ELW_8P; pHOWR_CA_ ELW_2P; pCx. stig_CA_ JSC_15P; pSOSP_CA3P- *P. homopolare*; pSPTO_CA_ ELW_6P- *P. cathemerium*	NA/+	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
	pCx.tars_CA_ JSC_14P; hAn. punc_CA_ JSC_1H; Cx.tars_CA_ JSC_2H	NA/–	NA	USA	NA	NA	NA	+	
Culex tarsalis	p SPTO_CA_ ELW_6P-*P. cathemerium*; pHOFI_CA_ ELW_8P-*P. cathemerium*	+/NA	Sporozoite	USA	NA	NA	NA	+	Carlson et al. (2018)
Culex hortensis	pLINN1	–/–	NA	Italy	*Homo sapiens; Podarcis muralis*	NA	+	NA	Martínez-de la Puente et al. (2015b)

Culiseta annulata	pKYS11	–/–	NA	Turkey	NA	NA	+	+	Inci et al. (2012)
Culiseta inornata	–	–/–	NA	USA	*Odocoileus virginianus; Bos taurus; Felis catus*	NA	–	NA	Mehus and Vaughan (2013)
Culiseta particeps	pSOSP_CA3P- *P. homopolare*; SPTO_CA_ ELW_6P- *P. cathemerium*	NA/–	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
Coquillettidia perturbans	–	–/–	NA	USA	*Odocoileus virginianus; Bos taurus; Homo sapiens; Neovison vison*	NA	–	NA	Mehus and Vaughan (2013)
Coquillettidia richiardii	–	–/–	NA	Portugal	*Sturnus unicolor; Homo sapiens*	–	–	–	Ventim et al. (2012)
Coquillettidia richiardii	pLINN1	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)
Armigeres subalbatus	pGALLUS01	–/–	NA	Japan	NA	+	+	–	Kim and Tsuda (2012)
Mansonia titillans	pPAMIT01; pMaTIT01	–/–	NA	Brazil	*Homo sapiens*	NA	+	–	Ferreira et al. (2016)
Mansonia pseudotitillans	pTUMIG03	–/–	NA	Brazil	–	NA	+	–	Ferreira et al. (2016)
Mansonia uniformis	pTacy7	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)
Anopheles sinensis	pTacy7	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)

(continued)

Table 6.1 (continued)

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						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Anopheles barbumbrosus	*Plasmodium juxtanucleare*	–/–	NA	Philippines	NA	+	NA	NA	Chen et al. (2015)
Anopheles maculipennis	–	–/–	NA	Italy	*Gallus gallus; Canis lupus familiaris; Equus asinus; Equus caballus; Lepus europaeus; Bos taurus; Capra hircus; Felis catus; Homo sapiens; Vulpes vulpes*	NA	–	NA	Martínez-de la Puente et al. (2015b)
Anopheles darling	*Plasmodium vivax*	–/–	NA	Peru	*Gallus gallus;* other Galliformes; *Canis familiaris; Homo sapiens; Sus scrofa; Capra hircus; Rattus* sp.; non-human primates	–	–	+	Moreno et al. (2017)
Anopheles mascarensis	pPV12	–/–	NA	Madagascar	*Foudia omissa; Saxicola torquatus; Philepitta castanea; Ploceus nelicourvi; Nesillas typica; Bernieria madagascariensis; Hypsipetes Madagascariensis; Phedina borbónica; Foudia madagascariensis; Copsychus albospecularis; Motacilla flaviventris*	NA	–	+	Schmid et al. (2017)

Anopheles claviger	–	–/–	NA	Portugal	*Sturnus unicolor; Ovis aries*	–	–	–	Ventim et al. (2012)
Anopheles atroparvus	–	–/–	NA	Portugal	*Canis familiaris*	–	–	–	Ventim et al. (2012)
Anopheles freeborni	hAn. free_CA_JSC_3H	NA/–	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
Anopheles punctipennis	han. punc_CA_ JSC_1H	NA/–	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
Aedes albopictus	pSYAT05	–/–	NA	Italy	*Passer montanus; Turdus merula; Gallus gallus; Homo sapiens; Erinaceus europaeus*	NA	+	NA	Martínez-de la Puente et al. (2015b)
	pTs143h	–/–	NA	Japan	*Bos taurus; Felis silvestris; Mus* sp.*; Homo sapiens*	NA	+	NA	Tanigawa et al. (2013)
Aedes flavopictus	–	–/–	NA	Japan	*Bos taurus; Felis silvestris; Homo sapiens*	NA	–	NA	Tanigawa et al. (2013)
Aedes plumbeus	pLINN1	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)
Aedes japonicus	–	–/–	–	Japan	*Bos taurus; Homo sapiens*	NA	NA	NA	Tanigawa et al. (2013)
Aedes nipponicus	–	–/–	–	Japan	*Bos taurus; Homo sapiens*	NA	–	NA	Tanigawa et al. (2013)
Aedes subalbatus	–	–/–	–	Japan	*Bos taurus*	NA	–	NA	Tanigawa et al. (2013)
Aedes vexans	pKayseri1	–/–	NA	Turkey	NA	NA	+	+	Inci et al. (2012)

(continued)

Table 6.1 (continued)

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Aedes vexans	–	–/–	NA	USA	*Odocoileus virginianus; Bos taurus; Canis familiaris; Felis catus; Homo sapiens; Oryctolagus cuniculus; Zenaida asiatica*	NA	–	NA	Mehus and Vaughan (2013)
Aedes vexans	pAn.punc_CA_JSC_1H; Ae.vexa_CA_JSC_4H	NA/–	NA	USA	NA	NA	NA	+	Carlson et al. (2015)
Aedes vexans	pLINN1	–/–	NA	Austria	NA	+	NA	NA	Schoener et al. (2017)
Aedes quadrivittatus	–	–/–	NA	Mexico	*Sturnira hondurensis; Homo sapiens*	NA	NA	–	Abella-Medrano et al. (2018)
Aedes excrucians	–	–/–	NA	USA	*Odocoileus virginianus; Bos taurus; Homo sapiens; Procyon lotor; Geothlypis trichas*	NA	–	NA	Mehus and Vaughan (2013)
Aedes triseriatus	–	–/–	NA	USA	*Odocoileus virginianus; Bos taurus; Canis familiaris; Felis catus; Homo sapiens*	NA	–	NA	Mehus and Vaughan (2013)
Aedes canadensis	Unspecified *Plasmodium* and *Leucocytooon* lineages	–/–	NA	USA	*Odocoileus virginianus; Felis catus; Turdus migratorius*	NA	+	NA	Mehus and Vaughan (2013)

Aedes flavescens	–	–/–	NA	USA	*Odocoileus virginianus; Felis catus; Canis familiaris*	NA	–	NA	Mehus and Vaughan (2013)
Aedeomyia squamipennis	pPAN2; pPAN4; pPAN5; pPAN6; pPAN8; pPAN9	–/–	NA	Panama	*Turdus grayi; Thraupis episcopus; Sporophila americana; Cyanerpes cyaneus*[b]	+	+	NA	Loaiza and Miller (2013)
Wyeomyia adelpha	–	–/–	NA	Mexico	*Homo sapiens*	NA	NA	+	Abella-Medrano et al. (2018)
Ochlerotatus cantans	pGRW4-*P. relictum*	–/–	Ookinetes	Lithuania	NA	NA	–	NA	Valkiūnas et al. (2015)
Ochlerotatus cantans	pSGS1; pGRW11; hSISKIN1; hDELURB1; hSW1; hRB1; hRB2	–/–	NA	Russia Baltic Sea	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016)
Ochlerotatus cataphyla	hRBS1; hSFC1	–/–	NA	Russia Baltic Sea	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016)
Ochlerotatus sticticus	hSISKIN1	–/–	NA	Russia Baltic Sea	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016)
Ochlerotatus caspius	pSPHUjJ; pDonana06; pDonana08; pDonana09;	–/–	NA	Spain	*Gallus gallus; Passer domesticus; Turdus merula; Equus caballus; Homo sapiens;* other mammals	NA	+	NA	Muñoz et al. (2012); Ferraguti et al. (2013a)
Ochlerotatus caspius	–	–/–	NA	Italy	*Gallus gallus; Felis silvestris; Equus asinus; Equus caballus; Bos taurus; Canis familiaris; Homo sapiens*	NA	–	NA	Martínez-de la Puente et al. (2015b)

(continued)

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Psorophora discrucians	hPSDIS01-*H. syrnii* hPsDIS02	–/–	NA	Brazil	*Homo sapiens*	NA	+	–	Ferreira et al. (2016)
Uranotaenia alboabdominalis	hUal1; pUal1; pWA46	–/–	NA	Madagascar	*Hypsipetes madagascariensis*	NA	–	+	Schmid et al. (2017)
Uranotaenia sp.	hUan1; h1252; pUan1; pWA46; pP31; pmosquito132	–/–	NA	Madagascar	*Foudia omissa; Xanthomixis cinereiceps; Nesillas typica; Hypsipetes madagascariensis; Copsychus albospecularis; Zosterops maderaspatanus; Monticola sharpei; Ploceus nelicourvi; Foudia madagascariensis; Xanthomixis zosterops; Saxicola torquatus; Tylas eduardi; Philepitta castanea; Pseudobias Wardi*[b]	NA	–	+	Schmid et al. (2017)
Culicoides impunctatus	hCIRCUM01-*H. noctuae*	+/–	Sporozoite	Russia Baltic Sea	NA	NA	+	+	Bukauskaitė et al. (2015)

Culicoides impunctatus	pSGS1; hTURDUS2; hSISKIN1	–/–	NA	Russia Baltic Sea	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016)
Culicoides impunctatus	hSFC9-*H. balmorali;* hPARUS1-*H. majoris;* hYWT1-*H. motacillae;* hPFC1- *H. pallidus*	+/–	Sporozoite	Russia Baltic Sea	NA	NA	NA	NA	Žiegytė et al. (2017)
Culicoides impunctatus	pLINN1-*P. matutinum*	–/–	–	Lithuania	NA	NA	NA	+	Bernotienė et al. (2019)
Culicoides punctatus	–	–/–	NA	Bulgaria	*Cervus elaphus; Homo sapiens*	NA	–	NA	Bobeva et al. (2015)
Culicoides punctatus	hTURDUS2	–/–	NA	Russia Baltic Sea	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016)
Culicoides punctatus	hWW2-*H. majoris*; hTURDUS2-*H. minutus*; hPFC1-*H. pallidus*; hHAWF1-*H. tartakovskyi*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides pictipennis	pLINN1; *Haemoproteus minutus*; *Haemoproteus pallidus;* hSYAT03; hSYAT35	–/–	NA	Germany	*Homo sapiens; Turdus merula; Erithacus rubecula*	NA	+	+	Santiago-Alarcon et al. (2012b, 2013)

(continued)

Table 6.1 (continued)

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Culicoides pictipennis	hTURDUS2	–/–	NA	Russia Baltic Sea; Bulgaria	NA[a]	NA	+	NA	Bernotienė and Valkiūnas (2016); Bobeva et al. (2013)
Culicoides pictipennis	–	–/–	NA	Bulgaria	*Bos taurus; Cervus elaphus*	NA	–	NA	Bobeva et al. (2015)
Culicoides pictipennis	hTUPHI01-*H. minutus*; hSYAT01-*H. parabelopolskyi*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides circumscriptus	pRinshi-1; pGAL-2012; pP15; pDelurb5; pSPHUjJ; pAFTRU5; pDonana10; hBUBIBI01	–/–	NA	Spain	*Anas acuta; Anas strepera; Aythya ferina; Bubulcus ibis; Ciconia ciconia; Egretta garzetta; Fulica cristata; Gallinula chloropus; Himantopus himantopus; Marmaronetta angustirostris*	+	NA	NA	Ferraguti et al. (2013b)
Culicoides circumscriptus	hHAWF2; hCULCIR1	–/–	NA	Bulgaria	–	NA	+	NA	Bobeva et al. (2013)
Culicoides circumscriptus	hCIRCUM01	–/–	NA	Bulgaria	*Asio otus*	NA	+	NA	Bobeva et al. (2014)
Culicoides circumscriptus	–	–/–	NA	Bulgaria	*Asio otus; Pica pica; Turdus merula*	NA	–	NA	Bobeva et al. (2015)

Culicoides circumscriptus	hTURDUS2-*H. minutus*; hGAGLA05; hAEFUN03	–/–	NA	Spain	NA	+	NA	NA	Veiga et al. (2018)
Culicoides alazanicus	hCIRCUM01	–/–	NA	Bulgaria	*Asio otus*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	hTUPHI01	–/–	NA	Bulgaria	*Columba palumbus*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	hDELURB01	–/–	NA	Bulgaria	*Delichon urbica*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	hSFC01	–/–	NA	Bulgaria	*Muscicapa striata*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	hORORI01	–/–	NA	Bulgaria	*Oriolus oriolus*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	hORORI02	–/–	NA	Bulgaria	*Oriolus oriolus*	NA	+	NA	Bobeva et al. (2014)
Culicoides alazanicus	–	–/–	NA	Bulgaria	*Anthus trivialis; Ardea purpurea; Asio otus; Columba palumbus; Delichon urbica; Ixobrychus minutus; Luscinia luscinia; Muscicapa striata; Oriolus oriolus; Parus major; Phylloscopus trochilus; Pica pica; Sylvia borin; Turdus merula; Turdus philomelos; Homo sapiens*	NA	–	NA	Bobeva et al. (2015)
Culicoides festivipennis	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2012b, 2013)

(continued)

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						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culicoides festivipennis	hTURDUS2	–/–	NA	Czech Republic	–	+	NA	NA	Synek et al. (2013)
Culicoides festivipennis	hCIRCUM01	–/–	NA	Bulgaria	*Asio otus*	NA	+	NA	Bobeva et al. (2014)
Culicoides festivipennis	hCIRCUM03	–/–	NA	Bulgaria	*Pica pica*	NA	+	NA	Bobeva et al. (2014)
Culicoides festivipennis	–	–/–	NA	Bulgaria	*Anthus trivialis; Asio otus; Nycticorax nycticorax; Oriolus oriolus; Passer domesticus; Passer hispaniolensis; Passer montanus; Pica pica; Streptopelia decaocto*	NA	–	NA	Bobeva et al. (2015)
Culicoides festivipennis	hROBIN1-*H. attenuatus*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides clastrieri	–	–/–	NA	Germany	*Homo sapiens; Tadorna ferruginea; Turdus philomelos*	NA	–	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides deltus	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides dewulfi	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides chiopterus	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2012b)

Culicoides kibunensis	hCUKI1; hTUPHI1; hTURDUS2	–/–	NA	Czech Republic	–	+	NA	NA	Synek et al. (2013)
Culicoides kibunensis	hSYAT01; hSYAT02; hSYAT03; hSYAT07; hSYAT35	–/–	NA	Germany	*Sylvia attricapilla; Erithacus rubecula; Homo sapiens*	NA	+	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides kibunensis	hPFC1-*H. pallidus*	–/+	Sporozoite	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides kibunensis	hTURDUS2-*H. minutus;* hSISKIN1-*H. tartakovskyi*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides obsoletus	–	–/–	NA	Germany	*Homo sapiens; Bos taurus*	NA	–	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides obsoletus	hROBIN1-*H. attenuatus*; hRB1-*H. lanii*; hTURDUS2-*H. minutus*; hSISKIN1-*H. tartakovskyi*; hSFC4; pGRW11-*P. relictum*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides pallidicornis	–	–/–	NA	Germany	*Homo sapiens; Bos taurus*	NA	+	NA	Santiago-Alarcon et al. (2012b, 2013)

(continued)

Table 6.1 (continued)

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						Whole unengorged vectors	Engorged females or unengorged abdomen parts	Thoracic parts of vectors	
Culicoides paolae	NA	NA	NA	Spain	*Homo sapiens; Coracias garrulus; Falco tinnunculus; Passer domesticus; Turdus merula; Upupa epops; Streptopelia decaocto; Linaria cannabina*	NA	NA	NA	Veiga et al. (2018)
Culicoides paolae	hH1CG.1-*H. coraciae*; hTURDUS2-*H. minutus*; pSYAT05-P. vaughani	NA	NA	Spain	NA	+	NA	NA	Veiga et al. (2018)
Culicoides poperinghensis	hSYAT02	–/–	NA	Germany	*Homo sapiens*	NA	+	NA	Santiago-Alarcon et al. (2012b)
Culicoides pulicaris	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2012b)
Culicoides scoticus	hSYAT01; hSYAT02	–/–	NA	Germany	*Homo sapiens; Equus caballus*	NA	+	NA	Santiago-Alarcon et al. (2012b, 2013)

Culicoides scoticus	hLULU1-*H. balmorali*; hTURDUS2-*H. minutus*; hHAWF1-*H. tartakovskyi;* pTURDUS1-*P. circumflexum;* pGRW11 & pSGS1-*P. relictum*	–/–	–	Lithuania	NA	NA	–	+	Bernotienė et al. (2019)
Culicoides semimaculatus	hCCF2	–/–	NA	Germany	*Homo sapiens; Erithacus rubecula*	NA	+	NA	Santiago-Alarcon et al. (2012b, 2013)
Culicoides segnis	hCUKI1; hTUPHI1; hCCF4; hROFI1	–/–	NA	Czech Republic	–	+	NA	NA	Synek et al. (2013)
Culicoides newsteadi	–	–/–	NA	Germany	*Homo sapiens*	NA	–	NA	Santiago-Alarcon et al. (2013)
Culicoides nubeculosus	hCIRCUM01-*H. noctuae* hCULCIB01-*H. syrnii*	+/NA	Sporozoites	Russia Baltic Sea	–	NA	NA	+	Bukauskaitė et al. 2015
Culicoides nubeculosus	hTURDUS2-*H. minutus*; hYWT2-*H. motacillae*; hROBIN01-*H. attenuatus*	+/NA	Sporozoites	Lithuania	NA	NA	NA	NA	Bukauskaitė et al. (2018)
Culicoides nubeculosus	hPHSIB2-*H. homopalloris*	+/NA	Sporozoites	Lithuania	NA	NA	NA	NA	Chagas et al. (2018)

(continued)

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Culicoides cf. *griseidorsum*	–	–/–	NA	Bulgaria	*Acrocephalus palustris; Coccothraustes coccothraustes; Luscinia megarhynchos; Pica pica; Cervus elaphus*	NA	–	NA	Bobeva et al. (2015)
Culicoides occidentalis	–	–/–	NA	USA	*Bos taurus; Canis familiaris; Equus caballus; Equus asinus; Lepus californicus; Capra hircus; Odocoileus hemionus; Ovis aries; Sus scrofa domesticus; Dromaius novaehollandiae*	NA	–	NA	Hopken et al. (2017)
Culicoides variipennis complex	–	–/–	NA	USA	*Bos taurus; Canis familiaris; Equus caballus; Equus asinus; Lepus californicus; Odocoileus hemionus; Ovis aries; Sus scrofa domesticus; Dromaius novaehollandiae*	NA	–	NA	Hopken et al. (2017)
Culicoides biguttatus	–	–/–	NA	USA	*Odocoileus virginianus*	NA	–	NA	Hopken et al. (2017)
Culicoides crepuscularis	–	–/–	NA	USA	*Haemorhous mexicanus*	NA	–	NA	Hopken et al. (2017)

Culicoides reevesi	–	–/–	NA	USA	*Odocoileus hemionus*	NA	–	NA	Hopken et al. (2017)
Culicoides stellifer	–	–/–	NA	USA	*Odocoileus virginianus*	NA	–	NA	Hopken et al. (2017)
Culicoides utahensis	–	–/–	NA	USA	*Odocoileus hemionus*	NA	–	NA	Hopken et al. (2017)
Simulium meridionale	*Leucocytozoon schoutedeni*; *L. gentili*; *L. buteonis*	–/–	NA	USA	*Gallus gallus*; *Buteo jamaicensis*[b]	+	NA	NA	Jones et al. (2015)
Simulium silvestre	lCOLBF01; lSTOCC13; lPARUS25; lCB1; lEUSE2; lTABI09; lCOLBF09; lTUMER02; lCNEORN01; lZOLEU02; lCOLBF14; lTRPIP2; lEMPALN01; lBUVIR03; lHGR1; lTROED09; lCOLBF24; lCARFLA04; lSTUR1; lEUSE1; lHYPHI28		NA	USA	*Dendragapus obscurus; Corvis brachyrhynchos*	+	NA	NA	Murdock et al. (2015)

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Simulium exulatum	lSTOCC13	–/–	NA	USA	–	+	NA	NA	Murdock et al. (2015)
Gigantodax misitu	lHEVE01; lGIMIS_01	–/–	NA	Colombia	*Homo sapiens*	NA	+	–	Lotta et al. (2016)
Simulium furcillatum	–	–/–	NA	Colombia	*Grallaria* sp.; *Equus caballus*	NA	–	–	Lotta et al. (2016)
Simulium bicoloratum	lSIBIC_01	–/–	NA	Colombia	–	NA	+	–	Lotta et al. (2016)
Simulium cormonsi	–	–/–	NA	Colombia	*Grallaria ruficapilla*	NA	–	–	Lotta et al. (2016)
Simulium furcillatum	–	–/–	NA	Colombia	*Grallaria* sp.; *Equus caballus*	NA	–	–	Lotta et al. (2016)
Simulium ignescens (complex)	–	–/–	NA	Colombia	*Basileuterus nigrocristatus*	NA	–	–	Lotta et al. (2016)
Simulium muiscorum	lSIMUI_01	–/–	NA	Colombia	*Atlapetes pileatus*	NA	+	+	Lotta et al. (2016)
Simulium vernum	lNEVE1; lBT2; lEUSE2; lPARUS4; lPARUS25	–/–	NA	Czech Republic	*Aegolius funereus*[b]	+	NA	NA	Synek et al. (2016)
Simulium vernum	lPERATE04; lWW6; lPARUS14; lZOCAP05; lTUPHI06; lGAGLA06	–/–	NA	UK	*Cyanistes caeruleus*[b]	NA	+	+	Woodford et al. (2018)
Simulium angustipes	lNEVE1	–/–	NA	Czech Republic	*Aegolius funereus*[b]	+	NA	NA	Synek et al. (2016)

Simulium cryophylum	lPERATE04; lWW6; lNEVE01; lMTUR2; lPARUS21; lCOLIV04; lGAGLA06; lSERSER04	–/–	NA	UK	*Cyanistes caeruleus*[b]	NA	+	+	Woodford et al. (2018)
Simulium aureum	lPERATE04; lWW6; lMTUR2; lPARUS4	–/–	NA	UK	*Cyanistes caeruleus*[b]	NA	+	+	Woodford et al. (2018)
Simulium chumpornense	lLEUCOTH1; lLEUCOTH2; lLEUCOTH3; lLEUCOTH4; lLEUCOTH5	–/–	NA	Thailand	–	+	NA	NA	Jumpato et al. (2019)
Simulium asakoae	lLEUCOTH6 lLEUCOTH7; lLEUCOTH8; lLEUCOTH9; lLEUCOTH10; lLEUCOTH11; lLEUCOTH12; lLEUCOTH13; lLEUCOTH14; lLEUCOTH15; lLEUCOTH16	–/–	NA	Thailand	–	+	NA	NA	Jumpato et al. (2019)
Greniera denaria	lCOLBF01	–/–	NA	USA	–	+	NA	NA	Murdock et al. (2015)
Olfersia spinifera	*Haemoproteus iwa*	–/+	NA	Ecuador (Galapagos)	*Fregata minor*[b]	NA	NA	+	Levin and Parker (2012, 2014)

distribution of extant species to upper elevation environments with fewer mosquitoes and prolonged or stalled *Plasmodium* developmental periods in the vector (Warner 1968; van Riper et al. 1986, Lapointe et al. 2010). In the past decades, many studies have been conducted to understand vector's habitat use, dispersion, susceptibility, and capability to transmit *P. relictum* in Hawaii, and these aspects were summarized by LaPointe et al. (2012). Consequently, this chapter focuses on other tropical areas, but further discussion on the Hawaiian malaria system is addressed in Chapters 1 and 8.

6.4.1 Methods to Collect Avian Haemosporidian Vectors

Many methods have been applied to capture blood-feeding dipterans for avian haemosporidian studies. Most used the CDC Miniature Light Trap and similar models, and the BG Sentinel® trap. These traps consist on a motor-operated suction fan that draws and keeps dipterans into a container. Carbon dioxide source such as dry ice is the most common bait used to attract target insects into these traps (Ferraguti et al. 2013a, 2013b; Murdock et al. 2015; Chen et al. 2015). Alternatively, solutions containing sugar and yeast (*Saccharomyces cerevisiae*) can also be used to produce CO_2 (Abella-Medrano et al. 2018), as well as formulated lures that mimic the chemical cues of vertebrate cutaneous tissue. Other baits such as UV or LED light bulbs can be used solely (Bobeva et al. 2013; Lotta et al. 2016) or augmented with CO_2 or artificial lures (Santiago-Alarcon et al. 2013; Veiga et al. 2018). These methods are efficient to capture unfed mosquitoes, biting midges, and black flies, but engorged females are occasionally captured as well. Hippoboscid flies spend almost all of their adult life on their hosts (Arcoverde et al. 2009; Waite et al. 2012b), meaning that they should be directly collected from captured birds (Levin and Parker 2014). Active collection of resting insects with nets or aspirators and methods that use humans as baits to attract mosquitoes and black flies, such as Shannon traps, have been used in avian haemosporidian studies as well (Ferreira et al. 2016; Abella-Medrano et al. 2018; Jumpato et al. 2019). Biting midges can also be captured in sticky traps set inside nest boxes but in much lower proportion than when using CDC traps with UV or incandescent light bulbs augmented with CO_2 (Veiga et al. 2018). Additionally, resting boxes have been proven as an efficient method to collect engorged ornithophilic mosquitoes, providing opportunities for epidemiological studies associating parasites and other pathogens to the blood meal source (Egizi et al. 2018).

A paper by Carlson et al. (2015) highlights the importance of using a variety of sampling methods (gravid traps, red boxes, nets, Ehrenberg pigeon traps, Encephalitis Virus Surveillance [EVS] traps; see also Moreno et al. 2017 for the use of barrier screens) in order to get a thorough representation of the mosquito diversity. The authors showed that if only one type of trap were used, they would have missed important putative vectors that are more attracted to specific traps and that might dominate the transmission dynamics of specific *Plasmodium* parasites. It is

also important to sample biting insects at different seasons, ideally monthly (Santiago-Alarcon et al. 2013) and during different periods of the day because many species are active only at midday or during dusk or during the night (Abella-Medrano et al. 2015).

6.4.2 *Molecular Approaches to Investigate Putative Haemosporidian Vectors*

Studies focusing on avian haemosporidian vectors usually collect hundreds or even thousands of bloodsucking dipterans. Consequently, different approaches have been applied for parasite detection, which depend on the research questions and on available financial resources to conduct the experiments. In the field, collected insects are usually stored at 4 °C to −196 °C (the temperature of liquid nitrogen) depending on available conditions. Alternatively, biting midges, black flies, and louse flies can be stored in 70%–95% ethanol at ambient temperature until they reach the laboratory to be placed in −20 °C to −80 °C (Levin and Parker 2014; Bobeva et al. 2014; Jumpato et al. 2019). However, mosquitoes should never be initially stored in tubes containing ethanol because this impairs species identification due to the loss of taxonomically informative parts of the insects (i.e., scales and setae). In the laboratory, insects are identified using taxonomic keys (see Chap. 5) or molecular barcoding (Rozo-Lopez & Mengual 2015; Bernotienė et al. 2019). Engorged females are usually processed separately for blood meal source identification (see section below). In this case, the abdomen can be separated from the rest of the mosquito body for the blood meal analysis, while thoraxes can be processed for the detection of haemosporidians (Abella-Medrano et al. 2018).

Unfed insects can be analyzed for the presence of haemosporidians in the whole individual insect (Jumpato et al. 2019) or by extracting DNA from thorax and abdomen from the same individual in separate reaction tubes (Schmid et al. 2017). Another common approach is to use pools of whole insects for DNA extraction (Loaiza and Miller 2013; Chen et al. 2015). Alternatively, pools can be formed by grouping thoraxes and abdomens separately for haemosporidian detection (Ferreira et al. 2016; Lotta et al. 2016). If investigators decide to analyze pools, insects should be grouped by species, trap employed, area, and date of collection. The analysis of abdomens and thoraxes separately relies on the premise that parasite DNA detected in these segments would likely be derived from oocysts and sporozoites, respectively. However, a given Diptera species cannot be implicated in parasite transmission based only on PCR-based techniques using whole insects or even using separate body parts without dissection. This is because parasite DNA can stay viable for long periods in nonvector insect parts, including thorax. However, recent approaches detecting mRNA via high-throughput (next-generation) sequencing provide promising perspectives in the determination of avian haemosporidian vectors using molecular methods (see section below).

Molecular studies on avian haemosporidian vectors can reveal parasite lineages not previously detected in birds sampled in the same areas where insects were sampled (Ferreira et al. 2016; Ferreira Junior et al. 2017; Schmid et al. 2017), refining our knowledge on local parasite diversity. This may be explained by the fact that bird sampling techniques are heavily biased toward understory species, smaller bodied species, and aerial birds, potentially missing parasites in canopy dwelling, large-bodied, or ground bird species. Indeed, mosquitoes may have access to birds occupying different niches and would be exposed to parasites specific to undersampled bird groups such as the ones using aquatic environments (Egizi et al. 2018) or that preferentially fly at the canopy level.

6.4.3 Confirmation of Vector Competence

One important finding from our data in Table 6.1 is that despite the fact that Diptera have generalist feeding preferences (e.g., Hellgren et al. 2008; Santiago-Alarcon et al. 2013; Bobeva et al. 2014; Abella-Medrano et al. 2018), experimental studies have demonstrated developmental deficiencies and abortive infections when haemosporidians infect fly species belonging to nonvector families (Valkiūnas et al. 2013; Gutiérrez-López et al. 2016). This suggests that even when haemosporidian host switching must be common across different Diptera families, there is a degree of parasite specialization (e.g., *Plasmodium* – Culicidae, (*H. Parahaemoproteus*) – Ceratopogonidae).

Vector competence is determined by biotic (e.g., parasite and dipteran species and genetic variants, vector immunity and behavior) and abiotic factors (e.g., temperature, humidity, geographic location), which modulate the epidemiology of host–vector–parasite associations. A competent vector of haemosporidian parasites is one in which the parasite completes sporogony, forming viable sporozoites, which are in turn injected into other hosts during subsequent blood feedings (Valkiūnas 2011).

Some studies applying molecular tools have detected *Plasmodium* DNA in blood fed (Bobeva et al. 2014) and in unfed biting midges (Bernotienė et al. 2019), while other authors reported *Haemoproteus* DNA in unfed Culicidae mosquitoes (Njabo et al. 2011; Ferreira et al. 2016; Schmid et al. 2017). Such incompatible interactions most likely lead to abortive infections, in which the parasite develops into oocysts that do not fully mature to form sporozoites. For instance, Valkiūnas et al. (2013) observed degenerating *Haemoproteus* oocysts until 15–17 days post-infection (dpi) in mosquitoes midguts, with detectable parasite DNA in thorax and in abdomen segments during this period. This study clearly shows that haemosporidian can undergo sexual stages, forming ookinetes and early oocysts in noncompetent vectors. It is important to bear in mind that abortive *Plasmodium* infections also occur in some mosquito species, demonstrating that haemosporidian parasites may have a limited vector range even within Diptera families that usually serve as competent vectors (Palinauskas et al. 2015; Valkiūnas et al. 2015). As PCR techniques amplify

parasite DNA regardless of its life stage, dipterans cannot be confirmed as competent vectors based solely on molecular detection of parasite genes via PCR and Sanger sequencing.

It is important to determine vector competence via experimental infections to better understand avian haemosporidian ecology. To do so, vector candidates are allowed to feed on parasitized birds and successful experiments have been conducted using donor birds with gametocytemia between 0.02% and 0.1% for *Plasmodium* and *Haemoproteus* (Kazlauskienė et al. 2013; Valkiūnas et al. 2015; Žiegytė et al. 2016). Experiments using laboratory-reared insects are limited due to the small number of bloodsucking species kept under such conditions. One interesting way to test a higher diversity of putative vectors is to expose infected birds to free-living dipterans (Valkiūnas et al. 2013; Bukauskaitė et al. 2015). Briefly, investigators wearing gloves and with arms protected from insect bites hold subject birds in areas with high densities of candidate vectors. Wild insects are allowed to feed on the bird, and this usually occurs in feather-free areas. When the insects start blood feeding, the head of the bird is carefully placed into small insect cages. After completion of blood ingestion, engorged females fly off the birds, the bird is removed, and the cage is sealed and then kept under controlled laboratory conditions.

Around 40 engorged insects should be obtained in these experiments to follow parasite development, and dissections are conducted at intervals targeting each parasite stage. Ookinetes can be visualized in the midgut content at 1–2 dpi in *Plasmodium* infections (Žiegytė et al. 2014), while in *H.* (*Parahaemoproteus*) this stage is visible as early as 1 h post-infection (Bukauskaitė et al. 2018) and generally disappears after 12 hours post-infection (hpi) (Žiegytė et al. 2017). For this analysis, the midgut should be extracted and smashed into a glass slide and processed with Giemsa staining in a similar way as for blood smear staining (Valkiūnas 2005). Oocysts are visible in competent hosts between 2 and 7 dpi for *H.* (*Parahaemoproteus*) and between 5 and 15 dpi for *Plasmodium* parasites. The midgut from experimentally infected insects should be removed and stained with 2% mercurochrome solution for oocyst visualization (Kazlauskienė et al. 2013; Žiegytė et al. 2017; Bukauskaitė et al. 2018). Dissection of salivary glands can be done in parallel in the same insects tested for the presence of oocysts, as parasite development is usually asynchronous, with immature and mature oocysts co-occurring over the course of infection. Sporozoites are visible in the salivary glands after 5–10 dpi in biting midges (Žiegytė et al. 2014; Bukauskaitė et al. 2015; Žiegytė et al. 2017; Bukauskaitė et al. 2018) and after 5 dpi for *Plasmodium*-infected mosquitoes (LaPointe et al. 2010; Kazlauskienė et al. 2013; Valkiūnas et al. 2015). After dissection, the salivary glands are crushed to form a thin smear, which is fixed with methanol and stained with Giemsa. Thorax remnants of dissected vectors can be used for PCR analysis followed by sequencing to confirm parasite identity (Kazlauskienė et al. 2013).

Plasmodium DNA can be detected in saliva obtained from mosquitoes, as demonstrated in a study conducted with *Culex pipiens* feeding on naturally infected birds (Gutiérrez-López et al. 2016). For that, mosquito salivation can be induced by removing the legs and wings from each mosquito, while its proboscis is introduced in small capillary tubes containing fetal bovine serum; salivation can also be

stimulated by the injection of 2% pilocarpine into the mosquito thorax. DNA is then extracted from this material and subjected to PCR and sequencing. This method allowed confirming vector competence for *Plasmodium* parasites, while authors demonstrated that the same mosquito species is not competent for *Haemoproteus* transmission.

Kim and Tsuda (2015) proposed a method to identify avian *Plasmodium* vectors with mosquitoes infected in the wild. Female mosquitoes were captured and kept alive until the dissection of the midgut and salivary glands. The midgut was first analyzed for the presence of oocysts, and, when present, the salivary glands from corresponding mosquitoes were dissected. Salivary glands come in pairs, so one of the salivary glands was stored for DNA extraction and the other was smeared into a glass slide for Giemsa staining and microscope visualization of sporozoites. This allowed the molecular characterization of viable sporozoites in free-living mosquitoes, identifying two species as vectors of three parasite lineages each.

Carlson et al. (2015) dissected 3083 mosquitoes to describe *Plasmodium* parasites in free-living mosquitoes. Authors separated abdomens from thoraxes and extracted the salivary glands from each insect, keeping these three parts in separate tubes. First, they checked the thoraxes for the presence of haemosporidians DNA, and, in case of positivity, the respective salivary glands and abdomens were also subjected to PCRs. From 76 positive thoraxes, they detected *Plasmodium* DNA in 56 and in 57 salivary glands and abdomens, respectively. Five species of *Culex* had thoraxes positive for *Plasmodium*, but only three species (*Cx. stigmatosoma*, *Cx. tarsalis*, and *Cx. restuans*) had positive salivary glands and were considered vectors. Furthermore, Carlson et al. (2016) successfully completed *Plasmodium* life cycle in competent mosquito species by using a method for short-term storage (48 h) of infected blood with low *Plasmodium* parasitemias (e.g., <0.05%), which are the type of infection levels found in nature (parasitemia <0.1%). Briefly, the method consists of collecting blood using syringes that already contain an anticoagulant (e.g., citrate phosphate dextrose adenine solution, CPDA), an aliquot of blood was used to prepared blood smears in the field, and the rest of blood was divided into two parts: one part was left in the syringe stored in plastic bags with ice and the other was stored in lysis buffer for molecular diagnostics. Then, instead of doing quick stain in the field, the smears are stained in the laboratory with Giemsa as described in Valkiūnas (2005), parasitemia is determined microscopically, and if ≤1%, the blood is stored 48 h at a maximum temperature of 4 °C; finally, the blood can be used to infect naïve hosts intravenously (Carlson et al. 2016).

To confirm vector competence in wild-caught biting midges, Bernotienė et al. (2019) dissected parous females (bloodsucking insects looking for hosts after completing the first gonotrophic cycle) and applied both microscopic and molecular detection of haemosporidians. The anterior part of the thorax (were the salivary glands are located) of captured females was crushed onto a glass slide to prepare a smear for subsequent Giemsa staining and microscopic visualization. The remnants of each biting midge were stored in 96% ethanol for molecular identification of the parasites via PCR and Sanger sequencing. Only smears from PCR-positive insects were analyzed under the microscope. *Haemoproteus* sporozoites were detected in

two *Culicoides kibunensis*, while genetic sequencing of these parasites revealed the same parasite species (*Haemoproteus pallidus*), confirming vector competence. Furthermore, 10 *Haemoproteus* and 4 *Plasmodium* genetic lineages were detected in seven biting midge species, but no sporozoites were visualized.

Although experimental infections may be necessary to confirm vector competence, the approaches by Carlson et al. (2015, 2016), Kim and Tsuda (2015), and Bernotienė et al. (2019) can determine mosquito and biting midges species as natural avian haemosporidian vectors with high confidence. Viable sporozoites can be detected in the salivary glands of mosquitoes for more than 30 dpi (Kazlauskienė et al. 2013), meaning that vectors may persist as infective, and can be diagnosed as so for most of their adult life span, even if only one infected blood meal is ingested. Such methods to detect parasites in the salivary glands of dipterans can be useful for large-scale field studies and can quickly expand our knowledge on avian haemosporidian vectors.

Lindner et al. (2019), using high-throughput mRNA sequencing, showed that genes upregulated in sporozoites during the oocyst stage differed from genes upregulated during the sporozoite infective stage in the salivary gland in mammal *Plasmodium* species. This is a promising approach to investigate vector competence in avian haemosporidians. For instance, mRNA from such upregulated genes during the infective sporozoite stage may be present in higher proportions in competent avian haemosporidian vectors when compared to abortive infections in nonvectors. To our knowledge, haemosporidian gene expression has never been evaluated in dipterans during abortive infections, opening opportunities for the development of studies applying high-throughput RNA sequencing during parasite development in competent and in noncompetent vectors. The detection of genes expressed exclusively by avian haemosporidian sporozoites during the infective stage would ultimately enable confirmation of vector competence using molecular methods.

6.4.4 Blood Meal Analysis and Vertebrate Host Preference

Most studies on avian haemosporidians determine blood meal sources by sequencing fragments of vertebrate mitochondrial genes from engorged females. In general, universal primers are employed to enable gene amplification from a broad range of vertebrates, and sequencing results are compared to public databases such as GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Barcode of Life Data Systems (http://www.boldsystems.org/) to identify the blood meal source (Martínez-de la Puente et al. 2015a, b). However, success rates of host detection decrease with the progression of blood digestion (Martínez-de la Puente et al. 2013; Reeves et al. 2016). With less than 10 studies during the first decade of the twenty-first century, the field has expanded to more than 38 studies investigating vector-feeding preferences in the past 8 years (Table 6.1). Even more importantly, some of those studies have determined feeding preferences and avian haemosporidians infecting insects, along with the dynamics of insects' abundances and diversity

throughout the year (Ferraguti et al. 2013a; Loaiza and Miller 2013; Santiago-Alarcon et al. 2013; Zélé et al. 2014; Abella-Medrano et al. 2018) and across years (e.g., Kim and Tsuda 2012; Chen et al. 2015).

Many of the studied mosquito and biting midges species are considered mammophilic, and yet they have been infected with avian haemosporidians and/or detected with avian blood in their abdomens, indicating that many vector species have broad host preferences (Santiago-Alarcon et al. 2012b; Martínez-de la Puente et al. 2015a, 2015b; Abella-Medrano et al. 2018). For example, *Anopheles darlingi* is the main human malaria vector in Panama, and it is considered highly anthropophilic; however, blood meal analyses showed that, in addition to humans, this mosquito commonly fed on chickens, turkeys, dogs, pigs, goats, and rats, with 30% of blood meals composed of more than one host (Moreno et al. 2017). Blood-feeding specificity can vary geographically as described for *Culex pipiens* in Switzerland (Glaizot et al. 2012) and France (Larcombe and Gauthier-Clerc 2015) compared to its more general feeding preferences in Spain (Martínez-de la Puente et al. 2016). Furthermore, some species of biting midges can take blood meals from humans, domestic animals, and wild birds, as demonstrated in urban greenspaces in Europe (Santiago-Alarcon et al. 2012b; Santiago-Alarcon et al. 2013; Martínez-de la Puente et al. 2015a, 2015b). Interestingly, the review by Martínez-de la Puente et al. (2015a, 2015b) based on blood meal analysis indicated that *Culicoides obsoletus*, *Culicoides punctatus*, and *Culicoides scoticus* were primarily mammophilic species, but these dipteran species were found harboring *Haemoproteus* DNA in high frequencies (Bernotienė et al. 2019). This reveals that avian haemosporidian detection in bloodsucking insects is an important tool to understand vector-feeding habits, and it is especially important to indicate bridge vectors for pathogen transmission among birds and humans in tropical areas (see Chaps. 5 and 14). Generalist feeding behavior was found in *Culicoides* species from different parts in the United States as well, but in this case, no human blood meals were detected (Hopken et al. 2017).

Simuliidae insects also have broad host preferences, and a single species can transmit different species of *Leucocytozoon* (Barrow et al. 1968; Fallis and Bennett 1962; Hellgren et al. 2008). Such broad host preferences might aid parasites to host switch and develop novel zoonoses. For example, a species of *Simulium* has been involved in the transmission of nematodes (genus *Onchocerca*) from farm animals to people (Fallis 1964). Recent studies using molecular methods have shown that even when black flies can feed on a large array of hosts, such hosts are closely related, suggesting some degree of phylogenetic restriction in host usage (Malmqvist et al. 2004; Hellgren et al. 2008). It is important to note that mammophilic black flies can serve as vectors of *Leucocytozoon simondi* and *Leucocytozoon fringillinarum* when feeding on birds (Fallis and Bennett 1962; Desser and Yang 1973), and they can change host preferences based on seasonal availability (Fallis 1964).

Hippoboscid flies seem to be bird specialists, but they are able to parasitize species from a broad phylogenetic composition. For example, some species can be found on birds from different families in Mexico (Ibáñez-Bernal et al. 2015), and there are records for a single fly species, *Ornithomya anchineuria*, being detected in 17 bird species captured in the United States (Davis 1998). In general, bloodsucking

insects are host generalists, which agrees with the low specificity through evolutionary history of haemosporidians with their avian hosts (Ricklefs et al. 2004; Santiago-Alarcon et al. 2014; see Chap. 12). Thus, it is important to acquire as many details as possible on host–vector–parasite interactions at the local scale and among different geographical locations.

6.4.5 *Distribution of Avian Haemosporidian Vectors*

Tropical areas with high avian diversity, such as Madagascar, Brazil, Colombia, and Mexico, have been studied just recently for the identification of haemosporidian vectors (Table 6.1). Schmid et al. (2017) identified five lineages of the genera *Haemoproteus* (2.15% prevalence) and seven lineages from *Plasmodium* (2.87% prevalence) infecting several mosquito species in Madagascar. *Anopheles mascarensis* and three species of the genus *Uranotaenia* had thoraxes positive for *Plasmodium* DNA, sharing the same parasite lineages with more than 10 bird species sampled on the island. This link between parasites, vertebrate, and dipteran hosts increases the confidence to point these as important avian malaria vectors in Madagascar. In Brazil, a study conducted in a seasonally dry tropical forest tested 12 different mosquito species for the presence of haemosporidians. *Mansonia titillans* and *Mansonia pseudotitillans* were positive for avian *Plasmodium* parasites, identifying a novel malaria lineage; *Culex* spp. were positive for *P. gallinaceum*, and *Haemoproteus* parasite lineages were detected in *Psorophora discrucians* (Ferreira et al. 2016). The abundance and composition of mosquito species changed according to habitat type and season; putative vectors were more abundant in pasturelands but were also present at locations with different degrees of ecological succession. In Mexico, a study of the Culicidae assemblage as a function of land-use type showed that the composition of mosquito assemblages was similar across studied conditions, with differences in the abundance and dominance of assemblages as a function of season and land-use type. Most of the dominant mosquito species represented putative avian *Plasmodium* vectors, which also have generalist feeding behavior (Abella-Medrano et al. 2015, 2018). *Plasmodium* DNA was detected in thoraxes and in abdomens from engorged *Culex restuans*, mainly in an urban greenspace (Abella-Medrano et al. 2018). This mosquito species was indicated as a malaria vector in the United States (Carlson et al. 2015) and, therefore, may play a role in *Plasmodium* transmission in tropical areas of North and Central America.

Other studies in the tropics also revealed species that likely constitute competent haemosporidian vectors even though researchers did not evaluate captured females for the presence of sporozoites in the salivary glands. Pools of whole mosquitoes from the species *Aedeomyia squamipennis* and *Culex ocossa* have been consistently detected with a high diversity of *Plasmodium* lineages in Panama (Gager et al. 2008; Loaiza and Miller 2013). The high ornithophily of *A. squamipennis* makes this species likely to be the most important avian malaria vector in Panama, followed by *Cx. ocossa*, which feeds more readily on mammals (Loaiza and Miller 2013). In

Colombia, one pool of thoraxes from *Simulium muiscorum* was positive for *Leucocytozoon* DNA, while one abdomen pool from *Simulium bicoloratum* and one abdomen pool from *Gigantodax misitu* were also positive (Lotta et al. 2016). These black fly species are distributed across Colombian highlands, where *Leucocytozoon* prevalence is high in birds, making them candidate vector species. *Simulium chumpornense* and *Simulium asakoae* presented high positivity rates in Thailand for *Leucocytozoon* (29% and 7%, respectively) (Jumpato et al. 2019). Detected parasites are common in local backyard chickens, making these Simuliidae species candidate vectors, warranting further studies to confirm competence. These results show that there are plenty of opportunities to detect new haemosporidian vectors in the tropics. Efforts should be directed toward experimental infections and morphological demonstration of parasite stages in dipterans, and species consistently positive in PCR-based diagnostic tools could be primary targets for such competence studies.

Leucocytozoon parasites are normally associated with high-altitude environments (González et al. 2014), but this genus was recently detected using molecular tools in birds of the Amazonian rainforest at low prevalence (~ 5%) (Fecchio et al. 2018). Authors suggested that high temperature should be the main environmental factor restricting the common transmission of *Leucocytozoon* in lowland tropical habitats (but see Chap. 1 where it is clear that *Leucocytozoon* parasites are common across Africa). The transmission of *Leucocytozoon* parasites in areas where they are normally absent might be mediated by the presence of alternative insects (e.g., *Culicoides*), whenever their usual Simuliidae vectors are rare or absent (Garnham 1950).

Anthropogenic impacts such as forestry, urbanization, and fragmentation affect the ecology of host–parasite interactions (Santiago-Alarcon and Delgado-V 2017), usually in unexpected ways such as aiding the invasion success of nonnative species (Sehgal 2015; see Chap. 14 for a synthesis on avian haemosporidian research in reference to anthropogenic impacts). In this sense, the successful invasive tiger mosquito (*Aedes albopictus*) might be involved in *Plasmodium* parasite enzootic cycles in its invaded range, and potentially serve as a bridge between avian parasites and humans because it feeds mainly on people but also on birds (Martínez-de la Puente et al. 2015b). Urbanization is one of the major biodiversity threats, and it clearly modifies host–vector–parasite interactions (Hassell et al. 2017). For instance, a study on *Cx. pipiens* clearly showed preferences of some *Cx. pipiens* forms for urban conditions (molestus) compared to the more preserved areas preferred by the form pipiens (Martínez-de la Puente et al. 2016). Despite habitat preferences by some *Cx. pipiens* forms, they showed broad host preferences feeding on birds (21 species) and mammals (10 species, including humans), as well as being infected by parasites of the genera *Plasmodium* and *Haemoproteus* (Martínez-de la Puente et al. 2016). In Mexico, a study on Culicidae at locations with different land-use types found that the most abundant mosquito species (i.e., *Culex restuans*, *Aedes quadrivittatus*, and *Wyeomyia adelpha*) were feeding on humans, domestic animals, and bats adapted to urban conditions. Such mosquito species were also infected by avian *Plasmodium* lineages, mainly in urban and peri-urban areas (Abella-Medrano

et al. 2018). The potential role of Culicidae as a bridge among different animal parasites and humans is even clearer in a study in Hungary, where researchers found that mosquitoes from the genera *Culex*, *Ochlerotatus*, and *Aedes* were positive for mammal heartworms DNA (*Dirofilaria immitis*, *Dirofilaria repens*), mammal filarioid nematodes (*Setaria tundra*), and avian *Plasmodium* (Zittra et al. 2015; see alo Fryxell et al. 2014; Boothe et al. 2015; Ionică et al. 2017). Thus, anthropogenic alterations of natural environments might lead to novel zoonosis (Brearley et al. 2013) that may interact synergistically even between unrelated parasite groups (e.g., West Nile Virus and avian *Plasmodium*, Medeiros et al. 2016; but see Medeiros et al. 2014). Factors associated with urbanization (i.e., highly built cities where urban green space coverage is minimum or no present at all, losing the potential for dilution effects) have been related with an increase in pathogen emergence risk (Hassell et al. 2017).

6.5 Experimental Studies

6.5.1 Behavioral Effects of Haemosporidians on Vectors

Host manipulation by parasites has several ecological and epidemiological implications. In some situations, parasites can change host behavior to enhance their transmission from infected to uninfected hosts (Lefèvre et al. 2009). This is especially important in parasites with trophic transmission, because behavior manipulation can make their intermediate hosts more likely to be predated upon by the definitive host and permit the successful completion of the parasites' lifecycle (Lefèvre and Thomas 2008). In a similar way, many vector-borne pathogens can manipulate phenotypic traits of both vertebrate and invertebrate hosts to increase the chance of transmission. In this chapter, we will focus on behavior manipulation of dipterans that transmit haemosporidians. This manipulation can be indirect, for example, when infected birds influence vector attraction; or direct, when change in behavior is due to parasite development inside the vectors.

6.5.1.1 Indirect Behavior Manipulation of Vectors

Heteroxenous parasites manipulating uninfected vectors to be more attracted to infected vertebrates would increase parasite transmission, and this "parasite manipulation hypothesis" has been supported in malaria parasites. For instance, uninfected mosquitoes can be more attracted to humans with circulating gametocytes (the infective form to vectors) than to noninfected individuals or even when compared to individuals presenting only asexual (noninfective) stages in the bloodstream (Lacroix et al. 2005). Similar patterns were observed by Cornet et al. (2013a) using the avian malaria system with *Culex pipiens quinquefasciatus* and domestic

canaries (*Serinus canaria*) infected with *Plasmodium relictum*. Mosquitoes were more attracted to infected birds only during the chronic phase of infection (24 dpi), and no change in attractiveness was observed during the acute phase. Another study by Cornet et al. (2013b) also revealed that mosquitoes are more attracted by infected birds going through chronic infections, when compared to the uninfected group, with 60% of the mosquitoes taking blood from infected birds.

Experimental studies have found the opposite effect of vector attractiveness to infected birds as well. Lalubin et al. (2012) observed that birds naturally infected with *Plasmodium* spp. attracted less mosquitoes when compared to the uninfected control group. This study applied Y-shaped olfactometers, which allowed mosquitoes to be simultaneously exposed to body odors from both groups in a dual host choice without direct or visual contact with birds. The authors explained their findings as an alternative hypothesis for heteroxenous parasites, the "parasite avoidance." Avoidance to haemosporidians would be beneficial to the vectors as these parasites can pose deleterious fitness effects on blood-feeding dipterans (Valkiūnas and Iezhova 2004; Valkiūnas et al. 2014). These deleterious effects are especially prominent when ookinetes perforate the intestine epithelia of these insects in order to develop into oocysts under the basal lamina (Ferguson and Read 2002). Similar results were found in chickens infected with *Plasmodium gallinaceum* when exposed to *Aedes aegypti* under laboratory conditions (Freier and Friedman 1976). Elegant studies using free-living blue tits (*Cyanistes caeruleus*) showed that more *Culicoides* spp. were captured in nests in which birds infected with *Haemoproteus* were treated with an antimalarial drug, clearing or reducing intensity of infection (Tomás et al. 2008; Martínez-de la Puente et al. 2009).

Gutiérrez-López et al. (2019) found random feeding patterns of mammophilic (*Ochlerotatus caspius*) and ornithophilic (*Culex pipiens*) mosquitoes toward *Plasmodium*-infected and -uninfected birds. Similarly, a study using pairs of noninfected and naturally infected birds moving freely inside the same cage found that mosquito feeding was randomly distributed between groups (Yan et al. 2018). However, these latter authors did find that mosquitoes fed more on birds with higher parasitemia when compared to birds with reduced parasitemia due to antimalarial treatment 7 days after receiving the drug. This result supports the host manipulation hypothesis because higher parasite loads in the vertebrate host may increase the success of parasite transmission. Together, these studies suggest that parasitemia may play a more important role than infection status in vector behavior manipulation, opening opportunities for future studies to approach vector–vertebrate–parasite interactions.

In both hypotheses (host manipulation and parasite avoidance), parasite-induced changes in bird behavior and in the production of volatile compounds would make them more or less attractive to vectors, respectively, and may change transmission dynamics in haemosporidians. Bird anti-mosquito behavior is an important defense mechanism that can reduce blood-feeding success by putative vectors (Darbro and Harrington 2007), suggesting that changes in this natural response would change feeding rates on infected birds. In Cornet et al. (2013a), birds were physically restrained inside the cages to control for behavioral changes between infected and

noninfected groups. In this case, increased mosquito feeding rates in chronically infected birds may be related to changes other than behavior manipulation. Many volatile compounds (i.e., odors) emanating from birds have been demonstrated to attract mosquitoes (Allan et al. 2006; Syed and Leal 2009), so changes in such compounds due to haemosporidian infection is a likely mechanism to influence parasite transmission cycle. Overall, vector attraction toward infected hosts may be due to an interplay between host manipulation and adaptive avoidance.

Behavior defenses such as preening are efficient to kill hippoboscid flies, as demonstrated by experiments using pigeons and the dipteran *Pseudolynchia canariensis*. Infested birds spend twice as more time preening than birds without flies. Moreover, preening birds killed twice as many flies over 1-week intervals than birds with impaired preening, eliminating 40% and 20% of the insects, respectively (Waite et al. 2012b). However, partial elimination of flies infected with *H. columbae* does not prevent pigeons from being infected, as birds with and without impaired preening presented similar parasite prevalence and parasitemia (Waite et al. 2014). Hippoboscidae flies spend between 70% and 90% of their lifetime, which can last up to 86 days, on the host, taking 20–80 minute bouts of blood twice daily (Arcoverde et al. 2009; Waite et al. 2014). This tight relationship between vectors and hosts provides enough chances for parasite transmission even if only a single infective vector survives.

6.5.1.2 Direct Behavior Manipulation of Vectors

Haemosporidians can manipulate vector behavior in a direct way. These parasites can influence host-seeking and feeding behaviors during their development inside mosquitoes, and the type of manipulation can be dependent on parasite life stage (Cator et al. 2012). Mosquitoes harboring only *Plasmodium* oocysts, the "preinfectious females," can be less attracted to hosts (Cator et al. 2013) and less likely to engage on blood feeding (Koella et al. 2002) when compared to uninfected mosquitoes. These same studies showed that sporozoite-infected (infectious) mosquitoes are more attracted and more motivated to probe for a blood meal when compared to oocyst-infected and uninfected females. After landing on a host, preinfectious mosquitoes are less persistent to complete the blood meal when compared to uninfected females. On the other hand, mosquitoes harboring sporozoites in their salivary gland are less likely to give up on feeding when compared to both uninfected and oocyst-infected females (Anderson et al. 1999). Additionally, infectious mosquitoes are more likely to complete a blood meal using more than one host (Koella et al. 1998). These experiments showed that host manipulation would reduce risky activities such as host-seeking and blood feeding when vectors are not yet infectious. The parasite in its transmissible form can manipulate mosquitoes to increase contact rates with vertebrates, which would increase parasite transmission among hosts (Cator et al. 2014).

Using an avian haemosporidian system, Cornet et al. (2013b) found that uninfected and sporozoites-infected mosquitos had similar feeding rates toward infected

birds housed together with an uninfected counterpart. The host manipulation by parasites acting directly on vectors would force infective mosquitoes to feed more frequently on uninfected birds. However, parasite effects on the vertebrate host, as discussed above, would make infected birds more attractive to mosquitoes regardless of infective status in this latter group. In fact, Cornet et al. (2013b) showed that infected birds attracted 60% of the total number of mosquitoes when housed together with uninfected birds. In this system, one explanation is that host choice by infected mosquitoes was driven by the parasite inside the vertebrate host, not by the parasite inside the vector.

A study in the Galapagos archipelago by Levin and Parker (2014) found that *Olfersia spinifera*, a hippoboscid fly, infected with *Haemoproteus iwa* had reduced activity, moving less between frigate birds when compared to noninfected flies. As this study was conducted with free-living birds and vectors, authors could not infer if this change was due to behavior manipulation or because of pathological effects on infected flies. The first hypothesis would suppress parasite transmission, but it cannot be disregarded.

6.5.2 *Detrimental Effects of Haemosporidians on Bloodsucking Dipterans*

6.5.2.1 Effects on Longevity, Fertility, and Survival

A parasite able to increase vector longevity would have more chances to be transmitted to an uninfected host. This can be achieved by suppressing the vector's reproductive fitness (i.e., number of produced eggs) in favor of a longer survivorship via reallocation of resources. Interestingly, most studies found that *Plasmodium* infections usually reduce (Ferguson and Read 2002) or do not affect vector longevity (Lefèvre and Thomas 2008). A relevant exception is the study by Vézilier et al. (2012), in which they showed that *Cx. pipiens* taking blood from *P. relictum*-infected birds survived longer (median of 1.3 days more) than mosquitoes fed on noninfected birds. Additionally, mortality rates after 14 days of blood ingestion were lower for *Plasmodium*-infected mosquitoes when compared to the control group. This study, using a vector–parasite system that naturally occurs in the wild, also showed that infected females produced fewer eggs than noninfected ones, providing a strong evidence of a trade-off between increased survival to the detriment of fecundity. Infected females with longer survival produced fewer eggs, bringing authors to suggest that this effect was due to energy allocation changes between both traits in order to maximize parasite transmission by making mosquitoes living longer. Furthermore, Pigeault et al. (2018) showed that mosquitoes taking one blood meal from *P. relictum*-infected birds with two subsequent blood-feeding cycles on noninfected birds presented reduced fertility when compared to mosquitoes that did not ingest infected blood during three feeding cycles. However, there was no difference in mosquito longevity and survivorship between groups.

Culicoides impunctatus had higher mortality rates after taking blood from birds infected with *Haemoproteus* (*Parahaemoproteus*) spp. when compared to midges feeding on uninfected birds (Valkiūnas and Iezhova 2004). Mortality was especially high between the first and fourth days after feeding, with a maximum of 25% of insects surviving this period in comparison to 80% in the control group. These effects were observed only in midges feeding on birds with high parasitemia (20 parasites per 1000 erythrocytes), since it was absent in the group feeding on birds with one parasite per 1000 erythrocytes (Valkiūnas 2005). Interestingly, high mortality can occur as early as 12 hours post exposure of *C. impunctatus* to *Haemoproteus* (*Parahaemoproteus*) *lanii* (Bukauskaitė et al. 2016). In this latter study, all biting midges were dead by 24 h post exposure.

Species of *H.* (*Parahaemoproteus*) can cause early mortality in mosquitoes as well. Mortality in *Ochlerotatus cantans* peaked between 1- and 3-day postfeeding on infected birds. After this initial period, mosquitoes feeding on birds with high parasitemias (from 3% to 9.3% of infected erythrocytes) had survival rates between 2.2- and 4.4-fold lower than mosquitos feeding on uninfected birds or on a bird with low parasitemia (0.01%) (Valkiūnas et al. 2014). These results reinforce the idea that insect damage is proportional to the number of formed ookinetes, the most common parasite form found at the first days of infection in bloodsucking insects. It is important to bear in mind that *Haemoproteus* parasites develop only up to the oocyst stage in mosquitoes without the formation of infective sporozoites (Valkiūnas et al. 2013; Gutiérrez-López et al. 2016), suggesting that haemosporidians have deleterious effects on bloodsucking insects in abortive infections. Survival of infected bloodsucking insects may be even lower under harsh environmental conditions in the wild (Žiegytė and Valkiūnas 2014). Higher mortality and reduced lifespan of infected mosquitoes, particularly during the adult stage, can have profound implications on human *Plasmodium* transmission (Smith et al. 2012), but it has never been investigated using avian haemosporidians as a model.

Parasites from the *Haemoproteus* subgenus also affect the fitness of hippoboscid flies. Females of *P. canariensis* feeding on pigeons infected with *H. columbae* had higher mortality rates when compared to the control group feeding on uninfected birds (Waite et al. 2012a). In this case, mortality increased only between the fourth and fifth weeks post exposure, showing possible late detrimental effect on vectors. Difference in mortality was not observed in male flies. Females exposed to infected birds had lower reproduction rates when compared to the control group as measured by the number of produced puparia over the course of 5 weeks (Waite et al. 2012a).

Colonies of Simuliidae flies have proved difficult to maintain in laboratory, although insects captured in the wild can survive for long enough to be subjected to experimental tests on fitness. Black flies feeding on ducks infected with *L. smithi* survived less than those feeding on uninfected birds (Davies 1953), suggesting a fitness cost of infection on *Simulium* flies. This study also showed that full-fed flies had higher mortality rates than half-fed flies even when both groups fed on uninfected birds. This difference between full and partial feeding also was observed on flies feeding on infected birds, precluding us from understanding if insect mortality is dependent on the number of ingested *Leucocytozoon* gametocytes.

6.5.2.2 Pathogenicity of Haemosporidian Infections on Bloodsucking Dipterans

Physical damage caused by haemosporidians in the head, thorax, and abdomen of insects after the ingestion of mature haemosporidian gametocytes is one of the major drivers of mortality during the initial postfeeding period (Valkiūnas et al. 2014). Additionally, *Haemoproteus* parasites damage the midgut wall of *Culicoides impunctatus* and migrate through the hemocoel forming parasite clumps in both thorax and abdomen, which likely interrupt hemolymph flow (Bukauskaitė et al. 2016). Ookinete invasion causes cell mortality in the midgut epithelia due to the loss of membrane integrity and due to an increase in programed cell death (apoptosis). This latter response seems to be part of a repair mechanism to restore midgut epithelia integrity, which in turn would promote mosquito survival depending on damage extension (Hurd and Carter 2004). These effects can cause the death of insects before the formation of oocysts and sporozoites, showing that ookinetes may be the most pathogenic haemosporidian developmental stage for dipterans. Tissue damage is proportional to the number of formed ookinetes, which is directly related to the gametocytemia in birds, explaining the high mortality rates of insects ingesting blood from birds with high parasitemias.

Late effects of haemosporidian infection in vectors include reduction in egg production (Gray and Bradley 2006; Vézilier et al. 2012; Pigeault and Villa 2018). These effects can be initiated when the dipteran ingests infected blood meal and during oocyst development on the basal lamina. Parasites trigger apoptotic processes in the ovary of females, causing the reabsorption of follicles, translating into the reduction of egg production (Hurd and Carter 2004; Hurd et al. 2006). One would expect that the potential reduced blood quality from haemosporidian-infected birds would lead to the reduction in egg production; however, a revision by Hurd (2003) provided no support for this effect. In fact, Pigeault and Villa (2018) showed that egg production remained compromised even when mosquitoes ingest blood from healthy birds for two gonotrophic cycles after taking a first blood meal from a *Plasmodium*-infected bird.

In summary, some evidence shows that mosquitoes are more attracted to vertebrates hosting parasites even when provided with a choice to feed on uninfected hosts, but this trend is mixed for avian malaria parasites. Behavior manipulation of insects can also be triggered by haemosporidians developing inside the vector, with some evidence supporting that these changes are dependent on the parasite stage. In general, blood-feeding rates may be determined by the interplay of physiological changes provoked by the parasite during development inside vectors and birds if both hosts are infected. Haemosporidian parasites inflict detrimental effects to bloodsucking dipterans, with a correlation between tissue damage and the number of circulating gametocytes in the blood source. Initial damage caused by ookinetes formed in the midgut can kill these insects within hours. In addition, the reduction in fecundity appears as the main long-term effect reported in the literature.

6.6 Knowledge Gaps and Future Directions

Although the number of studies on vectors of avian haemosporidians has increased during the past decade worldwide, it remains the least studied aspect of haemosporidians' life cycles, particularly in tropical areas. One aspect that stands out from our analyses is the isolation that exists among tropical countries and research groups from other regions in terms of collaborations for haemosporidian vector research (Fig. 6.3a,b), which might be due to the relatively recent formation of most research groups (<10 years). Thus, there is ample room to increase international collaborations among countries with active research. Furthermore, joint research initiatives must be encouraged to strengthen research cooperation between researchers from countries with ample access to research funding and those researchers from tropical areas.

Most experiments using avian haemosporidian vectors were conducted with parasite systems from temperate areas. Birds were, in general, either naturally or experimentally infected with parasites common in Europe and exposed to insects also from this area. Some experiments using parasites and vectors that would not coexist in the wild, like the *P. gallinaceum–Aedes aegypti* system, also helped to build up our knowledge about haemosporidian effects on vector–vertebrate interactions, but we still lack studies on tropical systems. Remarkable exceptions include the works by Levin and Parker (2012, 2014), conducted on the Galapagos archipelago using naturally infected birds and hippoboscid flies, and studies from the Hawaiian archipelago using invasive mosquito species and the only haemosporidian locally transmitted, *P. relictum* (LaPointe et al. 2005). With that, we still need to experimentally explore vector–parasite–vertebrate interactions in the tropics. Tropical areas have the highest haemosporidian (Clark et al. 2014) and Diptera diversity (Rueda 2008; see Chap. 5 for a review of tropical Diptera families relevant to haemosporidian research), providing countless opportunities for studies on avian haemosporidian vectors. Thus, future studies should consider integrating year-round vector sampling, in order to determine insect phenology, diversity, composition, and abundance structure together with haemosporidian detection. Studies should also focus on Ceratopogonidae, Hippoboscidae, and Simuliidae insects in the tropics, since these groups have received little attention when compared to Culicidae. No studies have evaluated haemosporidians in tropical Ceratopogonidae despite the fact that this Diptera family is also captured with traps applied for mosquito research.

Next steps should aim to expand the list of putative vectors in tropical areas. Kim and Tsuda (2012, 2015) demonstrated that mosquito species commonly detected with certain parasites using only molecular methods were later proven to be vectors for the same parasites when using microscopic visualization of viable sporozoites. This reinforces the fact that large-scale studies using only PCR-based techniques are important starting points, indicating insect species to which research focus could be directed to confirm vector competence. Experimental confirmation of vectors is another urgent task in the tropics, as it represents a major impediment for

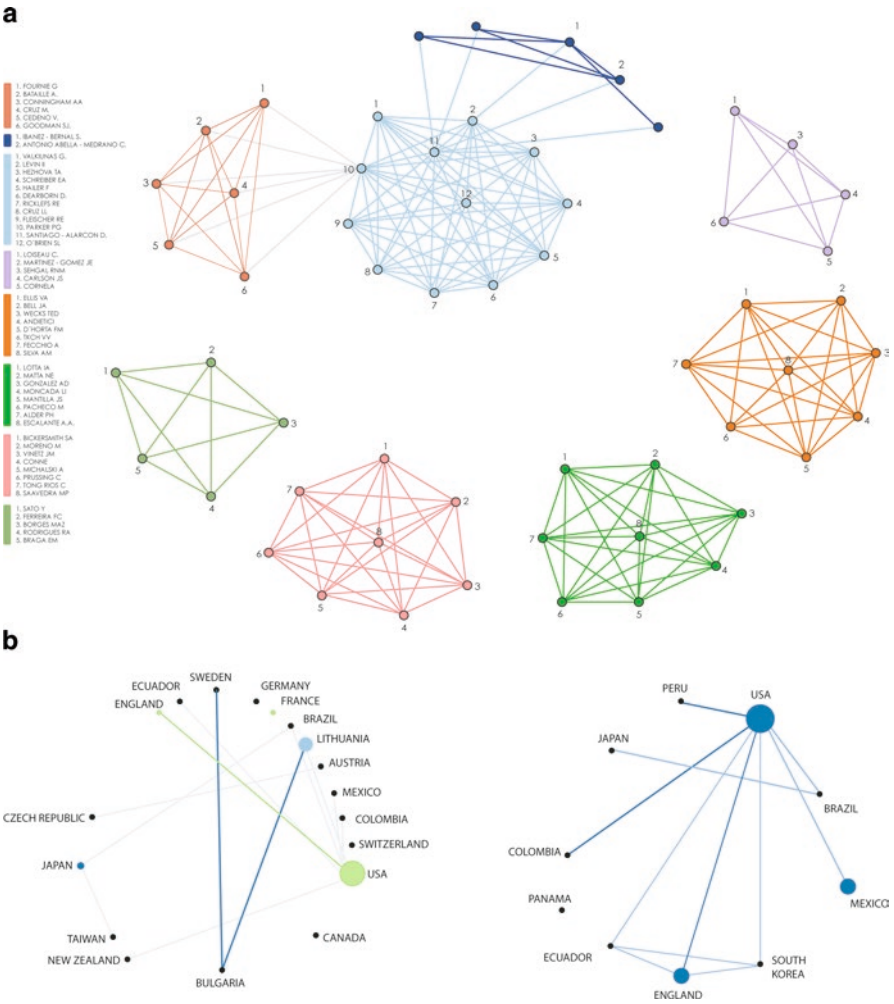

Fig. 6.3 Author collaboration network (**a**) and country collaboration network (**b**) on Diptera research around the world. The country collaboration network (**b**) includes collaborations around the world (left) and collaborations restricted to the Neotropical bioregion (right). Bibliometric analyses are described in Fig. 6.1 legend; here, in order to obtain all results from around the world, we conducted the same search as in Fig. 6.1 but removing the last section of the Boolean search that included terms referring to tropical areas

establishing laboratory colonies and strains of haemosporidians isolated from tropical environments. Therefore, future studies should aim to identify and isolate endemic parasites to determine natural avian haemosporidian vectors in the tropics.

Investigating within insect vector infection dynamics between unrelated parasite groups might lead to the development of vector control strategies; for example, black flies infected with the endosymbiont bacteria *Wolbachia* might interact

positively, negatively, or present no interaction with *Leucocytozoon* parasite infections, where the outcome depends on fly species (Woodford et al. 2018). Furthermore, insect vectors are not passive vessels, among their defense strategies, recent studies have shown that insects' microbiome (bacterial natural communities found in their midguts) strongly determines vectorial capacity for malaria infections. Mosquitoes with an intact microbiome had lower infection rates with *Plasmodium* compared to mosquitoes that were removed of their microbiota, suggesting that symbiont bacteria act as natural immunological barriers against pathogens (Dong et al. 2009).

Recent studies applying next-generation sequencing enabled the assemblage of the first genomes and transcriptomes of avian haemosporidians in their vertebrate hosts (reviewed by Videvall 2019; see Chap. 4 for a summary of molecular research and methods on avian haemosporidians). Recently, *P. relictum* (lineage DONANA05) genome was assembled from oocysts in highly parasitized *Culex* mosquitoes infected under laboratory conditions (Böhme et al. 2018). This approach retrieved better genomic results than contig results of *P. gallinaceum* obtained from bird blood, opening new opportunities to generate genomic data of avian haemosporidians using experimentally infected vectors. The only study so far conducted on bird transcriptional response to haemosporidian parasites revealed variation in gene expression during peak and decreasing parasitemia rates (Videvall et al. 2015). However, no study has tested vector response to avian haemosporidians (Videvall et al. 2019). Similarly, there is no transcriptome expression data from avian haemosporidians during their development inside the vector. Future transcriptomic studies should evaluate gene expression from both vectors and parasites during different developmental stages, particularly at target tissues such as midgut epithelium and salivary glands.

6.7 Conclusions

1. Wildlife parasites of the family Plasmodiidae have been used as model in research of human malaria for over 100 years. On the other hand, parasites of the family Haemoproteidae and Leucocytozoidae have been less studied despite their importance causing severe and even lethal diseases in some bird species.
2. Information about genetic lineages of avian haemosporidians has been rapidly accumulating, but lack of knowledge about their diversity in competent vectors prevents understanding the epizootiology of haemosporidioses.
3. PCR-based detection methods accelerate search of most probable vector species. However, not all PCR-positive samples can be determined as vectors due to possible abortive haemosporidian infections. Finding sporozoites in bloodsucking dipteran salivary glands is still critical for incriminating a specific insect as a vector, but high-throughput sequencing technologies offer promising perspectives to determine avian haemosporidian vectors.
4. Haemosporidian parasites cause negative effects on bloodsucking dipterans, and experimental observations are needed for a better understanding of the damage

caused by haemosporidian parasites in mosquitoes and in other bird-biting insects.

5. Despite the significant number of studies on vectors of avian haemosporidians published during the past decade, most studies have been conducted with parasite systems from temperate areas. Therefore, it is urgent to expand the number of studies on avian haemosporidian vectors in tropical areas.

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Chapter 7
Ecological Niche Modeling and Other Tools for the Study of Avian Malaria Distribution in the Neotropics: A Short Literature Review

David A. Prieto-Torres, Octavio Rojas-Soto, and Andrés Lira-Noriega

Abstract Identifying the mechanisms driving the distribution, diversity, and structure of parasite assemblages is critical to understand host–parasite evolution, community dynamics, and disease transmission risk. However, despite their global distribution, the broad-scale environmental factors that can affect avian haemosporidian transmission remain only partially understood across avian communities in the Neotropics. With the recent technological advances in satellite imagery, computer modeling, and molecular biology, we are now capable of studying infectious diseases in an integrated fashion over diverse spatial scales. From this perspective, ecological niche modeling (ENM) and species distribution modeling (SDM) represent useful tools to study vector-borne diseases, emphasizing the role of environmental factors in constraining their geographic distributions. Herein, we present a review of studies that have implemented modeling approaches, particularly correlative methods commonly used in ENM and SDM, to assess questions of either parasites, vectors, or host species in avian malaria. We identify that most commonly approached topics include the description of geographic distributions (biogeography), population demography, and structure of the host communities (ecology), and in low proportion, other important topics include climate change effects and potential risk for invasions. We observed that most studies were performed from local-to-regional scales and were concentrated mainly on vectors, followed by a combination of parasites and hosts. The correlative algorithm used was mainly Maxent; however, other statistical analyses included spatial regressions, smoothing procedures, and more conventional multivariate regressions developed chiefly on environmental

D. A. Prieto-Torres
Museo de Zoología, Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, Mexico

O. Rojas-Soto
Red de Biología Evolutiva, Laboratorio de Bioclimatología; Instituto de Ecología, A.C, Xalapa, Veracruz, Mexico

A. Lira-Noriega (✉)
CONACyT Research Fellow, Red de Estudios Moleculares Avanzados, Laboratorio de Biogeografía, Instituto de Ecología A.C, Xalapa, Veracruz, Mexico

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dimensions. To date, applications of these approaches to the understanding of the geography and ecology of vector-borne diseases are in early stages. Diverse challenges related to theoretical and empirical advances, as well as the need for more (organized) data, still remain poorly explored. We present an adaptation of the Biotic-Abiotic-Mobility (BAM) framework to describe new potential arrangements in the context of this complex epidemiological/epizootiological systems. We hope this review can be useful to provide the basic knowledge and guidance for modeling of ecological niches on avian haemosporidian systems.

Keywords Climate variability · Ecological niche · Environmental factors · Geographic distribution patterns · Spatial analyses · Vector-borne diseases

7.1 Introduction

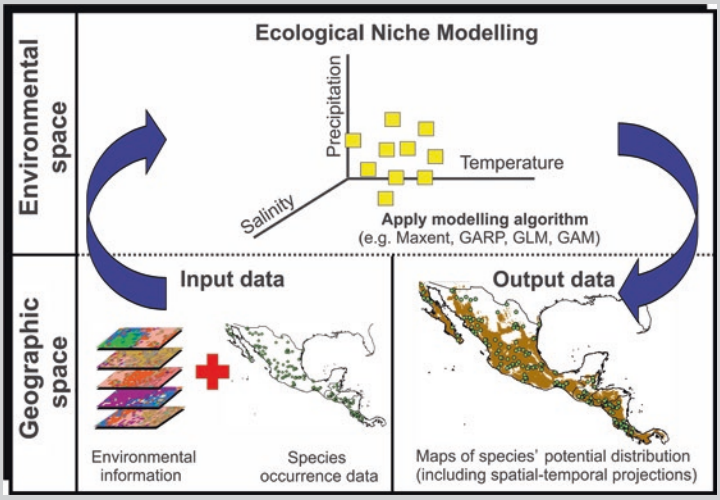
In a rapidly changing world with many newly emerging and geographically expanding pathogens and parasites, we must investigate factors implicated in the distribution of such organisms (Doussang et al. 2019). Infectious diseases are increasingly important, as they contribute to declining populations and mortality events of wildlife species (Jones et al. 2008; Ganser et al. 2016). Changes in climatic patterns will likely further impact in the distribution of disease vectors, increasing their frequency, expanding their geographic distribution and, consequently, affecting the ecological integrity of ecosystems (Atkinson et al. 2014; Fortini et al. 2015, 2017; see Chaps. 6, 10, 11, 13 and 14) or particular species (Fortini et al. 2017). For example, several cases of climate projections estimate a range loss higher than 50% for most species in the absence of effective vector controls, or increased disease resistance (e.g., Fortini et al. 2017). Likewise, previous studies have established a link between the deforestation patterns and the abundance of *Anopheles darlingi*, one of the most important malaria vectors in the Neotropics (e.g., Vittor et al. 2009, Herrera et al. 2012; see Chap. 6 for a review of vector ecology concerning avian haemosporidians of tropical regions). Indeed, there has been in recent years an increased interest in the development of accurate spatial predictions integrating environmental conditions conducive to pathogen proliferation (e.g., Daszak et al. 2000; Woolhouse and Gowtage-Sequeria 2005; Sehgal et al. 2011; Moens and Pérez-Tris 2016; see Chap. 14 for anthropogenic effects on vector-borne parasites). This information is also relevant to understand the evolution and ecology of parasites, as well as to determine hotspots of potential emerging infectious diseases (Daszak et al. 2000).

Despite an accelerated focus on describing host specificity for a multitude of parasites (e.g., Hellgren et al. 2009; Clark et al. 2018; Doña et al. 2018; Park et al. 2018; see Chap. 11), there are few empirical studies accounting for the environmental dependency by considering the host–parasite contact areas or understanding the distribution patterns of vectors and parasites (Canard et al. 2014). Despite the variety of theoretical and methodological approaches that have been recently applied to the analysis of the distribution of diverse disease vectors (e.g., Escobar et al. 2016;

Alkishe et al. 2017; Altamiranda-Saavedra et al. 2017), little information is available regarding the broad-scale environmental factors that can affect (and predict) the distribution and transmission of many vector-borne diseases (Pérez-Tris and Bensch 2005; Sehgal et al. 2011; see also Chap. 9 for an application of macroecology and networks to antagonistic interactions). This certainly seems to be the case for the haemosporidian parasites across avian communities in the Neotropics (Foley et al. 2010a; Galen and Witt 2014).

Ecological niche modeling (ENM) and species distribution modeling (SDM) are useful tools to predict the potential distribution of species (including parasites, vectors, and hosts) based on the relation between environmental variables associated with the sites where the species have been observed. This approach produces suitability maps that allow us to predict spatial predictions about the potential distribution of the target phenomenon or species (Peterson et al. 2011; Peterson 2014), as has been demonstrated in infectious diseases of birds (e.g., Ageep et al. 2009; Doussang et al. 2019). This approach also allows the visualization of how natural landscapes and climatic variables are associated with parasite transmission (Fuller et al. 2012a, 2012b), particularly in largely unsampled regions. Predictive maps that explain the potential distribution of these diseases can be used as early warning surveillance systems and as guides for management decisions (Ganser et al. 2016). On the other hand, the recent technological advances in satellite imagery, computer capacities, and molecular biology for lineage identifications, allow the study of infectious diseases over different spatial scales, by modeling environmental factors associated with vectors, hosts, and parasites (Kitron 1998; Sehgal et al. 2011; Eisen and Eisen 2011; Atkinson et al. 2014; Altamiranda-Saavedra et al. 2017) (Box 7.1).

Box 7.1 The General Diagram About the Implementation of ENM Approach (Modified from Martínez-Meyer 2005)



Both ***ENM*** and ***SDM*** are generated using two types of information (input data): (a) ***occurrence/absence records*** of species to be modeled and (b) descriptive variables that will define the ***species' niche*** in ***"environmental" space (E-space)***, which correspond to those conditions where a species can potentially be distributed in ***"geographic" space (G-space)***. Standard ways to obtain occurrence data is by recording geographic coordinates during fieldwork, bibliographic sources, and/or by retrieving information from digitized collections and open digital gazetteers like the Global Biodiversity Information Facility (***GBIF***). Likewise, the selection of environmental data to include as part of the models requires choosing an adequate number and that these variables are associated with the most important information for the species or natural entity analyzed; these, in turn, should correspond with the objectives of the study. There are mainly three types of variables that are commonly used: climatic and bioclimatic (i.e., variables derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables), topographic-edaphic, and remote sensing-derived variables. Most often, models rely on environmental variables that are more stable in relatively short periods of time and that are not directly modified or affected by the organism being modeled, which are called ***scenopoetic variables***; instead, there are fine resolution and coupled variables to the demographic processes of the organisms being modeled which are known as ***bionomic variables***. These represent two broad kinds of variables that can be used to classify the types of ecological niches being modeled (Peterson et al. 2011).

Once the information on the presences and variables has been defined, the most appropriate modeling technique should be selected. It is important to emphasize that there is no single best ***algorithm*** for all modeling purposes, and that choosing the right one may depend on the configuration of the analysis and type of data (i.e., presence-only, presence-absence, or presence-background information; Qiao et al. 2015). Several types of ***models*** (including statistical approaches) and algorithms can be used to perform ENMs, such as: Generalized Linear Models (GLM), Generalized Additive Models (GAM), Random Forest (RF), Boosted regression trees (BRT), BIOCLIM, GARP, and Maxent, as well as one relatively new approach to obtain consensus models (i.e., ***ensemble prediction***). The selected modeling technique or algorithm will establish a relationship between the presence or absence of information and the range of values of the set of variables where these points are located. This relationship is usually called the adjustment of the model or classification rule, which allows us to define the environmental space where suitability conditions for species could be found.

The final step in the generation of ENM and SDM is the projection of the defined ***suitability*** conditions on geographical space to define the potential ***distribution areas*** on a map. This continuous output can be converted to a

binary prediction after imposing a threshold over the suitability values above and below which it is assumed that suitable conditions exist or not, respectively. Models need to be evaluated statistically and geographically to test whether there is reliability. The process of model testing allows calculating indicators of model performance, such as the percentage of positives and negatives (i.e., "real" absences and presences of species) that are correctly predicted by the models; such values are typically summarized in what is called the ***confusion matrix***. Finally, a particular calibration of an ENM can be used to explore the relative magnitude of environmental variables (commonly known as ***model transferences***) in time (e.g., future climate conditions) and space (e.g., different world regions). This procedure has been very useful for assessing the effects of climate change and invasive risk on species and ecosystems.

Glossary for Box 7.1

- ***Absence records:*** Datasets containing "records" of places where sampling has occurred but the species has not been documented. A locality where a species has been reported as absent, or assumed to be, despite sampling efforts (but note that the species may inhabit these sites, if sampling is present but inadequate).
- ***Algorithm:*** A specific sequence of instructions for solving a problem or developing a task. It usually refers to the software used to calibrate ENMs.
- ***Bionomic variables:*** Variables of fine spatial and temporal resolutions that are typically coupled with the demographic processes of the species or entity being modeled (e.g., species interactions).
- ***Confusion matrix:*** A matrix relating rows summarizing distinct combinations of predicted presence (via a binary prediction) versus absence of a species (from occurrence records of the species, as well as absence, pseudoabsence, or background data), which are commonly used to calculate the omission error rate and commission error rate (including both true and apparent commission error).
- ***Distribution area:*** The geographical space that has been accessible to a species and where abiotic conditions and ecological interactions favor the individuals' presence (with intrinsic growth rate greater than zero) at different scales.
- ***Ecological niche modeling (ENM):*** Estimation of the different niches (fundamental, existing, potential, and occupied), particularly those defined using scenopoetic conditions. In practice, it is carried out via estimation of abiotically suitable conditions from observations of the presence of a species; such models can be used to estimate different distributional areas (the abiotically suitable area, potential distributional area, and occupied distributional area) by stating assumptions about factors in B and M, the latter area being the goal of species distribution modeling (SDM).

- ***Ensemble prediction:*** A consensus prediction of a niche or a distributional area made by combining results of different methods, alternative parameterizations of the same method, or multiple iterations of stochastic methods, to generate a composite value of suitability.
- ***Environmental data:*** Values for environmental variables (generally scenopoetic variables) used in ecological niche modeling. Typically, these variables must be a coincident raster grid for the study region employed in model calibration.
- ***Environmental space (E-space):*** A multi-dimensional space described by environmental variables and defined by "n" dimensional units or their transformations.
- ***GBIF****—the Global Biodiversity Information Facility*—is an international network and research infrastructure funded by the world's governments and aimed at providing anyone, anywhere, open access to data about all types of life on Earth. This includes a database on geographic records for all types of organisms from different sources (including museums, herbaria, and studies, among others).
- ***Geographic space (G-space):*** The space defined by latitude and longitude where environmental conditions and species are found.
- ***Model transferences:*** The application of a model (calibrated in one region) to another place in geography (*G-space*) and/or to another period (e.g., climate change conditions).
- ***Model:*** A simplified representation of some aspects of nature for the purpose of research.
- ***Occurrence record:*** Records of species' presence, especially voucher specimens in natural history museums and herbaria, but also including observational records from visual observations and auditory records (e.g., of birds, amphibians, bats).
- ***Scenopoetic variables (or conditions):*** Variables that are not consumed or affected by individuals of a species, which are typically limiting species distributions and metabolic requirements and are available at coarse resolutions (e.g., temperature and precipitation).
- ***Species distribution modeling (SDM):*** Application of niche theory to questions about real spatial distributions of species, typically in the present and obtained via estimation of the occupied distributional area from occurrence information for a species. It is supported by information of its relationship to environmental characteristics, along with their correlations with dispersal limitation and biotic interactions.
- ***Species niche:*** It is herein defined as the sum of all the environmental factors (including biotic and abiotic) of an "n" dimensional hyperspace acting on the organism distribution.
- ***Suitability:*** The degree to which the environment is appropriate for the species in question.

In the particular case of vector-borne parasites, several authors have suggested that vectors and hosts may promote parasite diversification and permit the coexistence of a larger range of parasite species (Krasnov et al. 2007; Poulin 2011; Clark et al. 2014; see Chaps. 11 and 12 for a thorough discussion on avian haemosporidian diversification). While much recent literature has focused on the spread of invasive vector species (such as *Aedes aegypti*, *A. albopictus*, and *A. atropalpus*), more studies are needed to understand vector–host–parasites distributions along climate gradients (Murray et al. 2015), as well as their relationship among different spatial scales. Although distributions of avian haemosporidian parasites can vary at macro and local scales (Wood et al. 2007; Cosgrove et al. 2008; Doussang et al. 2019), several uncertainties remain related to the role of the environment when vector and host distributions are considered at such scales. For example, analyzing how the environment influences the prevalence and diversity of haemosporidian parasites, including their interaction with hosts and vectors, will help to the understanding and prediction of their distributional and diversity patterns, including community assemblage and disease transmission risks (Pérez-Tris and Bensch 2005; Sehgal et al. 2011; Eisen and Eisen 2011; Fuller et al. 2012a, 2012b; Atkinson et al. 2014; van Hoesel et al. 2019). This information is particularly essential when considering the effect of rapid reduction of native habitats and their conversion to agriculture, livestock, and mining uses (Atkinson et al. 2014; Altamiranda-Saavedra et al. 2017).

A growing body of ENM/SDM studies on human malaria vectors have improved the understanding of the ecology and biogeography of this pathogen system, including the identification of suitable areas and environments (e.g., Foley et al. 2008, 2010b; Lambin et al. 2010; Sinka et al. 2010; Fuller et al. 2012a; Altamiranda-Saavedra et al. 2017). For example, specific data and models might be well suited for understanding the assembly of vector–hosts communities in a particular region, while being limited for generalizing management decisions across taxonomic groups in several regions (Wood et al. 2007; Cosgrove et al. 2008; Doussang et al. 2019). This means that appropriateness of a given dataset and modeling strategy needs to be analyzed based upon the type of question being addressed; therefore, best-practice standards and guidelines should be followed to support the evaluation, policy recommendations, and decisions (see, e.g., Araújo et al. 2019).

In Chaps. 5 and 6, the authors have reviewed current knowledge on the present taxonomic status, life cycle, and ecology of the dipteran vectors associated with avian haemosporidians. Herein, we present a review of studies focused on spatial and environmental questions assessed under correlative ecological approaches, including ENM and SDM and other statistical methodologies. Thus, we provide a general view on avian haemosporidian studies, based on the following questions: (i) *How have different modeling approaches been implemented considering natural landscapes and climatic variables to understand parasite transmission?* (ii) *Which are the best-practice standards in ENM and SDM approaches?* and (iii) *What are current challenges and the future opportunities in modeling avian haemosporidians?* From the reviewed literature, we observed a poor knowledge related to theoretical and empirical advances, as well as the need for more (organized) data. Additionally, we present an adjustment of the Biotic-Abiotic-Mobility (BAM)

framework (see Soberón and Peterson 2005) to describe an alternative potential arrangement within this framework, based on this complex epidemiological system.

7.2 Historical Implementation of ENM and SDM Approaches in Avian Malaria Studies

To analyze the current state of knowledge of ENM for these vector-borne pathogens, we performed a review of research articles on avian malaria. Literature search criteria included the keywords "avian malaria AND biogeography", "avian malaria AND ecological niche model*", "avian malaria AND species distribution model*", "avian malaria AND Neotropics", "modeling/modelling avian haemosporidians" including some of the cited references within articles found based on these keywords. We found 59 articles published between 2006 and early 2019. Next, we compiled all the information from these articles in a table including the following information: (a) year; (b) entity of study (i.e., parasites, hosts, vectors, and combinations of them); (c) geographic scale (i.e., local, national, regional, global), region and/or country; (d) theme addressed: biogeography and distribution, evolution, climate change, invasion risk, and ecology (e.g., community structure, habitat requirements, prevalence, dispersal, host range, host–parasite interaction, niche breadth); (e) algorithms (e.g., Maxent, GARP, GLM, GAM, GLMM); and (f) environmental variables used.

From our compilation of studies, we observed that research on avian malaria using ENM/SDM and other statistical methodologies has shown an increase in the last decade, where most contributions (54.2%) were published during the last 6 years (2013–early 2019). However, in comparison with studies related to other vector-borne diseases (e.g., human malaria, dengue, and chagas), avian malaria and related genera have not received much attention, probably because avian malaria is not a human pathogen that can currently represent a potential emerging infectious disease.

The studied entities or focal units of study (i.e., vector, host, and parasite) varied in each case (Fig. 7.1). Most studies (45.8%) focused on vectors, followed by a combination of parasite and hosts (32.2%), and few were focused exclusively on the parasite (5.1%). Even though our search was focused on cases of Neotropical avian haemosporidians, it turned out that other regions are better studied. For example, studies in countries from Asia encompass 30.7% of cases, followed by North America (20.3%, highlighting that half of those were focused exclusively in Hawaiian birds), Europe and Africa (both cases with 14.0% of studies). Studies focused in Neotropical countries (i.e., from Mexico to Argentina and Brazil, including the Caribbean islands) represented 17.4%, while only 3.5% of studies were performed in countries from Oceania. On the other hand, we observed that most studies (32.3%) were performed at local scales, followed by regional (23.7%) and national (22%) perspectives. The continental and worldwide levels of analysis represented only 15.3% and 6.7%, respectively (Fig. 7.1). This is quite relevant because

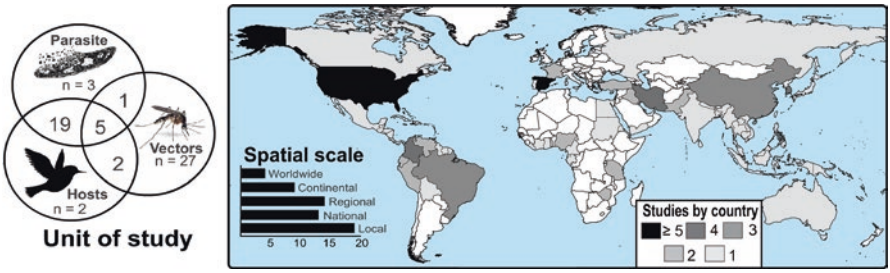


Fig. 7.1 Number of avian malaria studies implementing statistical and ecological niche modeling approaches. Herein, we characterized the proportion of cases for each unit of study analyzed, the studies by countries, and the geographical scale used

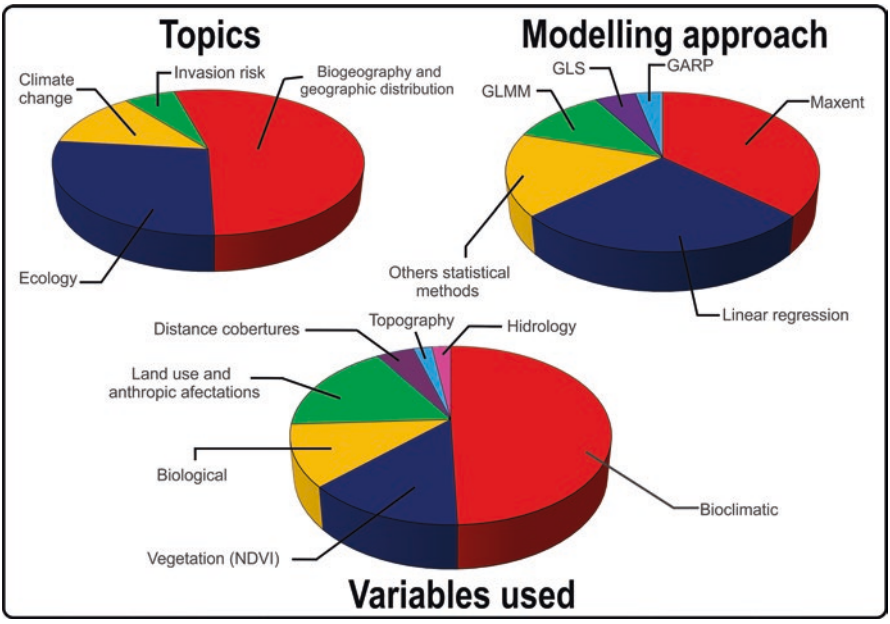


Fig. 7.2 General description for the 59 avian malaria studies implementing ecological niche modeling approaches analyzed herein, indicating the topic or focus of analysis, the modeling approach, and variables considered

different conclusions emerge from analyzing the transmission or prevalence of avian haemosporidians as scale changes (see Sect. 7.3).

The range of topics being covered varied greatly (Fig. 7.2). Most articles were centered around questions touching on some aspects of biogeography and geographic distribution (55.8% of articles), followed by studies on ecology (27.4%), climate change (12.6%), and invasion risk (4.2%). Despite the importance of each one of these topics, several articles were multidisciplinary in nature and their

approach combines more than one of these topics. The most frequent combination of topics and questions were those of biogeography and geographic distributions and ecology (28.9% of cases), followed by studies including the current geographic distribution and potential effects of climate change (15.3%) (Fig. 7.2).

Regarding different modeling approaches implemented by studies, 66.7% used correlative methods, while the rest used other statistical approaches such as ModelBuilderTM or Boosted Regression Trees. For those works implementing correlative methods, 56.4% used Maxent (Phillips et al. 2006) as a tool to perform ENMs, followed by other types of statistical approaches, mainly linear models (Fig. 7.2). Finally, in terms of the environmental variables used to model either some entities (i.e., parasites, vectors, hosts) or process (e.g., levels of anthropic impacts), bioclimatic layers were the most frequently used (49.5%), followed by vegetation-related variables, such as vegetation and Normalized Difference Vegetation Index [NDVI] (13.5%), land use, and anthropic information such as human population size or livestock (16.8%). Other biological variables were used such as the host presence information (11.2%), distance layers (e.g., distance to rivers or roads; 4.4%), topographic (2.3%), and hydrology (2.3%) (Fig. 7.2). Aside from climatic variables, most studies used a combination of climate-related variables with others such as elevation and vegetation information.

7.3 Implementing Best-Practice Standards in ENM/SDM for Avian Haemosporidian Studies: A Study Case with Neotropical Human Malaria

Despite the growing body of ENM/SDM literature, and the recent demand for their use in avian haemosporidian studies, no generally agreed-upon standards for best practices yet exist for guiding the building and evaluating the adequacy of these models. Thus, to provide a general perspective about the best-practice standards applicable to a variety of available data and modeling approaches, we show such a framework with detailed guidelines for scoring key aspects of the ENM/SDM approach used in avian haemosporidian studies. For this, we analyzed the published study by Altamiranda-Saavedra et al. (2017) about the "*Potential distribution of mosquito vector species in a primary malaria endemic region of Colombia*" to illustrate the implementation of ENM in this chapter. Although recommendations and best-practice standards for models in biodiversity assessments exist, it is important to recognize that the criteria for judging the data and models will differ according to the particular objectives (Schwartz et al. 2012; Araújo et al. 2019). Therefore, standards showed herein do not aim to govern or guide publishing of research on ENM and/or SDM in general, but rather focus on the applicability of these methods for avian haemosporidians assessments.

Altamiranda-Saavedra et al. (2017) applied ENM methods in order to estimate the potential distribution of three endemic human malaria vector species in northern

Colombia: *Anopheles nuneztovari*, *An. albimanus*, and *An. darlingi*. In addition, authors applied a niche overlap assessing hypotheses of niche similarity among the three vector species. The authors concentrated on evaluating the hypothesis that environmental heterogeneity is a driver for allopatric distributions of possible competing niche-related species (see Altamiranda-Saavedra et al. 2017 for a more detailed explanation), arguing that the dispersion rates and their ability to occupy diverse environmental situations may facilitate sympatry among the species of mosquitoes across environmental and geographic contexts (e.g., Laporta et al. 2011, 2015). Therefore, results may be useful for the design of malaria species-specific vector control interventions optimized for this important malaria region, especially considering the limited resources available for regular monitoring of vector species, vector-borne diseases, and control in a country like Colombia. In fact, maps based on vectors to predict the distribution of vector-borne diseases have been frequently used at broad spatial scales, with relatively fine-scale environmental factors to predict transmission dynamics of pathogens across the landscape (Pérez-Tris and Bensch 2005; Khatchikian et al. 2011).

In terms of the modeling development (Fig. 7.3), the first step was to generate predictor variables that are important in defining species' distribution, as well as the compilation of vectors' occurrence data. For the characterization of environmental variables, they used NDVI index obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) Terra satellite, from 2012 to 2014 and 16-day temporal resolution. The decision to use these variables to characterize the environmental variation and predict the more suitable environments for the vectors across the study region was based on the idea that spatial and temporal dynamics of vegetation could influence indirectly the mosquito reproduction and development (see Lourenco et al. 2011). For the occurrences, authors conducted sampling of vectors in or near human residences between December 2012 and March 2015, and the identification of collected vectors was performed using a morphological key and/or by PCR-RFLP-ITS2 and COI barcoding. They obtained a total of 40 localities of Urabaá – Bajo Cauca and Alto Sinuá region that were used to perform the ENM. It is important to clarify that there may be alternatives to retrieve occurrence information, such as records already available through the GBIF (https://www.gbif.org/) or VectorMap (http://vectormap.si.edu/). However, the use of alternative sources may be restricted by the availability and the quality of the information (Newbold 2010), which for cases such as malaria is scarcer than for other vector-borne human diseases.

It is important to note that authors discarded the use of alternative environmental information, such as bioclimatic variables from the WorldClim project (Hijmans et al. 2005; www.worldclim.org/) or topographic features from HYDRO1k project(USGS 2001) owing to the coarse spatial resolution available (approximately 1km^2). Nevertheless, the authors specified that NDVI should properly reflect rainfall as part of the vegetation photosynthetic processes. This shows that the selection of environmental variables is an important step. In all, 69 NDVI images were used. Procedures for ENM using the large set of environmental variables have been discussed extensively, including the fact that there may exist correlations among climate variables (e.g., Graham 2003; Peterson et al. 2011). In order to reduce

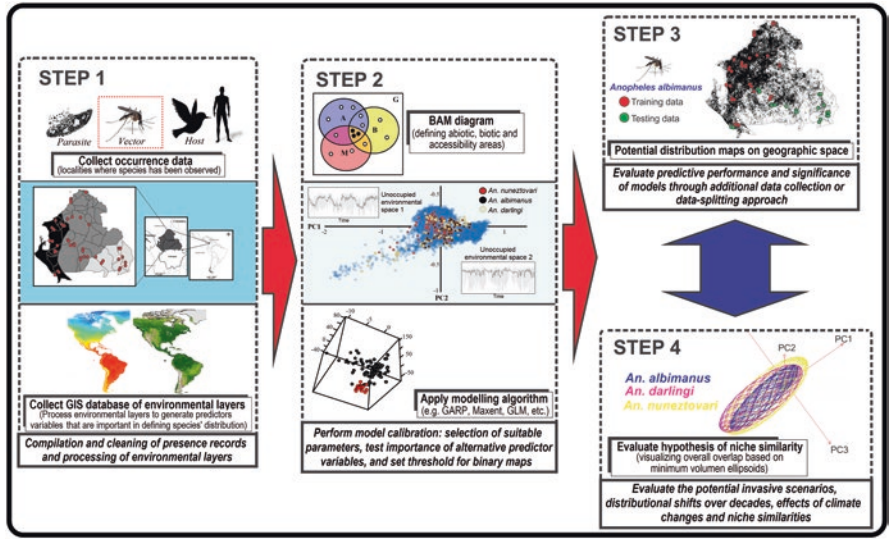


Fig. 7.3 Summary of steps and challenges in the ecological niche modeling process implemented by Altamiranda-Saavedra et al. (2017): estimation of potential distribution and test of niche similarity among three endemic human malaria vector species in northern Colombia. See text for a detailed explanation

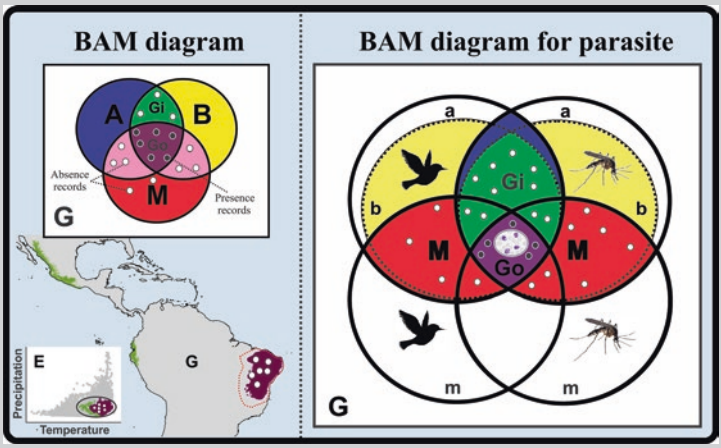
correlation among data layers, a principal components analysis (PCA) was performed using all images as variables. In this sense, model calibration and performance (steps 2 and 3 in Fig. 7.3) were tested for different combinations of principal components (PCs), considering only the first 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 components. Here, although authors did not discuss this point, it is important to consider that the use of PCA scores as variables in ENM represents an approach (which is not exempt from discussion) that facilitates the reduction of multicollinearity and model overfitting (Peterson et al. 2011). However, alternative methods could include the use of Pearson's correlation coefficient, selecting only those with none or low correlation (e.g., $r < 0.8$), as well as the jackknife test of variable importance performed by Maxent, used frequently to identify those variables with important individual effects (Wu 1986; Elith et al. 2011).

Having collated occurrence records and environmental variables, the next step was to calibrate the models (step 2 in Fig. 7.3) to characterize the species' ecological niche as a function of the environmental variables. This was performed using Maxent (Phillips et al. 2006), which estimates a target probability distribution by finding the probability distribution of maximum entropy (i.e., that which is most spread out, or closest to uniform), subject to a set of constraints that represent the incomplete nature of information about the target distribution. Detailed explanations for the proper implementation and interpretation of Maxent can be found elsewhere (e.g., Elith et al. 2011), and other algorithmic approaches exist that could have been used for this modeling problem, such as the Genetic Algorithm for Ruleset Production (GARP; Stockwell 1999) and BIOCLIM (Booth et al. 2014).

A key step during the modeling process is the definition of a region for model calibration, which is particularly relevant in algorithms like Maxent where the environmental background will highly impact the results (Barve et al. 2011). The model calibration region should include a relevant area in a biogeographic context for the species being modeled. In the example, the authors specified that based on the known distribution of the vector species across Colombia and considering the entire studied endemic region for human malaria, they would set the polygon for this country as hypothesis of the accessible area (or **M** *sensu* BAM framework; Soberón and Peterson 2005; Barve et al. 2011) for the three vector species. Nevertheless, in most of the ENM literature, it is frequently observed the use of a geographical mask based on the intersection of occurrence records with Terrestrial Ecoregions (Olson et al. 2001) or the Biogeographical Provinces (Morrone 2014) to define the areas for model calibration. Such consideration is based on the assumption that these regions may define the historical accessible area for each species in geographic space. Of interest and contrary to Altamiranda-Saavedra et al.'s work, we did not observe that the analyzed studies of avian malaria using ENM/SDM applied this hypothesis of the accessible area (**M**) in their models, which is opposite to following a good modeling approach practice, especially when the exercise is conducted at large geographic scales. Here, we propose an adaptation of the BAM framework that considers host traits as abiotic and biotic dimensions for avian haemosporidians (Box 7.2).

In the study by Altamiranda-Saavedra et al. (2017), models were calibrated for each species, with 10 bootstrapped replicates each and the median across replicates

Box 7.2 How Can the Biotic-Abiotic-Mobility Framework Be Incorporated in the Exploration of Avian Haemosporidians Distribution?



Initial discussions and models to represent the distribution of a species in space and then calculate the niches based on the environments were provided by Pulliam (2000) and Soberón and Peterson (2005). These last authors published a framework (known as "***BAM diagram***") describing the simultaneous influence of environmental abiotic conditions (or "***A***"), biotic interactions (or "***B***"), and dispersal (or "**M**") in shaping species' geographic distributions. In general terms, the set "A" represents regions in ***geographic space*** (or "***G***") where ***scenopoetic conditions*** (and existing resources) allow species' intrinsic growth rates to be positive; while set "B" represents those geographic regions where the interacting factors (mainly ***biotic interactions*** with other species) are favorable for the presence of the species. The third set, "M" (relating to movements of individuals of the species), corresponds to the ***accessibility areas*** to the species within a given time span. The intersections of these three factors produce two components defining the "***potential distributional area***" (Gaston 2003) of the species: the "***occupied distributional area***" (***Go***; where the species is present [see occurrence records] representing a proxy of the species' ***realized niche***) and the "***invadable distributional area***" (***Gi***; where the species is absent despite the favorable conditions).

Nevertheless, it is important to highlight that delineation of "M" should be based on biological characteristics of the species under analysis and on the sampling available for that species. Each species and each geographic situation requires a more customized parameterization. Thus, the configuration of the BAM diagram for the situation under consideration and the relation of elements of the BAM diagram in environmental space become critically important. From this perspective, and considering the complex epidemiological

system analyzed herein, we propose some considerations to adjust the BAM diagram for the study and definition of avian haemosporidian cases. These mainly consist in carefully interpreting the roles that vectors and hosts could represent for the abiotic, biotic and mobility sets, which have crucial theoretical and methodological implications while modeling avian haemosporidians.

As shown in the BAM diagram for the parasite case, *Go* depends on the biotic, abiotic, and mobility factors for both vectors and hosts (represented with white circles and lowercase letters; that is, "b" is the Biotic (B) component considering the vertebrate host and the Diptera vector, which are at the same time embedded in the abiotic (A) environment as represented by "a"). The dotted line representing "b" is smaller than "a" given that avian haemosporidians are not free-living organisms; thus, their biotic environment is restricted to the vertebrate and Diptera hosts, and consequently, the abiotic component "a" has an indirect effect on parasite occurrence via its hosts. Traditional ENM applications consider the B component to have negligent effects (the Eltonian noise hypothesis [Araújo et al. 2014]) when modeling species' geographic distributions under the BAM framework. However, we argue that biotic interactions play a critical role in parasitic relationships in nature, so they should be considered with caution in disease ecology (Johnson et al. 2019). This is important because the congruence or amount of overlap among commonly shared factors between vectors and hosts is 1) critical at each stage of the parasite's life cycle, its distribution, and transmission (see Rúa et al. 2005; Fuller et al. 2012b) and 2) easily affected by changes in scale.

Evidently, within A and M for both vectors and hosts, there is only a subset of areas where haemosporidian parasites could present positive intrinsic growth rates. Nevertheless, there will be areas that avian haemosporidians are not able to occupy because of present distributional constraints that cannot be overcome (e.g., elevation gradient that affects the life cycle, absence of vector or/and host). Barve et al. (2011) pointed out the crucial role of assumptions regarding M in niche modeling. These authors showed that models calibrated under different assumptions about M arrived at markedly different results, that the outcomes of model evaluations depended dramatically on which version of M was used; furthermore, the conclusions from model comparisons (Warren et al. 2008) were also dependent on assumptions regarding M. Thus, the modeling exercise for avian haemosporidians will depend on carefully thinking about the scale at which vectors and hosts are distributed, and on how abiotic, biotic, and mobility in each of these can determine the presence of the parasite.

Glossary for Box 7.2:

- ***Accessibility areas (M)*:** The biogeographic regions that individuals from a species have been capable of "testing" environmentally speaking; such regions are typically molded by factors that impede dispersal (movement) by individuals of a species (e.g., mountain chains or rivers).

- ***BAM diagram*:** A Venn diagram that displays the joint fulfillment in geographic space (G-space) of three sets of conditions that together determine a species' distribution: B, for biotic conditions; A, for abiotic conditions; and M, for movement of the species.
- ***Biotic interactions (B):*** Interactions between and among species—for example, competition, mutualism, and predation.
- ***Fundamental niche (FN):*** The set of all environmental states that permit a species to exist. Herein, we distinguish Eltonian fundamental niches from Grinnellian fundamental niches. The latter is the set of scenopoetic (non-interacting and non-linked) conditions that the species can tolerate.
- ***Invadable distributional area (Gi):*** Corresponds to those areas in the geographic space that the species could occupy if current distributional constraints were to be overcome.
- ***Occupied distributional area (Go):*** Those areas where the subset of the accessible region in which both scenopoetic and biotic conditions permit the species to maintain populations, and is synonymous with the "realized range" of Gaston (2003).
- ***Potential distributional area:*** The union of the occupied distributional area and invadable distributional area for a species—that is, the regions where the abiotic and biotic conditions are suitable. (Note that much of literature uses potential distribution in a different way, however, as a synonym of what we term the abiotically suitable area).
- ***Realized niche (RN):*** The set of all environmental states that would permit a species to exist in the presence of competitors or other negatively interacting species and restrictive factors.

was used as a basis for further analysis. No clamping or extrapolation options were disabled and the remaining parameters (i.e., regularization multiplier, prevalence, and features) were left as default. However, it is important to note that the calibration phase of models is critical; thus, more recent applications (such as ENMval and kuenm R packages) are exploring these parameter values in considerable detail obtaining the best models based on significance, performance, and simplicity (Muscarella et al. 2014; Cobos et al. 2019). In a first approach, to explore the robustness and predictive capabilities of the data (step 3 in Fig. 7.3), the models were generated using 50% of the locality records as training data (i.e., to calibrate the models), while the rest of data were used as testing points (i.e., for internal model evaluation). However, the final species' models were performed using all available data. In this sense, the algorithm used localities of species records and environmental conditions to perform a certain number of iterations (500 in this case) before reaching a convergence limit. The logistic output produces a map of habitat suitability, ranging from 0 (unsuitable) to 1 (perfectly adequate; Phillips et al. 2006; Phillips and Dubik 2008). All maps were converted to binary via a conservative least presence thresholding approach (i.e., "Minimum Training Presence"),

consisting of the lowest predicted value corresponding to any occurrence record of the species in the calibration dataset. It is important to note that there is no rule to set these thresholds, because its selection depends on the quality of the data used, and will vary from species to species. Detailed explanations for the proper implementation and interpretation of thresholds options in ENM could be consulted in Peterson et al. (2011) and Liu et al. (2013).

Before model predictions can be interpreted or used for any application, the predictive performance and significance need to be evaluated (step 3 in Fig. 7.3). A test using the receiver operating characteristic (ROC) curve is implemented by default in Maxent where the area under the curve (AUC) is measured with values that range from 0 to 1. However, due to the diverse critics to this test (see Lobo et al. 2008; Peterson et al. 2008 for a detailed explanation), Peterson et al. (2008) proposed the use of a modification of this test named as partial ROC. This method gives greater weight to omission errors (i.e., a false negative) and measures model performance using AUC ratios with values ranging from zero to two, where values above one indicate that models performed better than a random model ratio (AUC ratios >1.0). Bootstrap resampling was performed with 1000 iterations and with replacement of 50% of the original data points. In addition, omission rates were used as criteria to select optimal models for each species based on the evaluation of statistical significance when compared with null expectations, which was achieved by resampling 50% of the points. The partial-area ROC tests were performed using 50% of the unique occurrence data points for independent model evaluation (i.e., testing).

Finally, authors evaluated a hypothesis of niche similarity (step 4 in Fig. 7.3) among the three mosquito species following three approaches: (a) inspecting the loading values of each raw variable (16-day composite NDVI) on each of the first two principal components, and how they related to monthly rainfall averages in the study area; (b) using background similarity tests by overlaying predictions using the Schoener's D metric, with values ranging from 0 (no overlap) to 1 (complete overlap) (see Warren et al. 2008); and (c) visualizing overall overlap based on minimum volume ellipsoids for the species in three PCA dimensions considering the Jaccard index as a numerical estimation of environmental overlap among species (see Qiao et al. 2016, 2017). These analyses allowed to obtain a better characterization of how vegetation dynamics contained in NDVI related to suitability for each species, and, at the same time, a better understanding of the dispersal capacity of these species and their ability to colonize different ecosystems across many environmental and geographic contexts.

7.4 What Are Current Challenges and the Future Opportunities in Modelling Avian Haemosporidians?

The implementation of modeling approaches in studies of limiting factors and prediction of distribution of avian haemosporidians, including the association with hosts and vectors, has seen increasing number of applications during the last years.

These recent studies have been conducted to answer multiple kinds of questions, mostly to characterize current distributions and the potential spread of disease, at multiple scales across several regions and ecosystems worldwide, mostly in North America, Eurasia, and several countries of South America. This is probably a consequence of the broad applicability that ENMs possess to understand ecological requirements of species, aspects of their biogeography, predict geographic distributions, identify areas for potential risk, select areas for conservation, and forecast effects of environmental change, among others (Peterson et al. 2011; Araújo et al. 2019).

From our review, we identify six major challenges in successfully modeling of avian haemosporidians that are quite relevant for adequately assessing vector-borne parasites. The first is the proper taxonomic identification of parasites, vectors, and hosts. This is crucial not only to identify the entity being modeled (see Peterson et al. 2011), but also to be able to understand correctly the ecological and evolutionary associations and trends in the interactions among hosts, parasites, and vectors. This is more challenging perhaps for the parasite, followed by the vectors and probably less problematic for vertebrate hosts. Some studies have shown the advantage of using molecular biology techniques for this purpose (e.g., Altamiranda-Saavedra et al. 2017; see Chaps. 2 and 4 for the case of avian haemosporidians); however, they depend on having good databases derived from type specimens (e.g., COI barcodes), something that is mostly unrealistic for tropical areas particularly for vectors of nonhuman pathogens.

The second challenge is to have precise and complete information on occurrence databases (see Newbold 2010). A few efforts have been made on this aspect, mostly on the vectors (e.g., Foley et al. 2010a), but clearly there are also huge gaps on the parasites and hosts. Even if databases on birds are probably the most comprehensive among vertebrates worldwide, with highly accurate data, it is not enough to disentangle the potential distribution of avian haemosporidians. Researchers should avoid the temptation to pile occurrence data and environmental data into a niche modeling algorithm, press the button, and see what comes out (see Anderson 2015). Rather, occurrence data must be assembled carefully and comprehensively, and biases, uncertainties, and temporal characteristics must be pondered. Once the input data are assembled, and the models calibrated appropriately, outputs become considerably more rigorous.

Third and fourth challenges are the variables, and the scale and resolution that such variables better fit for the questions being asked. On this base are the conclusions and generalizations that can be made. Interestingly, the scale of analyses on which ENMs have been applied most commonly based on our review is local-to-regional, followed by larger scale analysis highlighting the broad applicability of these modeling techniques to look at the relationship between occurrence records and environmental characteristics at different scales (Overgaard et al. 2003; Foley et al. 2010b; Sinka et al. 2010; Fuller et al. 2012a; Altamiranda-Saavedra et al. 2017). This is probably because many studies aim at explaining avian malaria and its correlation with some environmental factors, which is commonly at local scales, where highest-quality data are typically available for either vectors, parasites or

hosts. From this perspective, it is important to note that variables directly affecting a species' physiology are preferred since their relationships with its geographic distribution are assumed to be stable across spatiotemporal scales (Foley et al. 2010b; Sinka et al. 2010; Fuller et al. 2012a; Anderson 2017). For instance, the slope and aspect of surface and the availability of water can be associated with anopheline habitats and their breeding sites in dry environments at local scales (Ageep et al. 2009; Fuller et al. 2012b). Recent studies showed that temperature, precipitation, and elevation can explain much of the variation in the distribution of *An. albimanus* in Latin America and the Caribbean (Sinka et al. 2010; Fuller et al. 2012b). This aspect is currently seeing fast advances with the incorporation of remote sensing information (Zellweger et al. 2019). As was observed in several of the publications, incorporation of high-resolution environmental surrogates, such as NDVI layers, appears to be crucial for analyzing vector-borne diseases like malaria (Foley et al. 2010b; Laporta et al. 2011; Cornuault et al. 2013a, 2013b; Ricklefs 2013; Altamiranda-Saavedra et al. 2017; Hundessa et al. 2018a, 2018b). Similarly, changes in land use and vegetation cover can also facilitate (or prevent) the spread of haemosporidian vectors (Patz et al. 2004; Vittor et al. 2009; Stresman 2010; Fecchio et al. 2018, 2019). According to Peterson (2014), ideal models of disease transmission should be based on remotely sensed datasets (e.g., Renner et al. 2016 who used laser ranging technology or LiDAR), rather than on climate data due to the lack of sufficient detail to provide genuinely helpful information in health applications (see Pérez-Rodríguez et al. 2013). Under some circumstances, no alternatives are available, but satellite imagery is invariably richer in genuine information that is measured on real-world landscapes, rather than interpolated from frighteningly sparse weather station-based data.

Another important complication in the case of avian haemosporidians is that even if we have an idea on what environmental conditions favor the transmission of the disease, we lack knowledge on the influence of several environmental factors on host communities that determine the prevalence of the parasite. In fact, the assemblage of a host or vector community does not guarantee a good prediction of parasite prevalence. Due to the complexity of the avian haemosporidian life cycles, it is difficult to draw an easy modeling framework, and even the reasoning and configuration of the BAM diagram framework (Soberón and Peterson 2005, see Box 7.2) can be challenging, because the factors within each set of conditions in B, A or M may change depending on the unit being modeled and the scale of the study. It is even further complicated because the interactions among avian haemosporidians, hosts, and vectors remain poorly understood (see Chaps. 6, 10, 11, 14, 15, and 16). Such interaction processes may be even more complex if we think about the general processes governing host specificity, in which case, we should assess both ecological and phylogenetic relationships of potential host species, in efforts to identify barriers to host range expansions (Poulin and Mouillot 2005; Hoberg and Brooks 2008; Clark et al. 2014, 2018; see Chap. 11 for an in-depth synthesis of avian haemosporidian specialization and dispersal). It seems possible to assume that this dynamic interplay may be influenced by the geography and evolutionary history of the landscape, where vector–host–parasite interactions take place (Ricklefs et al.

2004, 2014; Rivero and Gandon 2018). Since biotic interactions lie at the core of disease systems, neglecting interacting species and their role in parasite dynamics (maintenance, reproduction, and transmission) may lead to failure to forecast disease distributions (see Johnson et al. 2019). Parasite transmission is strongly influenced by interactions among infected and susceptible hosts, which can be altered by host behavior and demography (Peterson 2014; Johnson et al. 2019).

The statistical exploration of local environmental conditions linked to avian haemosporidians can be the starting point to select environmental predictors at other scales (i.e., results from local scale studies can be used to inform and parameterize coarse scale studies). For instance, globally, *Haemoproteus* exhibits greater lineage diversity than *Plasmodium*; but this pattern differs in South America, where a higher avian host diversity coupled with low *Plasmodium*-host specificity leads to greater lineage diversity of *Plasmodium* than *Haemoproteus* (Clark et al. 2014). However, the actual mechanism of diversification (see Chap. 12) and the broad-scale environmental factors that can affect their transmission remains only partially understood (Balls et al. 2004; Foley et al. 2010a, b; Lachish et al. 2011a, b). Opportunities exist for gaining a more comprehensive understanding of the interactions between environmental change and vector potential invasion, using different types of space-time models that can simulate environmental change or species distributions (e.g., Peterson 2009; Chaves and Koenraadt 2010).

Historical studies about ecological requirements of species and the forecasting of distribution of vector-borne disease have mainly been used at local spatial scales with relatively fine-scale environmental factors (Khatchikian et al. 2011; Ganser et al. 2016). Tools used for these analyses include spatial regressions, smoothing procedures, and more conventional multivariate regressions, all developed in "environmental" dimensions. For instance, ENM analyses of anopheline species (subgenus *Nyssorhynchus*) in Amazonian Brazil revealed diversification in habitat use: *An. triannulatus* is a generalist, whereas *An. oryzalimentes* and *An. janconnae* are specialists (Mckeon et al. 2013). ENMs were also used to predict distributions of *An. bellator*, *An. cruzii*, and *An. marajoara* of the Riviera Valley in southern Brazil, which revealed specific associations with land cover types (Altamiranda-Saavedra et al. 2017). Finally, low tolerance to dry environments was documented for *An. darlingi*; projected climate change would significantly reduce its suitable habitat mainly in Amazonian biomes, influencing both its distribution and abundance, in contrast to species of the *albitarsis* complex (Laporta et al. 2015).

Another challenge remains on the lack of a clear hypothesis about the areas that have been accessible (i.e., **M** in the BAM framework; Soberón and Peterson 2005) to the species (or entity) being modeled. This problem is not particular of avian haemosporidians, but rather an overall challenge during modeling ecological niches. However, defining the right accessibility area for model calibration in avian haemosporidians, given that it comprehends a series of interactions between hosts-parasites-vectors, complicates things. As mentioned in Box 7.2, this area is quite important because it indicates what the relevant environmental background is, and because it has huge influence on the performance of several modeling algorithms and on the significance of the model (Barve et al. 2011; Owens et al. 2013). The

accessible area in the case of avian haemosporidians may change as the entity being modeled changes (i.e., parasite, host, and/or vector; Box 7.2). For example, if we focus on the parasite, this implies that its accessibility area must be restricted to some part of the accessible area of the host and some part of the accessible area of the vector. However, this accessible area may also change with the scale of analysis (Lira-Noriega et al. 2013). It is not the same to concentrate our modeling efforts at a particular landscape, as opposed to over a continental region; in the first case, most of the landscape can be assumed as accessible to either the vector or the host, but that may not be the case at the continental level. However, the definition of this accessible area will be crucial for the right interpretation of the model.

7.4.1 Future Opportunities and Directions

The literature is full of examples of research on outbreaks of a given disease, in which the relative risk of infection is assessed for a series of potential risk factors (Daszak et al. 2000; Woolhouse and Gowtage-Sequeria 2005; Sehgal et al. 2011; Peterson 2014; Escobar et al. 2016; Alkishe et al. 2017; Altamiranda-Saavedra et al. 2017). With the ecological and geographic perspectives explored in this chapter, a broader viewpoint should be possible. This perspective might be more than simply an examination of which environmental factors are important for the proper modeling of species' niches and distributions. More in-depth studies might assess environmental correlates of key vector species' distributional ecology, including calculation of which factors are included (or excluded) in the geographical areas from the model's development (Peterson 2014).

Several additional steps remain to be explored in order to create better predictive maps of haemosporidians distributional patterns and transmission risk. We emphasize three crucial ones; although in all instances, good examples exist of what to do and what not to do, best practices are not always possible, feasible, or easy. First, wildlife-disease exploration requires the development of specific functionalities. One germane application is related to "time-specific" ecological niche models, which could begin to capture the essence of the temporal dynamics of species' distributions including parasite-vectors and potential hosts (Pérez-Rodríguez et al. 2014). For these cases, occurrence data should be characterized in latitude, longitude, and time, and the occurrences would be related to environmental datasets that are similarly specific in time to produce models for a particular point in time. However, it is important to note that a major bottleneck and challenge for this field is precisely the availability of high-quality occurrence data for vector species and avian haemosporidians—unlike for the case of human malaria (Foley et al. 2010a). Likewise, these models could then, in theory, be projected to other time periods to anticipate temporal dynamics of species' distributions. Initial explorations have been developed successfully (e.g., Peterson 2009, 2014; Tonnang et al. 2010; Pérez-Rodríguez et al. 2014; Alimi et al. 2015), but considerable additional exploration is needed.

Second, the niche specialization for a multitude of organisms is not fixed, but it is predicted to vary in response to environmental heterogeneity (Fecchio et al. 2018, 2019). A growing body of anecdotal and theoretical evidence suggests that parasites are not the exception (Hoberg and Brooks 2008; Agosta et al. 2010; Araujo et al. 2015). However, the actual mechanism of diversification and the broad-scale environmental factors that can affect their transmission remains only partially understood (Balls et al. 2004; Lachish et al. 2011a, b; Pérez-Rodríguez et al. 2014). In this sense, studies focused on the effects of climate change on avian haemosporidians, which would not be subject to the confounding patterns of human movement and economics (e.g., Gwitira et al. 2015; Ren et al. 2015; Chahad-Ehlers et al. 2018), would greatly contribute to our understanding of the impacts of changing ecological conditions on natural disease systems (Patz et al. 2004, 2008; Béguin et al. 2011; Mendenhall et al. 2013; Ren et al. 2015). It is a priority to identify which are the variables that determine and constrain distributions of disease vectors and host species, especially considering that risk of *Plasmodium* and *Haemoproteus* infection in birds is expected to increase with increasing temperatures on a global scale (Garamszegi 2011).

Finally, phylogenetic analyses are needed to reconstruct the evolutionary pathways of certain species (see Chaps. 3 and 12), and to assess whether or not current suspected hosts/reservoirs will expand in future scenarios, and whether this will result in transmission expansion (e.g., Ishtiaq et al. 2009; Svensson-Coelho and Ricklefs 2011; Mata et al. 2015). This last fact is very important considering that these changes in distribution may also affect the complex and dynamic networks of biotic interactions (Garamszegi et al. 2007; see Chap. 9). For instance, it will be relevant to analyze whether areas of high parasite prevalence are indicators of an increased abundance of vectors, increased transmission capacity, or decreased host resistance/immunity (Galen and Witt 2014; Pérez-Rodríguez et al. 2014; Zélé et al. 2014; Illera et al. 2017; Martínez et al. 2018; Pulgarín-R et al. 2018). The unresolved question that remains is whether, and to what extent, the characteristics of the landscape affect the prevalence of parasites transmitted by vectors, either directly or indirectly through the effects on hosts and/or vectors (Santiago-Alarcon et al. 2012; see Chaps. 9, 10, 13 and 14).

7.5 Conclusion

One of the major concerns is that most of the vector-borne diseases are associated with tropical environments. However, and despite that distribution limits of many haemosporidian vectors and parasites are associated with climatic conditions of temperature and precipitation, it is noteworthy that there is a poor representation of studies on avian haemosporidians in the tropics. Several studies have shown that climate variation influences the reproduction rates of parasites and the development of vectors and hosts, which in turn could affect the transmission of parasites and the exposure of parasites to new host species. Thus, the incorporation of diverse

methodologies and practical considerations, such as ENM and SDM, is needed to address the diversity of questions and challenges in disease-related topics. As our literature review showed, there is an imbalance on studies addressing aspects of avian malaria, especially those including ENM and/or SDM approaches because most of them are focused only on geographic distribution patterns. Other important issues that remain poorly explored are those describing the environmental relationships at different scales (in time and space), niche shift and specialization, as well as interactions among parasites, vectors, and hosts.

Although ENM/SDM approaches to the challenge of understanding the geography and ecology of disease transmission (including avian haemosporidians) could be considered in an early stage (Peterson et al. 2011; Peterson 2014; Johnson et al. 2019), several efforts show that niche modeling has a lot to offer to the field of both public and wildlife health and epidemiology. Typical spatial applications include mapping geographic patterns of disease transmission risk, identification of risk factors (spatially or not), and assessment of populations at risk of infection. However, ENM and SDM do not capture the full complexity of the phenomenon of disease transmission because they are fitted in purely geographic dimensions, and as such, the approach unravels complex ecological and distributional phenomena into broad spatial trends.

The ideas presented in this chapter are simply examples of a complex reality. In no case is a clear and detailed analysis available that crosses all the relevant scales and resolutions. Rather, the reader is left with tidbits and suggestive indications. As highlighted, most important to the authors are the fine delimitation of a BAM diagram in which hypotheses of sets of factors affecting the distribution of haemosporidians are established, including issues of scale. It is to be hoped that, as this field develops further, more and better examples will emerge. Overall, we hope that this review and conceptual essay can be useful to provide the basic knowledge and guidance for modeling of ecological niches of avian haemosporidian systems.

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Chapter 8
Island Biogeography of Avian Haemosporidians in the Neotropical Region

Juan E. Martínez-Gómez and Noemí Matías-Ferrer

Abstract Islands have provided numerous examples of unique biological patterns and also have been fundamental in the development of the theory of island biogeography and the identification of putative processes to explain the presence and numbers of species on islands. The equilibrium model explaining species richness on islands has been at the center of this theory. We conducted exploratory analyses of avian blood parasites (Haemosporida) in three Neotropical insular regions (West Indies, Galapagos, and the Revillagigedo Archipelago), aiming to contribute to the development of the theory of island biogeography for this group of parasites. To explore and synthesize current knowledge, we compiled published mtDNA cyt b sequences and geographical information. We discarded short sequences with less than 300 bp and those that did not coincide with the MalAvi reference sequences. We generated two matrices, the first with sequences of the genus *Haemoproteus* (n = 366, 346 bp) and the second with *Plasmodium* (n = 228, 443 bp) sequences, including sequences from the continental Americas as reference. We used lineages representing unique sequence haplotypes with at least one different base pair. We found a positive nonsignificant trend between the number of lineages and island size when including all islands in the study; it was significant only for the *Haemoproteus* genus in the West Indies. Taxon cycles are recognizable patterns resulting from processes by which taxa adapt to local and specialized conditions leading to restricted distribution and specialization of older taxa. Although *Haemoproteus* and *Plasmodium* in the West Indies showed a trend consistent with a taxon cycle-older lineages in a reduced number of islands — general linear model analyses were not significant. The small island effect (SIE) must be revised to understand what factors allow islands, such as Montserrat, Nevis or Socorro, to host disproportionately more lineages than expected from their size. The opposite pattern, where larger islands such as Santa Cruz and Fernandina host fewer lineages than expected also deserves study. Microorganisms, such as haemosporidian parasites, require comprehensive sampling on islands in a wider geographical range.

J. E. Martínez-Gómez (✉) · N. Matías-Ferrer
Red de Interacciones Multitróficas, Instituto de Ecología, A. C., Xalapa, Veracruz, Mexico

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8.1 Introduction

Islands have provided naturalists with unique scenarios to understand the evolution and ecology of species. Both Darwin (1859) and Wallace (1881) observed that species on islands were similar to the nearby mainland, that islands hosted a lower number of species but a higher proportion of endemics, that some taxa were absent from many islands, that dispersal was the main force to colonize oceanic islands, that the ocean was an effective barrier and that local speciation played an important role on islands. Darwin (1859) provided examples of species-area relationships by which islands have a lower number of species than continental areas of comparable size. Wallace (1881) considered that extinction processes, in addition to colonization and *in situ* speciation, were important in determining how many species live on islands; he underlined the need to generate comprehensive data sets for regions under study. Organisms on islands also develop peculiar changes such as gigantism, dwarfism, obsolescent legs and wings, and color changes, among others (Carlquist 1965). The study of island communities has also provided valuable information for the development of the theory of island biogeography by identifying putative processes to explain the presence and numbers of species on islands, such as assembly rules, ecological displacement, ecological release, and adaptive radiation (e.g., Carlquist 1974; Cody and Diamond 1975; Losos and Ricklefs 2009; Whittaker et al. 2017).

The equilibrium model, advanced to explain species richness on islands, has been of paramount importance in the development of the theory of island biogeography (Mac Arthur and Wilson 1963, 1967; Simberloff 1974). Two intuitive assumptions are behind its formulation: (1) more species are expected on islands as their surface areas increase and (2) fewer species are expected as distance to the continent increases. Incorporating immigration and extinction rates within a mathematical framework allows one to predict the number of species that should occupy an island of any given size and distance from the continent (Mac Arthur and Wilson 1963, 1967). In addition, the resulting dynamical equilibrium defining the number of species also implies niche saturation (earlier developments of the model can be found in Brown and Lomolino 1989, 1998). However, the model's appealing simplicity did not produce ample field verification. Since its inception, criticisms were put forward regarding its poor goodness of fit, and underlying assumptions or predictions (e.g., Simberloff and Wilson 1969; Simberloff 1976; Gilbert 1980; Boecklen and Gotelli 1984). As a consequence, this model has been considered a poor but, nevertheless useful theory (Simberloff 1976); the attempt to provide a mechanistic explanation for the organization of island communities constitutes its main virtue (Sauer 1969; Boecklen and Gotelli 1984; Wu and Vankat 1995).

Despite its limitations, the equilibrium model has persisted, allowing for revisions that stimulate the development of a new theoretical body. New concepts have appeared to explain biogeographical phenomena on islands (Walter 2004). For instance, historical losses caused by humans have been taken into consideration to explain present species numbers (Olson 1990), the inclusion of metapopulation dynamics allowed comparisons of species immigration–extinction dynamics (Hanski 1998; Morrison 2002), the addition of prey availability into classical formulas improved the assessment of trophic interactions (Gravel et al. 2011), the use of local plots to island-wide sampling yielded information on the role played by different scales (Karger et al. 2014), allometry afforded the inclusion of functional traits in the analyses (Jacquet et al. 2016), among other amendments. The equilibrium model of island biogeography has been extended to arbuscular mycorrhizae, bringing it to the realm of microorganisms (Glassman et al. 2017). Pathogen species richness was also observed to increase with island surface area and to decrease with distance to the mainland as expected from the basic assumptions of the equilibrium model (Jean et al. 2016). Nonetheless, Lossos and Schluter (2000) found that islands greater than 3000 km^2 no longer fit the equilibrium model. Instead, very large islands show patterns of species richness and diversity similar to continental regions.

A global analysis of cyt b sequences suggested that haemosporidia show diversity patterns like those of their hosts (Clark et al. 2014); thus, the equilibrium model could also be applied to blood parasites on islands. Reperant (2010) discussed some considerations when applying the equilibrium model to vector-borne diseases. She proposed that host demography, including population size, behavior, sex and sex ratio (e.g. Levin and Parker 2014), breeding system (e.g. Fecchio et al. 2013) may play a role comparable to island size and suggests their incorporation. The work with microorganisms adds an additional level of complexity that must consider reticulating phylogenies (Lomolino and Brown 2009) and approaches that employ analyses of genetic lineages instead of recognized taxonomic species (Crisp et al. 2011). Parasite lineages are associated to a certain number of islands and hosts (Fallon et al. 2003), thus suggesting a limited ability to move between hosts and locations, and an upper limit to the number of host species that can be infected. Parasite lineage turnover observed in Puerto Rico and St. Lucia indicates periodic colonization and extinction events (Fallon et al 2004). Ricklefs et al. (2011) found that parasite lineages in the Lesser Antilles are not associated to a particular host or a level of endemism, but that lineage richness was rather favored by the presence of migratory birds. *Plasmodium* lineages appear to be more generalist than *Haemoproteus* lineages (Beadell et al. 2009). The study of haemosporidia is of utmost importance because of their potentially detrimental impacts on island avifaunas (Warner 1968).

The exploration of species-area relationships in island groups allows the evaluation of two patterns associated with the classical equilibrium model. First, the small island effect (SIE) is a pattern where small islands have more species than expected from theory (Triantis and Sfenthourakis 2012). Under this scenario, small islands would host more species than the number expected from species-area regressions. Second, when genetic data are available for species living on island groups, it would

be possible to test for taxon cycles. Taxon cycles should result in a recognizable pattern where older taxa show a reduced geographic range as a result from adaptation to local and specialized conditions. On the other hand, younger lineages should occur widely (Ricklefs and Cox 1972; Ricklefs and Bermingham 2002). Alternatively, taxon cycles in haemosporidia should become evident if the number of host species function as a proxy for island size (as suggested by Reperant 2010). In this scenario, older lineages would be restricted to fewer hosts, or hosts with small populations. Loiseau et al. (2017) tested for the equilibrium model of island biogeography using lineage richness and explored the possibility of taxon cycles in islands of the Gulf of Guinea. They found support for the two basic assumptions of the equilibrium model mentioned above. Athough they did not incorporate time in their analyses, they found a high proportion of lineages exclusive to certain islands and fewer generalist lineages, a pattern suggestive of taxon cycles in avian blood parasites.

We conducted exploratory analyses of lineages of avian Haemosporida of the genera *Haemoproteus* and *Plasmodium* in three Neotropical insular regions. We described lineage richness, diversity, distribution, and affinities to adjacent continental areas. We applied generalized linear models (GLMs) using a Poisson model with a log link function to test for the traditional equilibrium model of island biogeography and checked for the small island effect (SIE) in the resulting models. In addition, we explored for taxon cycles through GLMs using genetic distance to the tree root, a proxy of lineage age, against the number of islands or avian hosts present.

8.2 Geographical Scenario

The Neotropical insular systems considered in this chapter are the West Indies in the Atlantic/Caribbean, the Galapagos Islands, and the Revillagigedo Archipelago in the Eastern Pacific Ocean (Fig. 8.1). A brief description of each system is presented below.

8.2.1 West Indies

The West Indies are comprised of more than 1000 islands created by andesitic vulcanism that includes the Bahamas, the Greater Antilles, and the Lesser Antilles (Woods and Sergile 2001). The Bahamas are islands of low elevation in the northern portion of the system; the Greater Antilles are continental fragments to the west of the Central American isthmus; the Lesser Antilles are islands in a volcanic arch in the Caribbean (Ricklefs and Bermingham 2008). The Bahamas platform, geologically very different to the Antilles, has maintained its relative position with North America since the Cenozoic (Mullins and Lynts 1977). The Greater Antilles are the oldest islands and probably emerged approximately 48 mya in the middle

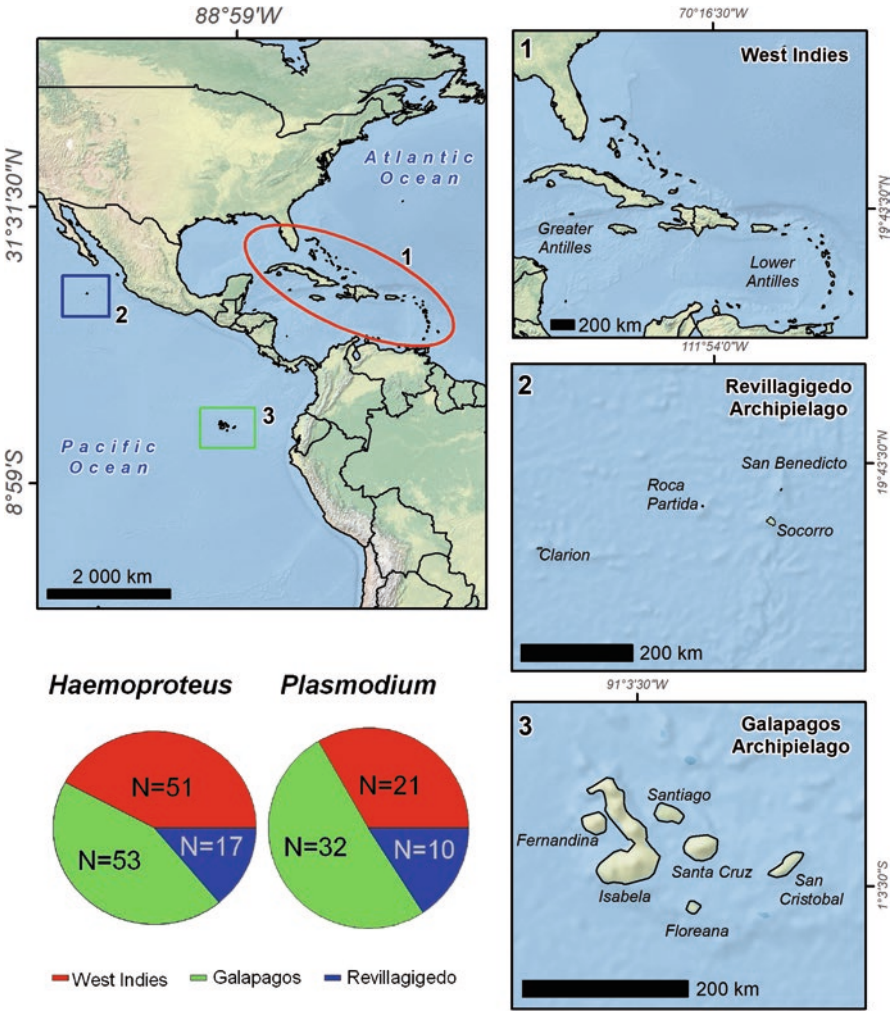


Fig. 8.1 Location of the West Indies, Galapagos, and the Revillagigedo Archipelago and proportional records of mitochondrial sequences (cyt b) of *Haemoproteus* and *Plasmodium* genera from each insular region. Some sequences may be from the same lineages

Eocene (Iturralde-Vinent and MacPhee 1999). The Lesser Antilles probably emerged 20 mya and have never had contact with the continent. With the exception of Antigua–Barbuda, St. Kitts–Nevis–St. Barthélemy, and Grenada–Grenadines that are united by shallow banks, the Lesser Antilles have never been united among themselves (Ricklefs and Bermingham 2008). At least 730 bird species have been recorded in the West Indies, of which 171 (23%) are endemic (Gerbracht and Levesque 2019); Planst species recorded in this region amount to 12,280 species of which 7446 (60%) are endemic (Acevedo-Rodríguez and Strong 2012).

8.2.2 Galapagos

The Galapagos Islands are found in the Eastern Pacific Ocean, about 1000 km from the coast of Ecuador. The archipelago has 45 named islands (Mueller-Dombois and Fosberg 1998). Of the 13 major islands and many rocky islets, Isabela is the largest and tallest island (4588 km^2 and 1707 m respectively; Wiggins and Porter 1971; Schatz 1998). The southeastern islands are the oldest, and those in the west such as Isabela and Fernandina are less than 1 million years and maintain a significant volcanic activity. The Galapagos were never connected to the continent (Simkin 1984). Climate is not tropical but has strong seasonality (Parker 2018). The warm and wet season occurs from January to June with April as the warmest moth; the cool and dry season occurs from July to December with september as the coolest moth: surface water is practically lacking; the archipelago is located in the region of the 500 mm isohyet of precipitation (Wiggins and Porter 1971; Mueller-Dombois and Fosberg 1998). At least 178 bird species have been recorded in Galapagos, of which 56 (31%) are endemic (Jiménez-Uzcátegui et al. 2012); of 1547 plant taxa recorded for the islands 245 (16%) are endemics (Jaramillo Díaz et al. 2017). Cold and upwelling ocean currents create an inversion layer creating arid conditions on the lower parts of the islands (Schatz 1998). Human presence occurred during the last 200 years and currently San Cristobal, Santa Cruz, Isabela, and Floreana are inhabited (Parker 2018).

8.2.3 Revillagigedo Archipelago

The Revillagigedo Archipelago has four islands: Socorro, Clarion, San Benedicto, and Roca Partida. They are located south of the Baja California Peninsula, Mexico, in the Pacific Ocean (Brattstrom 1990). These islands arose from the Mathematician's Ridge that has been active from 6.5 to 3.15 mya (Mammerickx et al. 1988) and are close to the Rivera and Cocos plates (Silver and Valette-Silver 1987). Socorro Island is the emerged portion of a large volcanic shield, that raised from the seabed at 3 km depth to its summit during the Pleistocene (Bryan 1966; Brattstrom 1990; Paoletti et al. 2016). Socorro Island is the largest and tallest island (132 km^2 and 1050 m respectively; Mueller-Dombois and Fosberg 1998; SEMARNAT-CONANP 2004); it hosts the largest number of plant and animal species in the archipelago. The Revillagigedo Archipelago hosts at least 135 bird species of which 16 (12%) are endemic (SEMARNAT-CONANP 2004) and 164 plant species, of which 38 (23%) are endemic (Levin and Moran 1989). Vegetation is xerophytic ranging from herbs and low scrub to forests on Socorro Island; surface water is lacking (Mueller-Dombois and Fosberg 1998). Its climate is arid and seasonal, with a marked hurricane season from June to November. Human settlements are recent (late 1950s) and they are restricted to naval facilities on Socorro and Clarion islands (Brattstrom 1990). Precipitation reaches 761 mm (Mueller-Dombois and Fosberg 1998). For our analyses, data were drawn solely from Socorro Island.

8.3 Haemosporidian Mitochondrial Lineage Diversity

The recognized number of existing taxonomic species represents an underestimate of extant genetic diversity of Haemosporida species, especially considering that many cryptic species have been found using genomic data (Bensch et al. 2009; see Chap. 4 for a review on molecular methods and advances on the genetic studies of avian haemosporidians). Our knowledge of genetic diversity in the neotropical insular systems under exploration is limited by the number and scope of available investigations. The West Indies have been the focus of several studies on the distribution, dispersal, abundance, and evolution of Haemosporida (Tables 8.1 and 8.2). Many mitochondrial lineages (cyt b) and species of avian parasites of the genera *Haemoproteus* and *Plasmodium* have been identified for this region. For the Galapagos and Revillagigedo archipelagos, studies were primarily oriented towards conservation and taxonomic issues (Levin et al. 2009, 2011; Santiago-Alarcon et al. 2010, 2014; Valkiūnas et al. 2010; Carlson et al. 2011, 2013; a thorough summary of work conducted on Galapagos during the last 20 years can be found in Parker 2018). Work on blood parasites of migratory birds has enriched sequence data banks by providing useful geographic and taxonomic connections between continental and insular systems in the Neotropics (e.g., Ricklefs et al. 2016, 2017).

To explore and synthesize current knowledge, we compiled mtDNA cyt b sequences and geographical information in published reports. Instead of taxonomic species, we used mitochondrial lineages of *Haemoproteus* and *Plasmodium* genera, noting the islands and host location. Additionally, sequences of haemosporidia from the continental Americas were also included to detect lineages shared between the islands and the mainland. Previous studies indicate that haemosporidia do not disperse easily across continents and many of them are confined to one host or suit of hosts (Valkiūnas 2005; Hellgren et al. 2007; Ricklefs et al. 2017; Pulgarín-R et al. 2018); thus, lineages from other geographic regions were not used.

We obtained a matrix of 532 sequences of *Haemoproteus* and 350 sequences of *Plasmodium,* of which 101 and 35 respectively, are haemosporidian lineages of the MalAvi database. After the initial alignment for each genus, we discarded several short sequences (less than 300 bp) and sequences not belonging to the cyt b region comnprised in the MalAvi database (Bensch et al. 2000, 2009). The fragment used in the analyses is a partial region of cyt b of 479 bp that allowed the recovery of the majority of *Haemoproteus* and *Plasmodium* haplotypes (Bensch et al. 2009). Many available sequences are incomplete; consequently, to avoid discarding a significant number of these sequences, we decided to use a shorter fragment of cyt b included within the 479 bp of MalAvi. As a result, the *Haemoproteus* matrix consisted of 365 sequences with a length of 397 bp, whereas the *Plasmodium* data matrix consisted of 229 sequences of 447 bp in length. To explore phylogenetic relationships of lineages, we performed a Maximum Likelihood analysis in PhyML 3.0 (Guindon et al. 2010), with a nucleotide substitution model GTR + I + G and 1000 bootstrap replicates for both *Haemoproteus* and *Plasmodium*, using *Leucocytozoon fringillinarium* as outgroup. In reference to lineages recognized in GenBank and MalAvi, we identified a total of 56 mitochondrial lineages of *Haemoproteus* and 35 of *Plasmodium* (Tables 8.1 and 8.2; Figs. 8.2 and 8.3).

Table 8.1 Summary of *Haemoproteus* (H) and *Parahaemoproteus* (P) mitochondrial lineages (cyt b)

ID		Lineage	Species	Archipelago	Other geographic region[a]	Genbank Access Number	References
H1	H	FREMIN01	*H. iwa*	GAL/WI	GAL/USA (HI)/BLZ/ PAN	JF833043, JF833044, JF833045, JF833048, JF833049, JF833050, JF833058	Levin et al. (2011), Levin and Parker (2013), Bastien et al. (2014), Santiago-Alarcon et al. (2014)
H2	H	CREFUR01	*H. jenniae*	GAL	ECU	JF833065, JF833065	Levin et al. (2011), Bastien et al. (2014)
H3	H	DR09DR25	*H. multipigmentatus*	WI		GQ395636	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H4	H	GDE13W	*H. multipigmentatus*	GAL		FJ462653, GU296221, JF833054	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)
H5	H	GDMA20W	*H. multipigmentatus*	GAL		GU296217, JF833056	Valkiūnas et al. (2010), Levin et al. (2011)
H6	H	GDSF7	*H. multipigmentatus*	GAL		FJ462672	Santiago-Alarcon et al. (2010)
H7	H	GDSF9	*H. multipigmentatus*	GAL		FJ462675, FJ462671	Santiago-Alarcon et al. (2010)
H8	H	GDSF9W	*H. multipigmentatus*	GAL	ECU	FJ462652, FJ462677, GU296219, GU296222, JF833051, JF833055, JF833066	Santiago-Alarcon et al. (2010, 2014), Valkiūnas et al. (2010), Levin et al. (2011)
H9	H	MICRO01	*H. multipigmentatus*	GAL	MEX/ECU/ GUA	FJ462651, FJ462659, FJ462660, FJ462678, FJ462679, FJ462680, GU296223, JF833059, MV_H_MICRO01	Santiago-Alarcon et al. (2010, 2014), Valkiūnas et al. (2010), Levin et al. (2011)
H10	H	MICRO02	*H. multipigmentatus*	GAL		GU296210, MV_H_MICRO02	Valkiūnas et al. (2010)
H11	H	SocH1	*H. multipigmentatus*	REV		JN788932, JN788945	Carlson et al. (2013)
H12	H	SocH11	*H. multipigmentatus*	REV		JN788942	Carlson et al. (2013)
H13	H	SocH12	*H. multipigmentatus*	REV		JN788943	Carlson et al. (2013)
H14	H	SocH13	*H. multipigmentatus*	REV		JN788944	Carlson et al. (2013)

H15	H	SocH16	*H. multipigmentatus*	REV		JN788947	Carlson et al. (2013)
H16	H	SocH17	*H. multipigmentatus*	REV		JN788948	Carlson et al. (2013)
H17	H	SocH18	*H. multipigmentatus*	REV		JN788949, JN788950	Carlson et al. (2013)
H18	H	SocH2	*H. multipigmentatus*	REV		JN788933	Carlson et al. (2013)
H19	H	SocH5	*H. multipigmentatus*	REV		JN788936, JN788946	Carlson et al. (2013)
H20	H	SocH6	*H. multipigmentatus*	REV		JN788937	Carlson et al. (2013)
H21	H	ZArDR491	*H. multipigmentatus*	WI	VEN	FJ462650, FJ462668	Santiago-Alarcon et al. (2010, 2014)
H22	H	ZEGAL05	*H. multipigmentatus*	WI/GAL	GUA/MEX	FJ462676, GU296211, GU296213, GU296214, GU296215, GU296216, GU296226, JF833042, JF833057, MV_H_ZEGAL05	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)
H23	H	ZEGAL06	*H. multipigmentatus*	GAL		FJ462674, GU296218, GU296220, GU296224, JF833052, JF833053, MV_H_ZEGAL07	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)
H24	H	COLBUC01	*H. paramultipigmentatus*	GAL		GU296227	Valkiūnas et al. (2010)
H25	H	COLPAS03	*H. paramultipigmentatus*	WI/REV	MEX/VEN	FJ462657, GQ141567, GQ395639, HM222486, JN788934, MV_H_COLPAS03	Latta and Ricklefs (2010), Santiago-Alarcon et al. (2010, 2014), Levin et al. (2011), Carlson et al. (2013), Ricklefs et al. (2014, 2016)
H26	H	COLPAS05	*H. paramultipigmentatus*	REV	REV/VEN	JN788939	Levin et al. (2011), Carlson et al. (2013), Santiago-Alarcon et al. (2014)
H27	H	GA02CI1	*H. paramultipigmentatus*	WI/REV	MEX	HM222487, JN788935	Latta and Ricklefs (2010), Carlson et al. (2013), Ricklefs et al. (2014, 2016, 2017)
H28	H	JA08	*H. sp1*	WI		KM598215	Ricklefs et al. (2014)

(continued)

Table 8.1 (continued)

ID		Lineage	Species	Archipelago	Other geographic region[a]	Genbank Access Number	References
H29	P	DR11DR29	*H. coatneyi*	WI	COL	GQ141565, GQ395637, MG766428	Outlaw and Ricklefs (2009), Latta and Ricklefs (2010), Pulgarín-R et al. (2018)
H30	P	SocH7	*H. cyanomitrae*	REV		JN788938	Carlson et al. (2013)
H31	P	NA15BRCO	*H. minutus*	WI	USA	GQ395665	Ricklefs et al. (2014)
H32	P	DR710	*H. picae*	WI		HM222464	Latta and Ricklefs (2010), Ricklefs and Outlaw (2010), Santiago-Alarcon et al. (2014), Ricklefs et al. (2014)
H33	P	PIPUB01	*H. picae*	WI	USA	GQ395666, GU252003, HM222472, MV_H_PIPUB01	Ricklefs and Outlaw (2010), Ricklefs et al. (2014)
H34	P	STAL2	*H. syrnii*	GAL		KF279523	Santiago-Alarcon et al. (2014)
H35	P	CI2J724	*H. vireonis*	WI	USA	GQ395632, GU251992	Ricklefs et al. (2014)
H36	P	GD3GD68	*H. vireonis*	WI	MEX/USA	GQ395649, MF077654	Ricklefs et al. (2014)
H37	P	Hap28	*H. vireonis*	WI	USA	AF465576	Ricklefs et al. (2014)
H38	P	LA26CCVO	*H. vireonis*	WI		GQ395663	Ricklefs et al. (2014)
H39	P	OZ13OZ01	*H. vireonis*	WI	USA	GQ395674, GU252006	Ricklefs et al. (2014)
H40	P	OZ17CCVO	*H. vireonis*	WI		AF465575	Ricklefs and Fallon (2002)
H41	P	VIOLI06	*H. vireonis*	WI	USA	GQ395673	Ricklefs et al. (2014, 2016)
H42	P	JA1J212	*sp10*	WI	Widespread	GQ141571, GQ141578, GQ395651, GQ395658, KM598212, KM598212	Levin et al. (2009), Outlaw and Ricklefs (2009), Ricklefs et al. (2014, 2016)
H43	P	NA09M65	*sp02*	WI	USA/VEN	AF465572, GQ395664	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014, 2016, 2017), Levin et al. (2009)
H44	P	ACCKGM63	*sp03*	GAL		GQ141557, GQ395631	Santiago-Alarcon et al. (2014)

H45	P	LA19MOMFU7	*sp04*	WI		AF465566, GQ141579, HM222470, HM222471	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014, 2016), Outlaw and Ricklefs (2009), Ricklefs and Outlaw (2010)
H46	P	CP24C	*sp05*	WI	Widespread	FJ462682, GU252000	Santiago-Alarcon et al. (2010), Ricklefs et al. (2014, 2016)
H47	P	DR02DR189	*sp05*	WI		AF465569, HM222460, HM222461	Ricklefs and Fallon (2002), Ricklefs et al. (2014, 2016, 2017)
H48	P	DR05DR9	*sp05*	WI		HM222462, HM222463, GQ141563, GQ395635	Latta and Ricklefs (2010), Levin et al. (2009), Ricklefs et al. (2014, 2016, 2017)
H49	P	DR2270	*sp05*	WI		GQ395638	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H50	P	JA06J729	*sp05*	WI		GQ141573, GQ395653, HM222468, HM222469	Levin et al. (2009), Ricklefs et al. (2014)
H51	P	JA2_J503	*sp05*	WI		GU251995	Outlaw and Ricklefs (2009)
H52	P	DR01DR20	*sp06*	WI		GQ395633	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H53	P	IIL2011nSE26F	*sp07*	GAL		JF833060, JF833061, JF833062, JF833063, JF833064	Levin et al. (2011)
H54	P	Hap36	*sp08*	WI		AF465584	Ricklefs et al. (2004, 2014), Ricklefs and Fallon (2002), Latta and Ricklefs (2010)
H55	P	H_LA16	*sp09*	WI		KM598217	Ricklefs et al. (2014)
H56	P	Hap31	*sp09*	WI/GAL		AF465579, GQ395686, KJ661283	Ricklefs and Fallon (2002), Levin et al. (2009)

West Indies (WI), Galapagos (GAL), Revillagigedo Archipelago (REV), and other geographical regions

[a]*COL* Colombia, *BLZ* Belize, *ECU* Ecuador, *GUA* Guatemala, *HI* Hawaii, *MEX* Mexico, *PAN* Panama, *SA* South America, *USA* United States, *VEN* Venezuela

Table 8.2 Summary of *Plasmodium* mitochondrial lineages (cyt b)

ID	Lineage	Species	Archipelago	Other geographic region[a]	Genbank Access Number	References
P1	SEIAUR01	*P. cathemerium*	WI/GAL	USA/MEX/ Widespread	AY377128, EU627827, HM222474, KC867655, KU212391, KU212392, KU212393 MV_P_SEIAUR01	Outlaw and Ricklefs (2009), Ricklefs (2010), Levin et al. (2013, 2016), Ricklefs et al. (2014), Perlut et al. (2018)
P2	GRW06	*P. elongatum*	WI	USA/ Widespread	DQ659588, FJ462669, FJ462685, GQ141570, GQ141590, GQ395650, GQ395675, MV_P_GRW06	Fallon et al. (2005), Levin et al. (2009, 2016), Outlaw and Ricklefs (2009), Santiago-Alarcon et al. (2010), Beadell et al. (2006)
P3	JA7J725	*P. elongatum*	WI	USA/URY/ GUY/BRZ/ COL	EU627843, GQ141574, GQ141594, GQ395648, GQ395654, GQ395680, MG766435, MG766438, MG766444, MG766446	Levin et al. (2009), Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013), Ricklefs et al. (2014, 2016, 2017), Pulgarín-R et al. (2018)
P4	BOBOVT-829176226	*P. homonucleophilum*	GAL	USA/ Widespread	AF465547, KC867673	Ricklefs et al. (2004), Levin et al. (2013, 2016), Perlut et al. (2018)
P5	BAEBIC02	*P. homopolare*	WI		AF465555, MV_P_BAEBIC02	Ricklefs and Fallon (2002), Svensson-Coelho et al. (2013)
P6	hap_53	*P. homopolare*	WI	USA	AF465553	Ricklefs and Fallon (2002), Svensson-Coelho et al. (2013)
P7	JH1238	*P. homopolare*	GAL	MEX/USA	AY172846, EU627845, JN792148, KC867674, KC867675, KC867676, KC867677, KX811228, MF077656	Levin et al. (2013, 2016), Ham-Dueñas et al. (2017), Marroquin-Flores et al. (2017)
P8	OZ06J620	*P. homopolare*	WI	USA/MEX	AF465554, GQ395670	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014), Levin et al. (2009), Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013)

P9	5IIL2016	*P. homopolare*	GAL		KU212398	Levin et al. (2016)
P10	ZArC427	*P. lutzi*	WI	MEX/USA	AY099032, FJ462681, KX811227	Perkins and Schall (2002), Santiago-Alarcon et al. (2010), Ham-Dueñas et al. (2017)
P11	EL02	*P. lutzi*	WI	USA	KM598210	Ricklefs et al. (2014)
P12	SocP11	*P. megaglobularis*	REV		HQ853678	Carlson et al. (2011)
P13	LVCP01Ven	*P. nucleophilum*	GAL	VEN	FJ462683	Santiago-Alarcon et al. (2010)
P14	LA20TRMB	*P. paranucleophilum*	WI		GQ141580, GQ395660, GU252015	Levin et al. (2009), Outlaw and Ricklefs (2009)
P15	LA24TRAI	*P. paranucleophilum*	WI		GQ395662, GQ141582	Levin et al. (2009), Outlaw and Ricklefs (2009), Ricklefs et al. (2014)
P16	LD_MC89	*P. sp1*	GAL		KC867672	Levin et al. (2013, 2016)
P17	PR2007	*P. sp2*	GAL		GQ395687	Levin et al. (2009)
P18	Dakota16	*P. sp3*	GAL/WI	SA	KC867668, KX811228, MF077656	Latta and Ricklefs (2010), Santiago-Alarcon et al. (2010), Levin et al. (2013, 2016), Ricklefs et al. (2014)
P19	PP1195	*P. sp3*	WI/GAL/REV		GQ395640, GQ395641, GQ395642, GQ395643, GQ395644, GQ395645, GQ395684, GQ395685, HQ853668, JF833046, JF833047, KC867665, KC867666, KC867667	Levin et al. (2009, 2013, 2016), Carlson et al. (2011), Palmer et al. (2013)
P20	SocP10	*P. sp4*	REV		HQ853677	Carlson et al. (2011)
P21	SocP2	*P. sp4*	REV		HQ853669	Carlson et al. (2011)
P22	SocP3	*P. sp4*	REV		HQ853670	Carlson et al. (2011)
P23	SocP4	*P. sp4*	REV		HQ853671	Carlson et al. (2011)
P24	SocP5	*P. sp4*	REV		HQ853672	Carlson et al. (2011)

(continued)

Table 8.2 (continued)

ID	Lineage	Species	Archipelago	Other geographic region[a]	Genbank Access Number	References
P25	SocP6	*P. sp4*	REV	AFR/ECU/EUR	EU810652, FJ404713, HQ853673	Carlson et al. (2011), Harrigan et al. (2014)
P26	SocP9	*P. sp4*	REV		HQ853676	Carlson et al. (2011)
P27	JA03J81	*P. sp5*	WI		HM222465, HM222466, HM222467	Ricklefs (2010), Ricklefs et al. (2014)
P28	hap_62	*P. sp6*	WI		AF465558	Ricklefs and Fallon (2002)
P29	LA06GUCF	*P. sp6*	WI	BRZ/USA/ECU	DQ659545, GQ141577, GQ395657, GQ395669	Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013), Ricklefs et al. (2014, 2016, 2017)
P30	YU4DR296	*P. sp6*	WI	MEX	GQ141600, GQ395691, GU252029	Outlaw and Ricklefs (2009), Levin et al. (2009), Latta and Ricklefs (2010), Ricklefs et al. (2014)
P31	JA09	*P. sp7*	WI		KM598216	Ricklefs et al. (2014)
P32	1 W3	*P. sp8*	GAL	USA	DQ659548, KC867650, KC867651, KC867653	Beadell et al. (2006), Levin et al. (2013, 2016)
P33	JA04	*P. sp8*	WI		KM598213	Ricklefs et al. (2014)
P34	PADOM09	*P. sp8*	GAL	USA/BRZ/NZL/URY/CRI	DQ659549, DQ838997, DQ838998, GQ141598, GQ395688, HM579783, JN819349, KC867648, KC867649, MV_P_PADOM09	Levin et al. (2009)
P35	Padom19	*P. sp8*	GAL		HM146903	Levin et al. (2016)

West Indies (WI), Galapagos (GAL), Revillagigedo Archipelago (REV), and other geographical regions

[a]*AFR* Africa, *BRZ* Brazil, *COL* Colombia, *CRI* Costa Rica, *ECU* Ecuador, *EUR* Europa, *GUY* Guyana, *MEX* Mexico, *NZL* New Zealand, *SA* South America, *URY* Uruguay, *USA* United States, *VEN* Venezuela

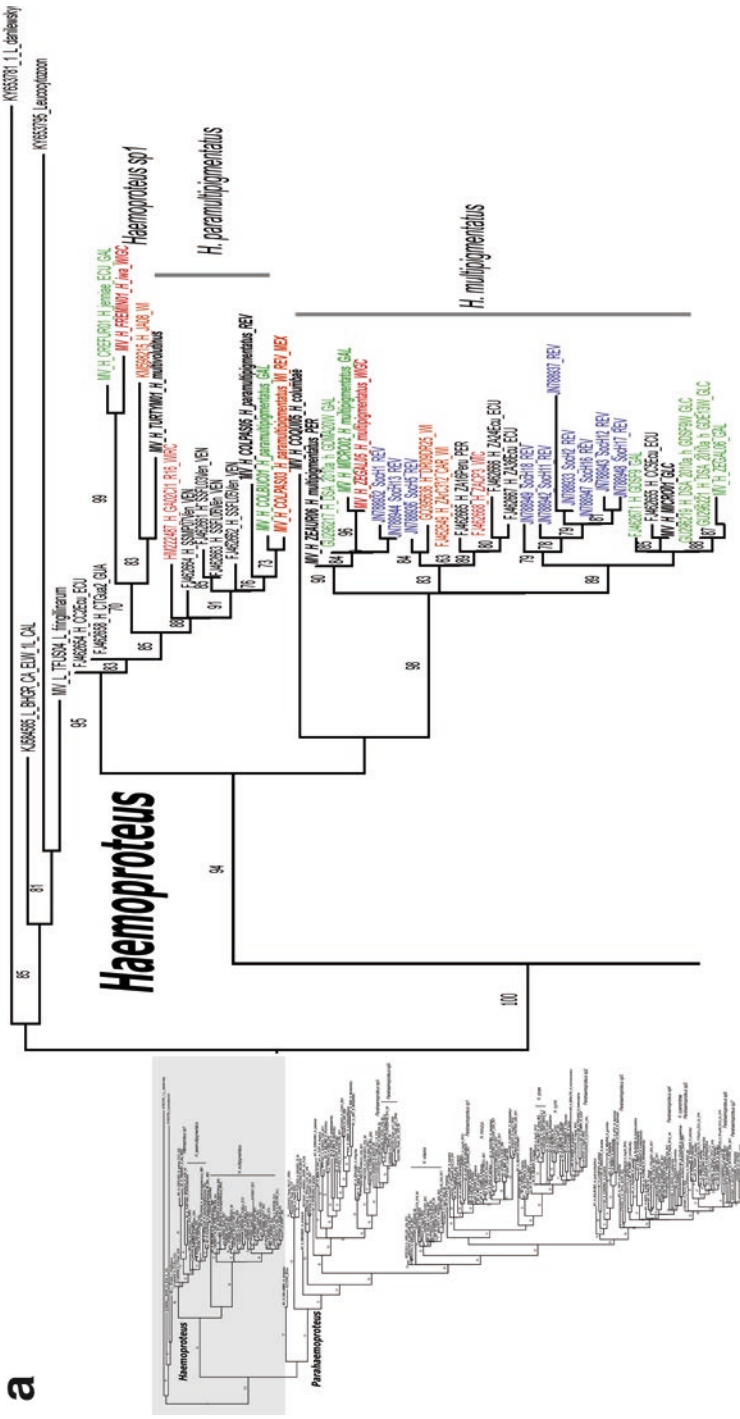


Fig. 8.2 Maximum likelihood phylogenetic reconstruction of relationships between *Haemoproteus* and *Parahaemoproteus* avian parasite lineages. Lineage labels are those recorded on the insular regions of study, West Indies (red), Galapagos (green), and Revillagigedo (blue). Taxonomic reference sequences obtained from MalAvi are indicated in bold italic. Continental lineages are in black labels. Numbers on branches are bootstrap support values

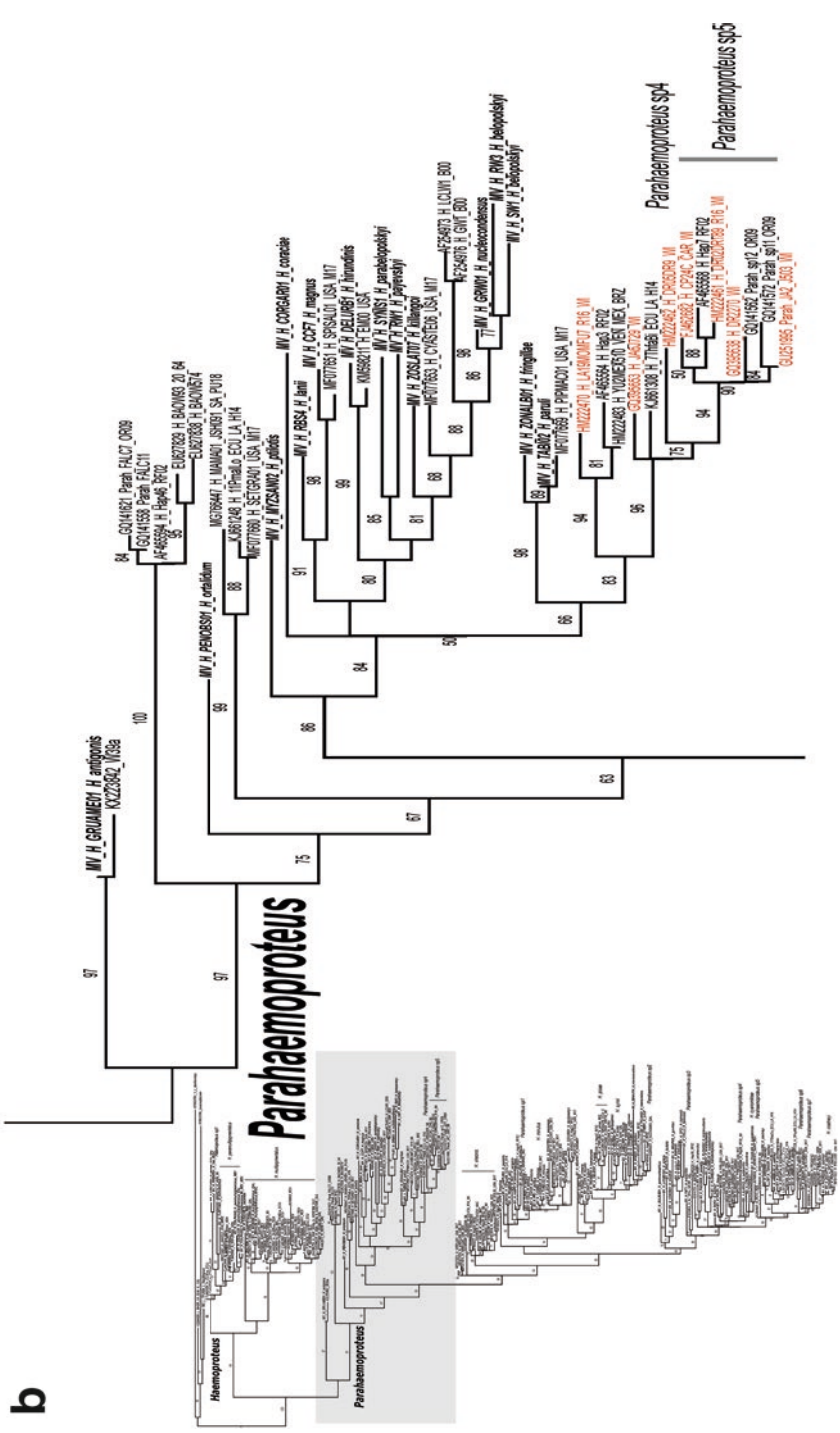


Fig. 8.2 (continued)

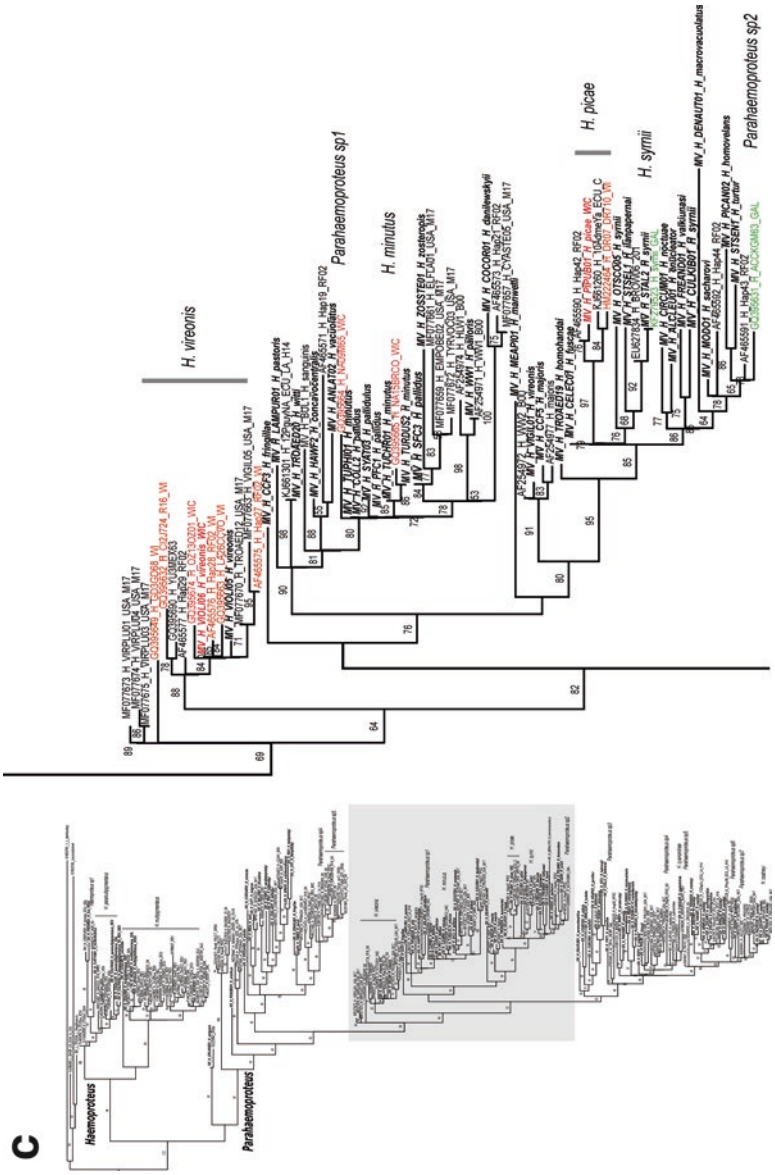


Fig. 8.2 (continued)

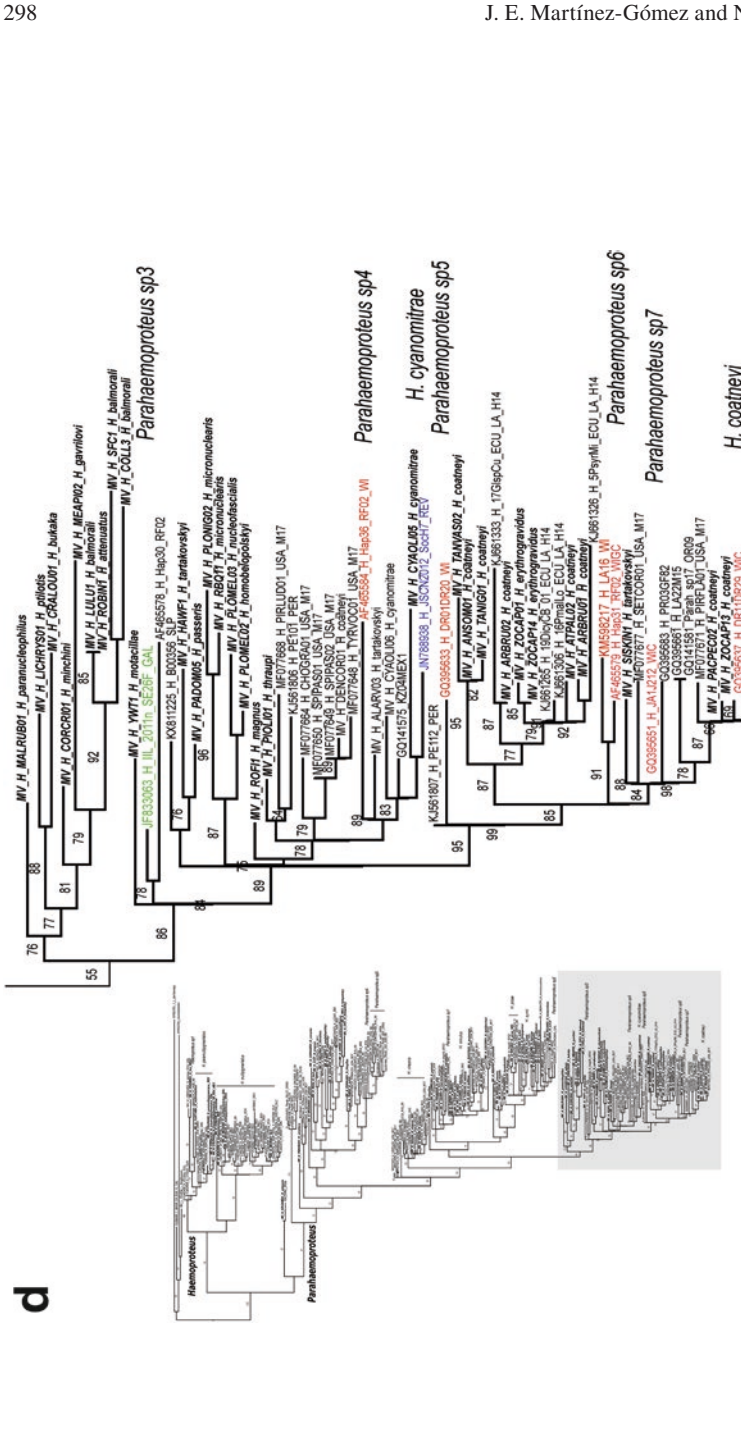


Fig. 8.2 (continued)

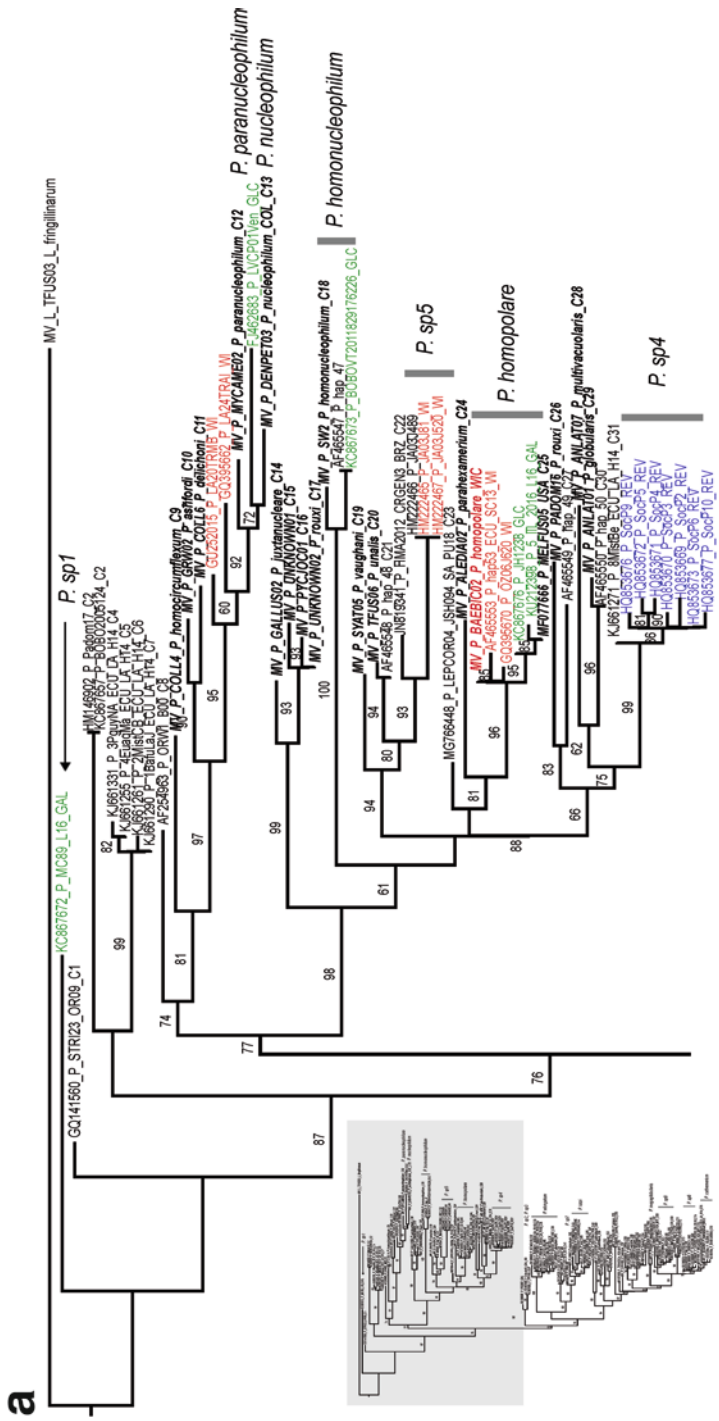


Fig. 8.3 Maximum likelihood phylogenetic reconstruction of relationships between *Plasmodium* avian parasite lineages. Lineage labels are those recorded on the insular regions of study, West Indies (red), Galapagos (green), and Revillagigedo (blue). Taxonomic reference sequences obtained from MalAvi are in bold italic. Continental lineages are in black labels. Numbers on branches are bootstrap support values

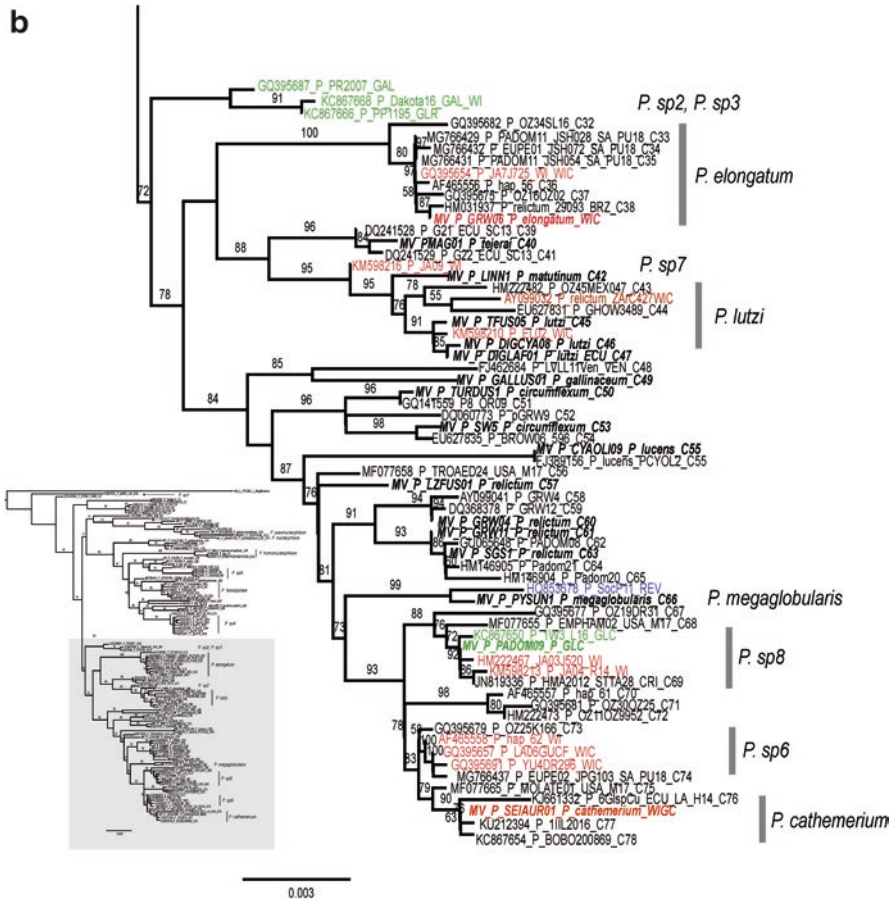


Fig. 8.3 (continued)

We employed Shannon-Wiener, Simpson, and Chao2 indices to estimate lineage diversity (α-diversity) of *Haemoproteus* and *Plasmodium* for each archipelago (Table 8.3); indices were obtained with the program EstimateS 8.0 (Colwell 2007), employing the number of lineages observed on each island of the archipelago. For the continent, the number of lineages observed per country was used. Mainland lineages not shared with any island were not used in diversity analyses. A total of 209 *Haemoproteus* and 124 *Plasmodium* lineages were included in the analysis. For both genera, diversity in the archipelagos was lower than continental diversity, with the exception of the Chao2 index for Galapagos. In all instances, the West Indies showed lower diversity values than Galapagos in spite of having the largest

Table 8.3 Diversity values for haemoporidian avian parasites in the West Indies, Galapagos, Revillagigedo and the continental Americas. Socorro Island was the only island sampled in the Revillagigedo Archipelago

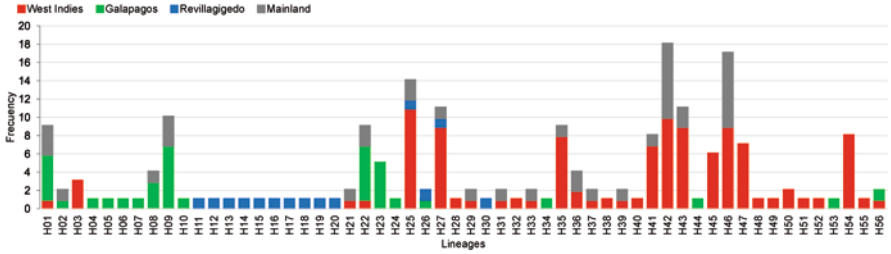
Region	Sample Size	*n* Lineages	*Shannon-Wiener's H* (S.D.)	*Simpson's D* (S.D.)	Chao 2 (S.D.)
Haemoproteus					
West Indies	109	31	2.75 (0.24)	13.01 (2.63)	22.08 (0)
Galapagos	37	16	3.21 (0.1)	18.2 (2.36)	124.62 (40.92)
Revillagigedo	14	14	3.42 (0.07)	22.25 (2.08)	90.34 (20.43)
Mainland	49	27	3.54 (0)	24.69 (0)	69.24 (6.75)
Plasmodium					
West Indies	33	16	2.27 (0.22)	7.66 (1.82)	13.65 (0)
Galapagos	26	11	2.78 (0.13)	11.51 (1.98)	55.74 (21.41)
Revillagigedo	9	9	3.04 (0.01)	15.21 (0.85)	71.56 (25.62)
Mainland	56	20	3.16 (0)	17.71 (0)	40 (3.6)

number of lineages. Values for the Revillagigedo Archipelago should be taken with caution because only Socorro Island was sampled; nevertheless, this island has the largest number of lineages in the study.

8.3.1 Haemosporidian Lineages and Their Islands

Of the 56 *Haemoproteus* lineages detected, 31 lineages were found in the West Indies, 15 are endemic to the archipelago, and 16 are shared with the continent. Three lineages are shared with Galapagos (H01, H22, and H56) and two with Socorro Island (H25 and H27). We found 17 lineages in Galapagos, of which five are found on the mainland. On Socorro Island, Revillagigedo Archipelago, we observed 14 lineages of which 11 are endemic, and two are shared with the mainland and one with Galapagos (Table 8.1; Fig. 8.4). We detected a total of 35 *Plasmodium* lineages in the three archipelagos (Table 8.2); one of them is present in the three insular systems (P19, Fig. 8.4). There are 18 in the West Indies, of which six are endemic to the region, 12 are shared with the continent, and two are shared with Galapagos and the continent (P01 and P18). We found 12 lineages in Galapagos, of which four are endemic to the archipelago, seven are shared with the continent, and two are shared with the continent and the West Indies. On Socorro Island we

H1	*H. iwa*	H12	*H. multipigmentatus*	H23	*H. multipigmentatus*	H34	*H. syrnii*	H45	*sp04*
H2	*H. jenniae*	H13	*H. multipigmentatus*	H24	*H. paramultipigmentatus*	H35	*H. vireonis*	H46	*sp05*
H3	*H. multipigmentatus*	H14	*H. multipigmentatus*	H25	*H. paramultipigmentatus*	H36	*H. vireonis*	H47	*sp05*
H4	*H. multipigmentatus*	H15	*H. multipigmentatus*	H26	*H. paramultipigmentatus*	H37	*H. vireonis*	H48	*sp05*
H5	*H. multipigmentatus*	H16	*H. multipigmentatus*	H27	*H. paramultipigmentatus*	H38	*H. vireonis*	H49	*sp05*
H6	*H. multipigmentatus*	H17	*H. multipigmentatus*	H28	*H. sp1*	H39	*H. vireonis*	H50	*sp05*
H7	*H. multipigmentatus*	H18	*H. multipigmentatus*	H29	*H. coatneyi*	H40	*H. vireonis*	H51	*sp05*
H8	*H. multipigmentatus*	H19	*H. multipigmentatus*	H30	*H. cyanomitrae*	H41	*H. vireonis*	H52	*sp06*
H9	*H. multipigmentatus*	H20	*H. multipigmentatus*	H31	*H. minutus*	H42	*sp10*	H53	*sp07*
H10	*H. multipigmentatus*	H21	*H. multipigmentatus*	H32	*H. picae*	H43	*sp02*	H54	*sp08*
H11	*H. multipigmentatus*	H22	*H. multipigmentatus*	H33	*H. picae*	H44	*sp03*	H55	*sp09*
								H56	*sp09*



P1	SEIAUR01	*P. cathemerium*	P10	ZArC427	*P. lutzi*	P19	PP1195	*P. sp3*	P28	hap_62	*P. sp6*
P2	GRW06	*P. elongatum*	P11	EL02	*P. lutzi*	P20	SocP10	*P. sp4*	P29	LA06GUCF	*P. sp6*
P3	JA7J725	*P. elongatum*	P12	SocP11	*P. megaglobularis*	P21	SocP2	*P. sp4*	P30	YU4DR296	*P. sp6*
P4	BOBOVT-829176226	*P. homonucleophilum*	P13	LVCP01Ven	*P. nucleophilum*	P22	SocP3	*P. sp4*	P31	JA09	*P. sp7*
P5	BAEBIC02	*P. homopolare*	P14	LA20TRMB	*P. paranucleophilum*	P23	SocP4	*P. sp4*	P32	1W3	*P. sp8*
P6	hap_53	*P. homopolare*	P15	LA24TRAI	*P. paranucleophilum*	P24	SocP5	*P. sp4*	P33	JA04	*P. sp8*
P7	JH1238	*P. homopolare*	P16	LD_MC89	*P. sp1*	P25	SocP6	*P. sp4*	P34	PADOM09	*P. sp8*
P8	OZ06J620	*P. homopolare*	P17	PR2007	*P. sp2*	P26	SocP9	*P. sp4*	P35	Padom19	*P. sp8*
P9	5IIL2016	*P. homopolare*	P18	Dakota16	*P. sp3*	P27	JA03J81	*P. sp5*			

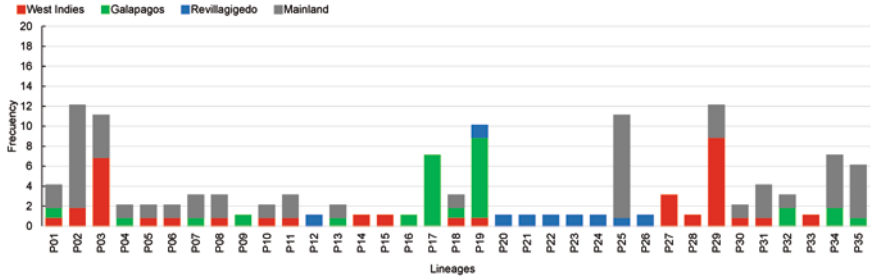


Fig. 8.4 Number of lineages of *Haemoproteus* (above) and *Plasmodium* (below) identified on the three Neotropical archipelagos under study

observed nine lineages, of which seven are endemic and one is shared with the continent (P25), and one (P19) is shared with the other two archipelagos (Fig. 8.4). Frequency of each lineage was obtained from the maximum number of islands in which the lineage was observed; for those lineages where the number of islands was not reported a number one was assigned. In the continent, values represent the maximum number of countries where the lineage has been reported.

8.3.2 Haemosporidian Lineages and Their Hosts

We observed that in the West Indies, 31 *Haemoproteus* lineages were found in 13 avian host families. Families hosting the major number of lineages were Thraupidae (10), Vireonidae (9), Columbidae (7), Icteridae (4), Mimidae (3), and Fregatidae (2). The remaining avian families (7) only hosted one lineage each. In Galapagos, there are 16 lineages in nine avian families. In descending order, Columbidae (9), Fregatidae, Laridae, Sulidae, and Spheniscidae have two each. The remaining three families host only one lineage each. On Socorro Island, Revillagigedo Archipelago, there were 14 lineages most of them in Columbidae (13) and Mimidae (2) (Table 8.4). We found 18 lineages of *Plasmodium* in 15 avian host families from West Indies. Thraupidae and Icteridae host 7 lineages each, Parulidae (4), Columbidae, and Paridae host two lineages each. The remaining 12 families only hosted one lineage each. In Galapagos we found 11 lineages in eight avian families. The family Icteridae hosted the majority of lineages (6), Parulidae (3), Spheniscidae and Columbidae (2), and Turdidae (1). On Socorro Island, we found eight *Plasmodium* lineages, but no avian hosts were detected, lineages were obtained from mosquito vectors (*Culex* sp. and *Aedes* sp.) (Table 8.5).

Table 8.4 Hosts and some vectors (*) of *Haemoproteus* (H) and *Parahaemoproteus* (P) mitochondrial lineages parasites from West Indies (WI), Galapagos (GAL), and the Revillagigedo Archipelago (REV)

ID		Lineage	Host family	Host species	Region	References
H01	H	FREMIN01	Fregatidae	*Fregata aquila, F. magnificens, F minor, *Olfersia sp.*	GAL/WI/ USA (HI)/ BLZ/PAN	Levin et al. (2011), Levin and Parker (2013), Bastien et al. (2014), Santiago-Alarcon et al. (2014)
H02	H	CREFUR01	Laridae	*Creagrus furcatus*	GAL/ECU	Levin et al. (2011), Bastien et al. (2014)
H03	H	DR09DR25	Columbidae	*Zenaida aurita, Z. asiatica, Columbina passerina*	WI	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H04	H	GDE13W	Columbidae	*Zenaida galapagoensis*	GAL	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)

(continued)

Table 8.4 (continued)

ID		Lineage	Host family	Host species	Region	References
H05	H	GDMA20W	Columbidae	*Zenaida galapagoensis*	GAL	Valkiūnas et al. (2010), Levin et al. (2011)
H06	H	GDSF7	Columbidae	*Zenaida galapagoensis*	GAL	Santiago-Alarcon et al. (2010)
H07	H	GDSF9	Columbidae	*Zenaida galapagoensis*	GAL	Santiago-Alarcon et al. (2010)
H08	H	GDSF9W	Columbidae, Laridae	*Columbina buckleyi, Zenaida galapagoensis, Creagrus furcatus,*	GAL/ECU	Santiago-Alarcon et al. (2010, 2014), Valkiūnas et al. (2010), Levin et al. (2011)
H09	H	MICRO01	Columbidae, Sulidae	*Geotrygon montana, L. rufaxilla, L. verreauxi, Zenaida macroura, Leptotila plumbeiceps, Zenaida galapagoensis, Z. auriculata, Columbina talpacoti, C. inca, C. cruziana, C. buckleyi, C. passerina, Sula granti, *Microlynchia galapagoensis*	GAL/MEX/ECU/GUA	Santiago-Alarcon et al. (2010, 2014), Valkiūnas et al. (2010), Levin et al. (2011)
H10	H	MICRO02		**Microlynchia galapagoensis*	GAL	Valkiūnas et al. (2010)
H11	H	SocH1	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H12	H	SocH11	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H13	H	SocH12	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H14	H	SocH13	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H15	H	SocH16	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H16	H	SocH17	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)

(continued)

Table 8.4 (continued)

ID		Lineage	Host family	Host species	Region	References
H17	H	SocH18	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H18	H	SocH2	Columbidae, Mimidae	*Zenaida macroura, Columbina passerina socorroensis, Mimus polyglottos*	REV	Carlson et al. (2013)
H19	H	SocH5	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H20	H	SocH6	Columbidae	*Zenaida macroura*	REV	Carlson et al. (2013)
H21	H	ZArDR491	Columbidae	*Zenaida auriculata, Z. aurita, Z. meloda*	WI/VEN	Santiago-Alarcon et al. (2010, 2014)
H22	H	ZEGAL05	Fregatidae, Columbidae	*Fregata magnificens, Leptotila plumbeiceps, Zenaida galapagoensis, Columbina talpacoti, *Microlynchia galapagoensis*	WI/GAL/GUA/MEX	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)
H23	H	ZEGAL06	Columbidae	*Zenaida galapagoensis*	GAL	Santiago-Alarcon et al. (2010), Valkiūnas et al. (2010), Levin et al. (2011)
H24	H	COLBUC01	Columbidae	*Columbina buckleyi*	GAL/ECU	Valkiūnas et al. (2010)
H25	H	COLPAS03	Columbidae	*Columbina passerina, C. p. socorroensis, C. talpacoti, Neothraupis fasciata,*	WI/REV/MEX/VEN	Latta and Ricklefs (2010), Santiago-Alarcon et al. (2010, 2014), Levin et al. (2011), Carlson et al. (2013), Ricklefs et al. (2014, 2016, 2017)

(continued)

Table 8.4 (continued)

ID		Lineage	Host family	Host species	Region	References
H26	H	COLPAS05	Columbidae	*Columbina passerina, Zenaida macroura*	REV/GAL	Levin et al. (2011), Carlson et al. (2013), Santiago-Alarcon et al. (2014)
H27	H	GA02CI1	Columbidae	*Columbina passerina, C. p. socorroensis C. talpacoti, Neothraupis fasciata*	WI/REV/MEX	Latta and Ricklefs (2010), Carlson et al. (2013), Ricklefs et al. (2014, 2016, 2017)
H28	H	JA08	Columbidae	*Leptotila jamaicensis*	WI	Ricklefs et al. (2014)
H29	P	DR11DR29	Thraupidae	*Coereba flaveola, Eucometis penicillata*	WI/COL	Outlaw and Ricklefs (2009), Latta and Ricklefs (2010), Pulgarín-R et al. (2018)
H30	P	SocH7	Mimidae	*Mimus polyglottos*	REV	Carlson et al. (2013)
H31	P	NA15BRCO	Turdidae	*Turdus migratorius, T. fumigatus*	WI/USA	Ricklefs et al. (2014)
H32	P	DR710	Todidae, Coraciiformes	*Todus subulatus*	WI	Latta and Ricklefs (2010), Ricklefs and Outlaw (2010), Santiago-Alarcon et al. (2014), Ricklefs et al. (2014)
H33	P	PIPUB01	Piciformes	*Colaptes sp., Melanerpes sp., Picoides sp.*	WI/USA	Ricklefs and Outlaw (2010), Ricklefs et al. (2014)
H34	P	STAL2	Strigidae	*Strix sp.*	GAL	Santiago-Alarcon et al. (2014)
H35	P	CI2J724	Vireonidae	Mostly *Vireonidae Vireo olivaceus, V. crassirostris, V. modestus*	WI/USA	Ricklefs et al. (2014, 2016, 2017)

(continued)

Table 8.4 (continued)

ID		Lineage	Host family	Host species	Region	References
H36	P	GD3GD68	Vireonidae, Troquilidae	Five species of Passeriformes, including *Vireo altiloquus, Trochilidae*	WI/MEX/USA	Ricklefs et al. (2014)
H37	P	Hap28	Vireonidae	*Vireo olivaceus, V. altiloquus*	WI/USA	Ricklefs et al. (2014)
H38	P	LA26CCVO	Vireonidae	*Vireo olivaceus*	WI	Ricklefs et al. (2014)
H39	P	OZ13OZ01	Vireonidae	*Vireo olivaceus*	WI/USA	Ricklefs et al. (2014)
H40	P	OZ17CCVO	Vireonidae	*Vireo antiloquus*	WI	Ricklefs and Fallon (2002)
H41	P	VIOLI06	Vireonidae	*Vireo olivaceus, V. altiloquus*	WI/USA	Ricklefs et al. (2014, 2016, 2017)
H42	P	JA1J212	Thraupidae, Icteridae	*Coereba flaveola, Icterus leucopteryx,* others	WI/ Widespread	Levin et al. (2009), Outlaw and Ricklefs (2009), Ricklefs et al. (2014, 2016, 2017)
H43	P	NA09M65	Mimidae	Mostly *Mimidae, Margarops fuscus, Dumetella carolinensis*	WI/USA/VEN	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014, 2016, 2017), Levin et al. (2009)
H44	P	ACCKGM63	Anatidae, Spheniscidae	*Anas crecca, Spheniscus mendiculus*	GAL	Santiago-Alarcon et al. (2014)
H45	P	LA19MOMFU7	Mimidae	*Margarops fuscus*	WI	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014, 2016, 2017), Outlaw and Ricklefs (2009), Ricklefs and Outlaw (2010)

(continued)

Table 8.4 (continued)

ID		Lineage	Host family	Host species	Region	References
H46	P	CP24C	Thraupidae, Icteridae, Columbidae	*Coereba flaveola, Icterus leucopteryx,* others *Thraupidae* species, *Columbina passerina*	WI/ Widespread	Santiago-Alarcon et al. (2010), Ricklefs et al. (2014, 2016, 2017)
H47	P	DR02DR189	Thraupidae, Vireonidae	*Loxigilla violacea, L. noctis, Tiaris bicolor, Vireo altiloquus*	WI	Ricklefs and Fallon (2002), Ricklefs et al. (2014, 2016, 2017)
H48	P	DR05DR9	Thraupidae	*Spindalis dominicensis*	WI	Latta and Ricklefs (2010), Levin et al. (2009), Ricklefs et al. (2014, 2016, 2017)
H49	P	DR2270	Thraupidae, Icteridae	*Coereba flaveola, Loxigilla noctis*	WI	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H50	P	JA06J729	Thraupidae, Vireonidae	*Coereba flaveola, Vireo olivaceus*	WI	Levin et al. (2009), Ricklefs et al. (2014)
H51	P	JA2_J503	Thraupidae	*Coereba flaveola*	WI	Outlaw and Ricklefs (2009)
H52	P	DR01DR20	Phaenicophilidae, Accipidtridae	*Phaenicophilus palmarum, Buteo lineatus*	WI	Latta and Ricklefs (2010), Ricklefs et al. (2014)
H53	P	IIL2011nSE26F	Sulidae	*Sula nebouxii*	GAL	Levin et al. (2011)
H54	P	Hap36	Mimidae	Mostly *Margarops (Margarops fuscatus)*	WI	Ricklefs et al. (2004, 2014), Ricklefs and Fallon (2002), Latta and Ricklefs (2010)
H55	P	H_LA16	Thraupidae	*Cyanerpes caeruleus, C. Cyaneus*	WI	Ricklefs et al. (2014)
H56	P	Hap31	Thraupidae, Icteridae, Spheniscidae	*Loxigilla noctis, Coreva flaveola, Spheniscus mendiculus*	WI/GAL	Ricklefs and Fallon (2002), Levin et al. (2009)

Table 8.5 Hosts and some vectors (*) of *Plasmodium* mitochondrial lineages parasites from West Indies (WI), Galapagos (GAL) and the Revillagigedo Archipelago (REV)

ID	Lineage	Host family	Host species	Region	References
P01	SEIAUR01	Icteridae, Strigidae, Passerellidae	32 species of Passeriformes, *Catharus ustulatus, Dolychonyx oryzivorus, Molothrus ater, Spiza americana, Ammodramus henslowii, Strix occidentalis*	WI/GAL/USA/ MEX/Widespread	Outlaw and Ricklefs (2009), Ricklefs (2010), Levin et al. (2013, 2016), Ricklefs et al. (2014), Perlut et al. (2018)
P02	GRW06	Columbidae	18 species, *Geotrygon montana*	WI/USA/ Widespread	Fallon et al. (2005), Levin et al. (2009, 2016), Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013), Santiago-Alarcon et al. (2010), Beadell et al. (2004)
P03	Dakota16	Parulidae, Spheniscidae	*Spheniscus mendiculus*, *Setophaga petechia*	GAL	Latta and Ricklefs (2010), Santiago-Alarcon et al. (2010), Levin et al. (2013, 2016), Ricklefs et al. (2014)
P03	JA7J725	Parulidae, 53 sp. Passeriformes	*Coereba flaveola,* 53 species of Passeriformes, *Loxigilla noctis, Tiaris bicolor, Columbina passerina, Vireo altiloquus, Setophaga petechia, Margarops fuscus, Eucometis penicillata*	WI/USA/URY/ GUY/BRZ/COL	Levin et al. (2009), Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013), Ricklefs et al. (2014, 2016, 2017), Pulgarín-R et al. (2018)
P04	BOBOVT-829176226	Icteridae, Passerellidae	*Dolychonyx oryzivorus, Melospiza georgiana*	GAL/USA/ Widespread	Ricklefs et al. (2004), Levin et al. (2013, 2016), Perlut et al. (2018)

(continued)

Table 8.5 (continued)

ID	Lineage	Host family	Host species	Region	References
P05	BAEBIC02	Paridae	*Parus bicolor*	WI/USA	Ricklefs and Fallon (2002), Svensson-Coelho et al. (2013)
P06	hap_53	Cardinalidae, Icteridade, Paridae, Parulidae, Thraupidae, Mimidae	*Passerina ciris, Cinclocerthia ruficauda,* Cardinalidae, Icteridae, Paridae, Parulidae, Thraupidae	WI/USA	Ricklefs and Fallon (2002), Svensson-Coelho et al. (2013)
P07	JH1238	Icteridae	*Molothrus ater, Dolychonyx oryzivorus, Geospiza fuliginosa, Amphispiza bilineata*	GAL/MEX/USA	Levin et al. (2013, 2016), Ham-Dueñas et al. (2017), Marroquin-Flores et al. (2017)
P08	OZ06J620	Passeriformes,	23 species Passeriformes, *Geothlypis trichas, Helmitheros vermivorus*	WI/USA/MEX	Ricklefs and Fallon (2002), Ricklefs et al. (2004, 2014), Levin et al. (2009), Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013)
P09	5IIL2016	Icteridae	*Molothrus ater, Dolychonyx oryzivorus*	GAL/USA/BRZ/ NZL/URY/CRI	Levin et al. (2016)
P10	ZArC427	Columbidae, Embericidae	*Zenaida aurita, Z. macroura, Amphispiza bilineata*	GAL/MEX	Santiago-Alarcon et al. (2010), Ham-Dueñas et al. (2017)
P11	EL02	Turdidae	*Turdus migratorius, T. plumbeus,* 6 additional species	GAL	Ricklefs et al. (2014)
P12	SocP11		**Culex sp.*	REV	Carlson et al. (2011)
P13	LVCP01Ven	Columbidae	*Leptotila verreauxi*	WI/USA	Santiago-Alarcon et al. (2010)

P14	LA20TRMB	Icteridae	*Molothrus bonariensis*	WI	Levin et al. (2009), Outlaw and Ricklefs (2009)
P15	LA24TRAI	Icteridae	*Chrysomus icterocephalus, Molothrus bonariensis*	GAL/VEN	Levin et al. (2009), Outlaw and Ricklefs (2009), Ricklefs et al. (2014)
P16	LD_MC89	Thraupidae,	*Geospiza fuliginosa*	WI	Levin et al. (2013, 2016)
P17	PR2007	Spheniscidae	*Spheniscus mendiculus*	WI	Levin et al. (2009)
P19	PP1195	Parulidae, Spheniscidae, Columbidae	*Spheniscus mendiculus*, *Setophaga petechia, Geospiza fortis, G. fuliginosa, Geotrygon montana, *Aedes sp.*	GAL	Levin et al. (2009, 2013, 2016), Carlson et al. (2011), Palmer et al. (2013)
P20	SocP10		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P21	SocP2		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P22	SocP3		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P23	SocP4		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P24	SocP5		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P25	SocP6	Nectariniidae	*Cyanomitra olivacea, *Aedes taeniorhynchus*	REV/AFR/ECU/EUR	Carlson et al. (2011), Harrigan et al. (2014)
P26	SocP9		**Aedes taeniorhynchus*	REV	Carlson et al. (2011)
P27	JA03J81	Turdidae	*Turdus aurantius, T. plumbeus*	WI/AFR/ECU/EUR	Ricklefs (2010), Ricklefs et al. (2014)
P28	hap_62	Thraupidae	*Loxigilla noctis*	WI	Ricklefs and Fallon (2002)
P29	LA06GUCF	Icteridae, Emberizidae, Tyrannidae, Mimidae, Thraupidae	*Coereba flaveola, Loxilla noctis, Tiaris bicolor, Elaenia martinica, Margarops fuscus, M. fuscatus*	WI	Outlaw and Ricklefs (2009), Svensson-Coelho et al. (2013), Ricklefs et al. (2014, 2016, 2017)

(continued)

Table 8.5 (continued)

ID	Lineage	Host family	Host species	Region	References
P30	YU4DR296	Parulidae, Thraupidae, Icteridae	Six species, Parulidae, Thraupidae, Icteridae	WI	Outlaw and Ricklefs (2009), Levin et al. (2009), Latta and Ricklefs (2010), Ricklefs et al. (2014)
P31	JA09	Thraupidae	*Euneornis campestris*	WI/BRZ/USA/ ECU	Ricklefs et al. (2014)
P32	1 W3	Icteridae, Parulidae	*Dolychonyx oryzivorus, Setophaga petechia, Myiarchus tyrannulus*	WI/MEX	Beadell et al. (2006), Levin et al. (2013, 2016)
P33	JA04	Thraupidae, Icteridae, Mimidae	11 species (Passeriformes): *Elaenia sp. Mimus sp. Quiscalus sp.*	WI	Ricklefs (2010), Ricklefs et al. (2014)
P34	PADOM09	Icteridae	*Dolychonyx oryzivorus*	GAL/USA	Levin et al. (2009)
P35	Padom19	Parulidae	*Setophaga petechia*	GAL	Levin et al. (2016)

8.3.3 Lineages per Area

To evaluate the relationship between lineage diversity versus island area, we fitted a GLM using the Quasi-Poisson family with a log link function; the number of lineages found on each island was used as the response variable, island surface was the explanatory variable. Analyses were performed separately for the Galapagos and the West Indies when exploring patterns in the archipelagos. Although the Revillagigedo Archipelago was not considered in regional analyses because information was available only for Socorro Island, it was included when fitting the overall model for all islands. Island size was obtained from http://islands.unep.ch/isldir.htm (December 2018). Analyses for *Haemoproteus* and *Plasmodium* were run separately in R (R Core Team 2013); graphics were created in Past 3.21 (Hammer et al. 2001).

Overall, all regressions showed a positive relationship between lineage and island surface area (Fig. 8.5; Tables 8.6 and 8.7). However, only the regression for *Plasmodium* in the West Indies yielded a significant result (Fig. 8.5; Table 8.7). Interestingly, Socorro Island in the Revillagigedo Archipelago hosted the largest number of *Haemoproteus* lineages (14) (Fig. 8.6; Table 8.1). Hispaniola hosted the largest number of lineages in the West Indies (12) and Española in the Galapagos (7). In the case of *Plasmodium,* Socorro Island hosted the largest number of lineages (9), followed by Isabela and Fernandina in the Galapagos (7), Floreana and Bartolome (4), and Jamaica, Guadeloupe, Martinique, and St. Lucia in the West Indies (3). The large number of lineages on Socorro Island requires additional investigation in the future to discard the possibility of sampling artifacts. Montserrat, Nevis, and Socorro show a rather large number of *Haemoproteus* lineages than expected from their size (10); these islands may constitute an example of the small island effect (SIE). Conversely, Santa Cruz and Fernandina represent an example opposite to the SIE by showing less diversity than expected from their surface area. Our analyses cannot go beyond a basic confirmation of the two conjectures that helped to derive the equilibrium model because there is no information on immigration and extinction rates in haemosporidians.

8.3.4 Taxon Cycles

To obtain a proxy for lineage age, we used patristic genetic distances from each lineage tip to the tree root represented by *Leucocytozoon fringillinarium*; we obtained genetic distances using Geneious Prime® 2019.2.1. We used patristic distance only for unique lineages present on the archipelagos under study. We explored the two possible scenarios consistent with taxon cycles mentioned above. The number of lineages found on each island was used as the response variable. Analyses for *Haemoproteus* and *Plasmodium* were run separately. We first assumed that older lineages would show a shorter genetic distance to the root tree while younger

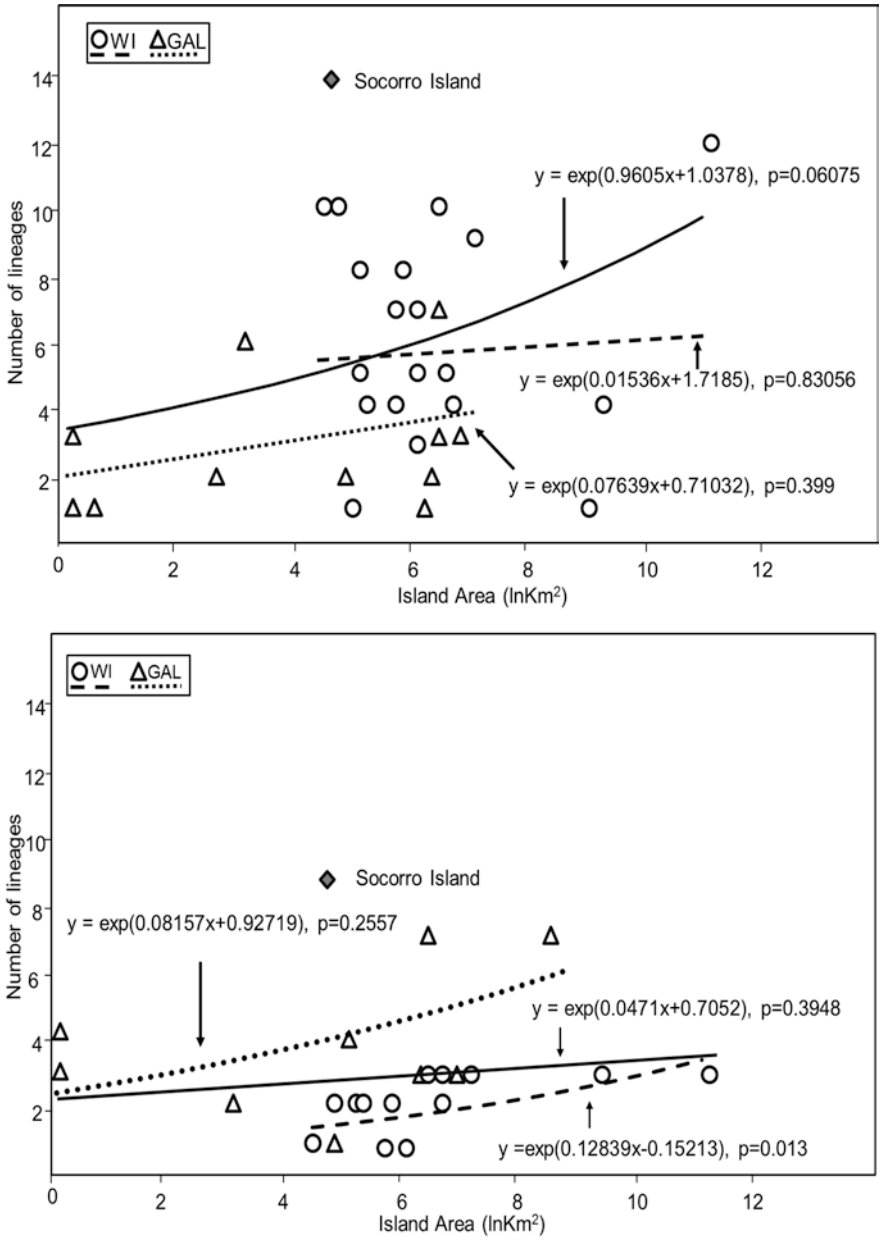


Fig. 8.5 Number of parasite haemosporidian lineages of *Haemoproteus* (above) and *Plasmodium* (below) from the Revillagigedo Archipelago (Socorro Island), Galapagos (GAL), and the West Indies (WI). The solid line represents the best-fit function when considering all islands together

Table 8.6 Generalized linear model (GLM) regression for the number *Haemoproteus* lineages versus island surface using a Quasi-Poisson model with a *log* link function

	Estimate	S. E.	*t*-value	*P*
West Indies–Galapagos				
Intercept	1.03	0.318	3.25	**0.0029**
ln Area (km^2)	0.096	0.049	1.95	0.06
Dispersion parameter: 1.87				
Null deviance: 62.02 (d.f. 29)				
Residual deviance: 54.72 (d.f.: 28)				
West Indies				
Intercept	1.71	0.473	3.62	**0.002**
ln Area (km^2)	0.015	0.07	0.217	0.83
Dispersion parameter: 1.7				
Null deviance: 32.25 (d.f.: 18)				
Residual deviance: 32.17 (d.f.: 17)				
Galapagos				
Intercept	0.71	0.443	1.6	0.144
ln Area (km^2)	0.076	0.086	0.886	0.399
Dispersion parameter: 1.4				
Null deviance: 12.59 (d.f.: 10)				
Residual deviance: 11.44 (d.f.: 9)				

Significant values in bold

Table 8.7 Generalized linear model (GLM) regression for the number of *Plasmodium* lineages versus island surface using a Quasi-Poisson model with a *log* link function

	Estimate	S. E.	*t*-value	*P*
West Indies–Galapagos				
Intercept	0.705	0.347	2.03	0.0546
ln Area (km^2)	0.047	0.054	0.868	0.394
Dispersion parameter: 0.99				
Null deviance: 20.48 (d.f.: 23)				
Residual deviance: 19.73 (d.f.: 22)				
West Indies				
Intercept	−0.152	0.317	−0.478	0.64
ln Area (km^2)	0.128	0.044	2.87	**0.013**
Dispersion parameter: 0.249				
Null deviance: 5.23 (d.f.: 14)				
Residual deviance: 3.35 (d.f.: 13)				
Galapagos				
Intercept	0.927	0.382	2.42	**0.045**
ln Area (km^2)	0.081	0.065	1.23	0.255
Dispersion parameter: 0.962				
Null deviance: 8.83 (d.f.: 8)				
Residual deviance: 7.27 (d.f.: 7)				

Significant values in bold

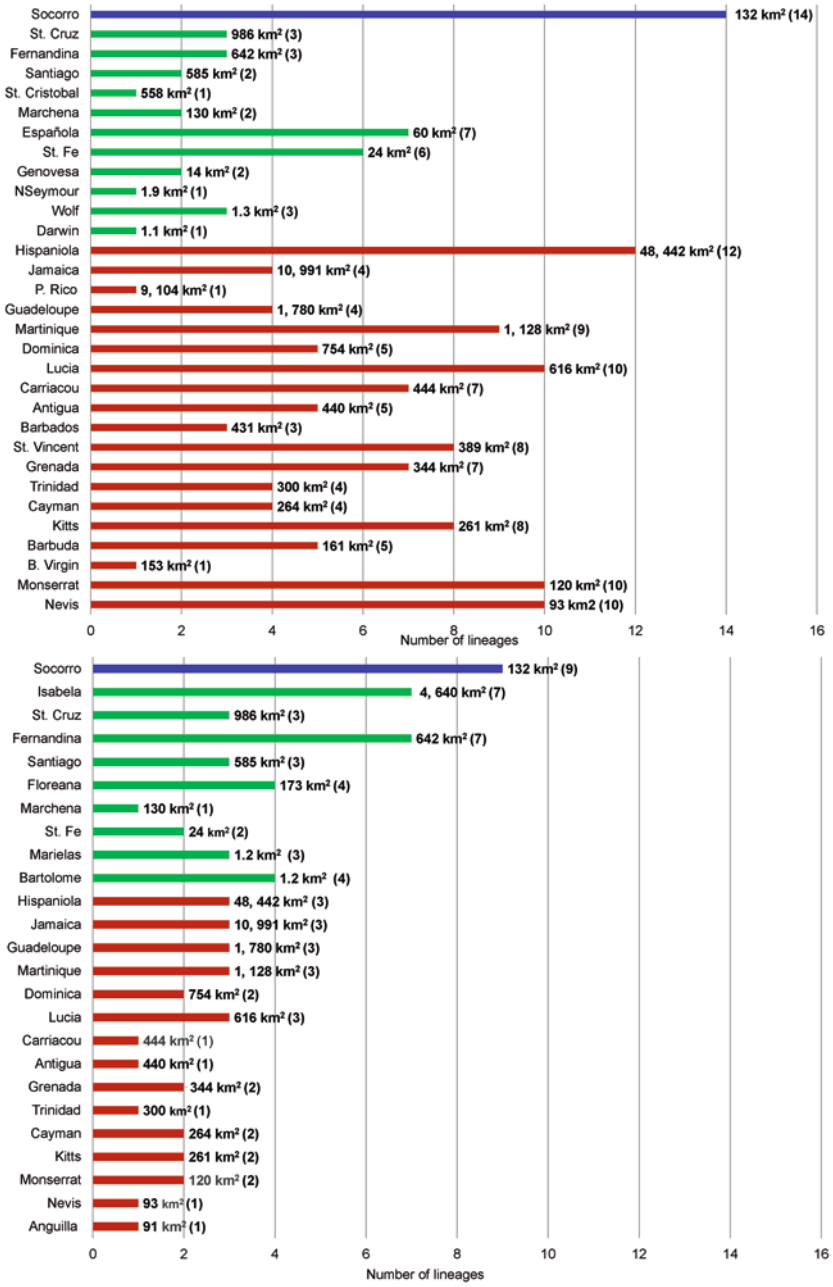


Fig. 8.6 Number of *Haemoproteus* (above) and *Plasmodium* (below) lineages recorded by island in the West Indies (red), Galapagos (green), and the Revillagigedo Archipelago (blue). The islands are arranged from largest to smallest size by archipelago. The number of lineages currently recorded by island is in parenthesis

lineages would show a larger genetic distance from tip to the root. Then, we fitted another model using the number of hosts infected by each haemosporidian lineage as the response variable. We multiplied genetic distance by −1 to have younger lineages to the left. Analyses were performed separately for the Galapagos and the West Indies. The Revillagigedo Archipelago was not considered because information is only available for Socorro Island. For taxon cycles, we did not fit an overall model because taxon cycles are contingent to the geological and evolutionary history of each island group. Observed patristic genetic distances ranged from 0.20 to 0.25 in *Haemoproteus*. In Galapagos, larger distances to the root were observed, suggesting an earlier origin. In *Plasmodium* genetic distances ranged from 0.38 to 0.54. For both, *Haemoproteus* and *Plasmodium* GLMs for the Galapagos and the West Indies showed opposite slope signs (Fig. 8.7). Although *Haemoproteus* and *Plasmodium* in the West Indies showed a trend consistent with a taxon cycle, analyses were not significant (Table 8.8). Most models of number of lineages and number of hosts produced a pattern like the one expected for taxon cycles, except for *Plasmodium* in the West Indies; none of these trends were significant (Fig. 8.8; Table 8.9).

8.4 Discussion

We explored available data to learn about the patterns of haemosporidian lineage diversity and richness on Neotropical islands. Phylogenetic trees for haemosporidians found on the three Neotropical archipelagos were assembled, despite differences in sequence region, quality, and length (Figs. 8.2 and 8.3). We used lineage diversity derived from these trees to explore the usefulness of the equilibrium model of species richness and taxon cycles. Overall, our results corroborated the basic assumption that larger islands have more species. However, information on colonization, extinction rates, or lineage turnover was unavailable. Most GLMs between number of lineages and island size were not significant; only the model fitted for *Plasmodium* from the West Indies was significant (Fig. 8.5; Table 8.6). Failure to corroborate the equilibrium model for species richness has been observed in other areas (e.g., Macaronesia, Illera et al. 2015). It was not possible to explore relationships within the Revillagigedo Archipelago because information was available for Socorro Island only. Nonetheless, it is noteworthy that Socorro Island had the largest number of lineages, a finding that requires additional study to discard the possibility of sequencing artifacts. A clear indication of the small island effect (SIE) was not detected presumably for two reasons: (1) regression model parameters were biased given that estimates were derived primarily from data points representing islands of medium size from different archipelagos (Fig. 8.5) and (2) island size varies a lot from archipelago to archipelago (Figs. 8.5 and 8.6; Tables 8.6 and 8.7); thus, the term “small” is rather dependent on the particular geographic layout of each island group.

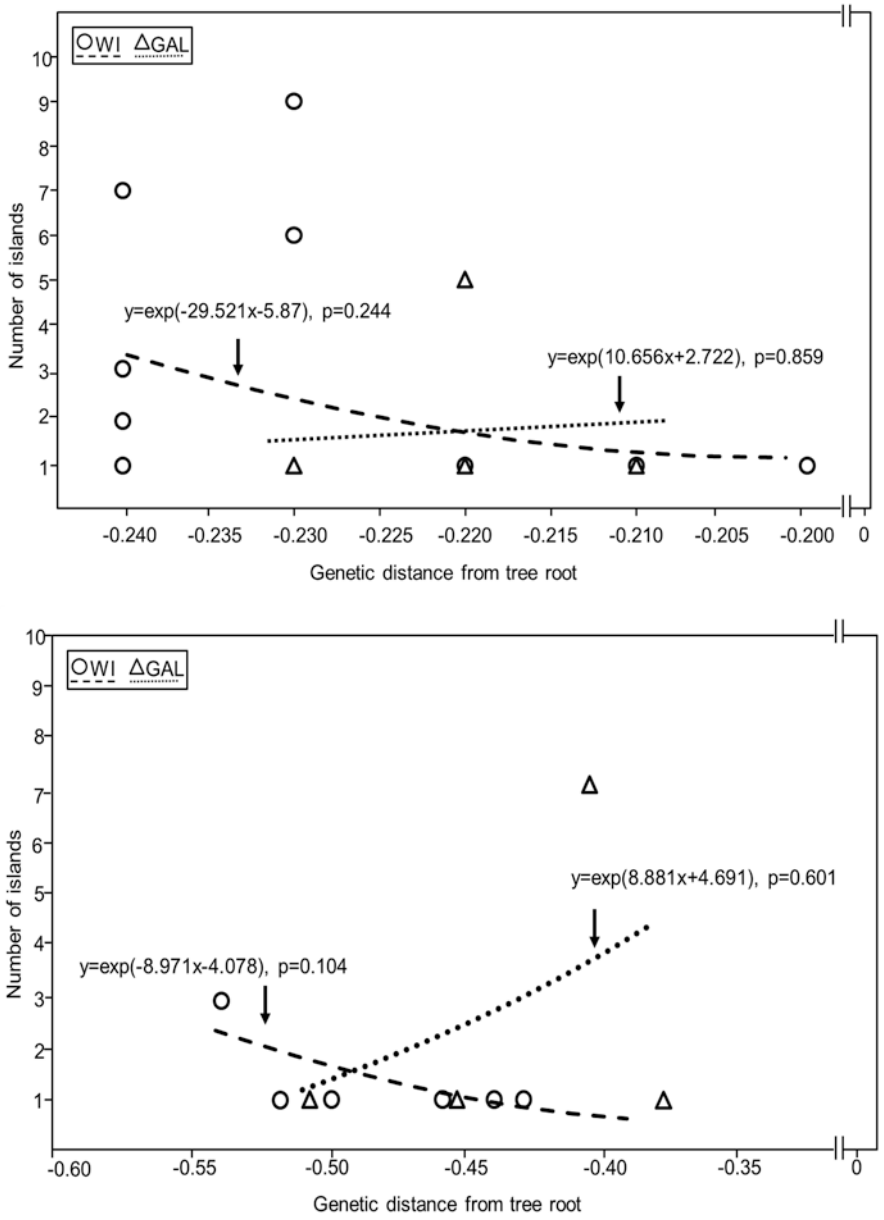


Fig. 8.7 Taxon cycle exploration through generalized linear models. Genetic distances of unique lineages versus number of islands for *Haemoproteus* (above) and *Plasmodium* (below) from the West Indies (WI) and the Galapagos (GAL)

Table 8.8 Taxon cycle exploration for *Haemoproteus* and *Plasmodium* lineages in the West Indies and Galapagos derived from generalized linear model (GLM) regressions using a Quasi-Poisson model with a *log* link function between genetic distance of lineages and the number of islands where the lineages were present

Haemoproteus	Estimate	S. E.	*t*-value	*P*
West Indies				
Intercept	−5.87	5.6	−1.04	0.314
Genetic distance	−29.52	24.19	−1.22	0.244
Dispersion parameter: 2.56				
Null deviance: 30.84 (d.f.: 14)				
Residual deviance: 26.64 (d.f.: 13)				
Galapagos				
Intercept	2.72	12.73	0.214	0.837
Genetic distance	10.65	57.66	0.185	0.859
Dispersion parameter: 1.38				
Null deviance: 6.53 (d.f.: 8)				
Residual deviance: 6.48 (d.f.: 7)				
Plasmodium	Estimate	S. E.	*t*-value	*P*
West Indies				
Intercept	−4.07	2.12	−1.92	0.127
Genetic distance	−8.97	4.28	−2.09	0.104
Dispersion parameter: 0.229				
Null deviance: 1.98 (d.f.: 5)				
Residual deviance: 0.944 (d.f.: 4)				
Galapagos				
Intercept	4.69	6.03	0.777	0.518
Genetic distance	8.88	14.43	0.615	0.601
Dispersion parameter: 3.86				
Null deviance: 8.91 (d.f.: 3)				
Residual deviance: 7.28 (d.f.: 2)				

Although trends consistent with taxon cycles were observed in some analyses of genetic distance *versus* number of islands or host numbers, respectively, they lacked statistical significance (Figs. 8.7 and 8.8; Tables 8.8 and 8.9). In regressions considering number of lineages and number of islands, the West Indies showed nonsignificant negative slopes consistent with taxon cycles. In Galapagos, for both *Haemoproteus* and *Plasmodium*, a nonsignificant positive slope was observed (Fig. 8.7; Table 8.8), which actually may be related to the age of an archipelago where not enough time has elapsed to detect a taxon cycle pattern. Regarding host specialization, most regressions showed trends consistent with taxon cycles, except one; however, none of the regressions were significant (Fig. 8.8; Table 8.9). Overall, *Haemoproteus* includes younger lineages that are widely distributed, while older lineages are restricted to fewer islands or fewer hosts (Figs. 8.7 and 8.8). Further studies are required to explore which conditions would produce opposite trends – older lineages present in more islands or number of hosts. In addition, it might be

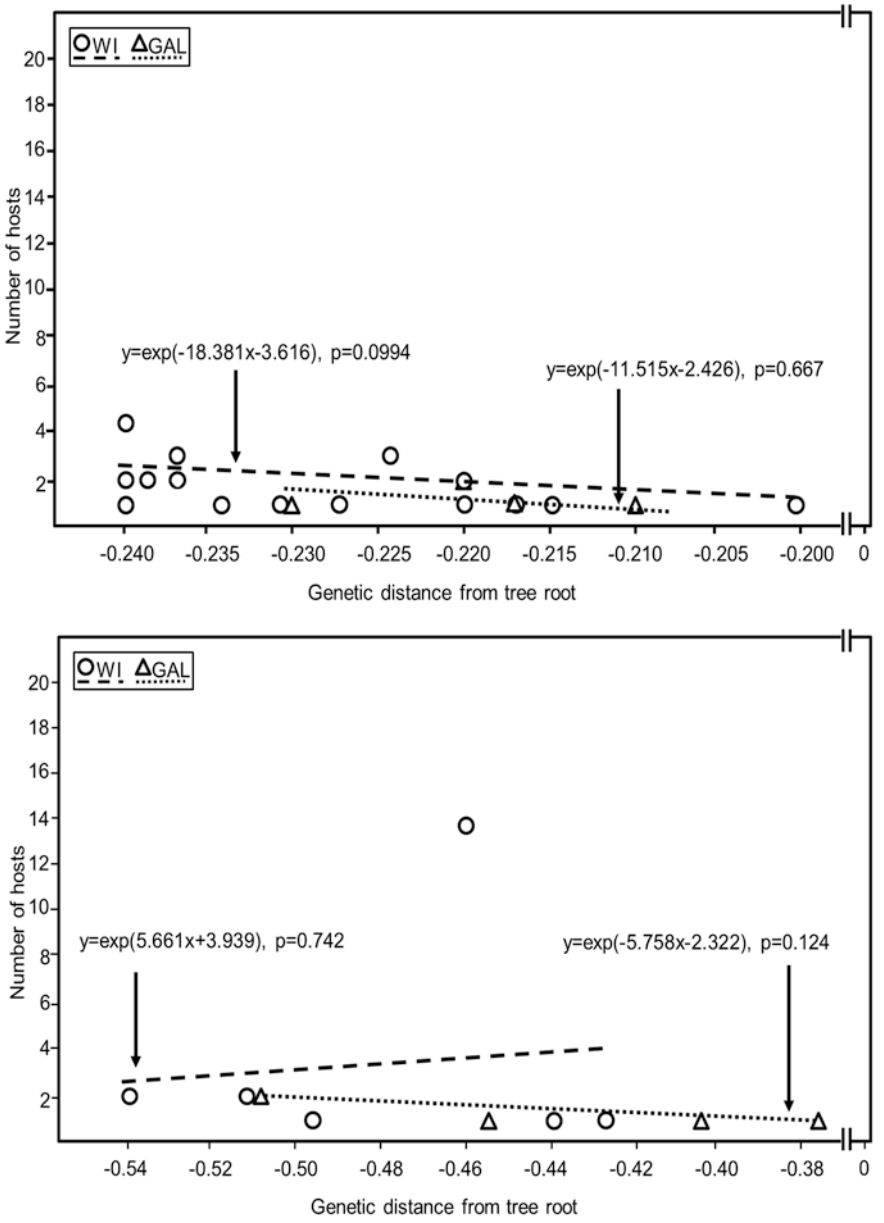


Fig. 8.8 Taxon cycle exploration through generalized linear models. Genetic distances of unique lineages for *Haemoproteus* (above) and *Plasmodium* (below) versus number of host species from the West Indies (WI) and the Galapagos (GAL) insular regions

Table 8.9 Taxon cycle exploration for *Haemoproteus* and *Plasmodium* lineages in the West Indies and Galapagos derived from generalized linear model (GLM) regressions using a Quasi-Poisson model with a *log* link function between genetic distance of lineages and the number of hosts where the lineages were present

Haemoproteus	Estimate	S. E.	*t*-value	*P*
West Indies				
Intercept	−3.61	2.38	−1.51	0.15
Genetic distance	−18.38	10.35	−1.77	0.099
Dispersion parameter: 0.399				
Null deviance: 6.39 (d.f.: 14)				
Residual deviance: 5.08 (d.f.: 13)				
Galapagos				
Intercept	−2.42	5.62	−0.431	0.681
Genetic distance	−11.51	25.45	−0.452	0.667
Dispersion parameter: 0.122				
Null deviance: 0.652 (d.f.: 7)				
Residual deviance: 0.626 (d.f.: 6)				
Plasmodium	Estimate	S. E.	*t*-value	*P*
West Indies				
Intercept	3.93	7.56	0.521	0.63
Genetic distance	5.66	16.03	0.353	0.742
Dispersion parameter: 8.39				
Null deviance: 26.82 (d.f.: 5)				
Residual deviance: 25.74 (d.f.: 4)				
Galapagos				
Intercept	−2.32	1.01	−2.29	0.149
Genetic distance	−5.75	2.24	−2.56	0.124
Dispersion parameter: 0.062				
Null deviance: 0.541 (d.f.: 3)				
Residual deviance: 0.13 (d.f.: 2)				

possible that patterns expected from taxon cycles are detectable at a certain age threshold and indistinguishable before. Also, it is possible that density dependent factors play an important role in determining how many lineages are found in each host species.

Extensive sampling is required to explore with greater detail the relationship between lineage age and the number of islands where it is found. The use of mitochondrial sequences from different sections of the cyt b gene, with a small overlapping section in common, affects the properties of our resulting phylogenies. Thus, there is a possibility that our results may be partially an artifact due to incomplete sampling and/or other biases (e.g., the use of a single molecular marker or low quality of available sequences). Ample sampling of haemosporidian diversity at these geographical locations will yield more, better, and longer sequences for future analyses. The study of the distribution of shared lineages may shed some light on the evolution of lineage diversity and potential processes of colonization. Shared

lineages of *Haemoproteus* are related to basal species such as *Haemoproteus multipigmentatus*. Lineages endemic to any of the insular regions all belong to the subgenus *Haemoproteus* and are basal suggesting an old age and possibly taxon cycles. On the other hand, the 15 lineages belonging to the subgenus *Parahaemoproteus* can be found across the phylogenetic tree. It seems that haemosporidians shared lineages are transmitted by introduced, migratory, or marine avian species, highlighting the importance of avian migration in the dispersal of haemosporidian parasites (e.g., Ricklefs et al. 2016, 2017).

The number of haemosporidian lineages in the West Indies was found to be rather large. This pattern suggests a combined action of age, a shorter distance to the continent, and a stepping stone layout used by migratory birds contributing to the high number of species detected in this archipelago. The reduced number of lineages in Galapagos, on the other hand, may be the result of its isolation from the continent. Although Socorro Island was the only island of the Revillagigedo Archipelago used in this study, the high number of lineages probably resulted from a combined contribution of a short distance to the continent and the regular presence of migratory birds. A more comprehensive study of hosts, vectors, parasites, and ecological conditions is required to better understand diversity patterns on islands, including why some islands host more or fewer lineages than those expected by their size.

As yet, there are few biogeographical analyses of avian haemosporidians in other archipelagos. *Haemoproteus* is widespread in continental areas, but it is absent or infrequent in some oceanic regions such as Madeira and the Canary Islands (Hellgren et al. 2011). In Australia, *Haemoproteus* was shown to exhibit a high lineage diversity (Clark et al. 2014). *Plasmodium* is a cosmopolitan genus, less diverse than *Haemoproteus*, except in South America, where it seems to have more lineages (Clark et al. 2014; see Chap. 1 for a thorough revision of haemosporidian prevalence across tropical regions according to studies conducted during the last century). The absence of a number of lineages in both Hawaii and French Polynesia is probably due to their isolation and absence of competent vectors, as well as by the presence of successful invasive parasites that may be superior competitors (e.g., avian malaria, *Plasmodium relictum*, in Hawaii). In addition, the presence of *Plasmodium* in Hawaii, French Polynesia, and New Zealand has been related to the effect of human activities (Ewen et al. 2012). In the Mascarene Archipelago, *Leucocytozoon* is more diverse than *Plasmodium,* assumed to be partially due to earlier colonization events and subsequent *in situ* diversification (Cornuault et al. 2013). In addition, it has been hypothesized that abiotic factors such as temperature and rainfall may help haemosporidian diversification (Clark et al. 2014), which, combined with isolation, can accelerate the differentiation process. Haemospordian diversity on islands may result in more stable communities than on the continent, but this not necessarily implies lower richness. In fact, depauperate parasite assemblages on islands are not a general pattern (Hellgren et al. 2011). Thus, the occasional arrival of novel lineages to islands via migratory birds or human activities, may ignite a diversification process if there are both competent vectors and hosts, where the life cycle of haemosporidians can be completed (see Chap. 2).

Species–area relationships for *Plasmodium* were found in Vanuatu, New Caledonia, and Loyalty in the Western Pacific; *Haemoproteus* did not show any relationship (Ishtiaq et al. 2010). Across the same region, Olsson-Pons et al. (2015) observed for *Plasmodium* a pattern concordant with isolation by distance and, at the same time, significant genetic variation among lineages but not among host families. They did not, however, observe isolation by distance in *Haemoproteus.* Olsson-Pons et al. (2015) attributed the differences between *Plasmodium* and *Haemoproteus* to geographical and environmental factors for *Plasmodium* and to host ecology for *Haemoproteus.* Overall, studies from other island regions have shown reduced haemosporidian lineage diversity compared to the closest continental mass (Hellgren et al. 2011; Baillie and Brunton 2011). However, in Macaronesia on the islands of the Gulf of Guinea *Plasmodium* lineage diversity was similar or greater to that observed on the continent (Illera et al. 2015; Loiseau et al. 2017). Furthermore, Clark and Clegg (2015) found for Heron Island that there was a higher prevalence and parasite diversity on the island than in the nearby mainland, though this was coupled with large variation over the years. In spite of a large probability of arrival through vagrant birds, *Haemoproteus* has a prevalence which suggests that host specificity determines both the colonization and persistence on the island. On the other hand, *Plasmodium* shows high prevalence and species diversity associated with environmental factors such as wind currents (Clark and Clegg 2015). Finally, in the Canary Islands on Tenerife, Padilla et al. (2017) found that *Plasmodium* lineages with high prevalence are those found in widely distributed hosts. Thus, based on these studies and our current analyses, we suggest that factors allowing relatively small islands to have a larger number of parasites than expected from theory include habitat availability, climatic factors, and host geographic distribution and movement patterns.

The equilibrium model of species richness was developed for vertebrates living on islands of the middle Pacific Ocean; patterns that inspired the model might differ in other regions (Steadman 2006). The current number of lineages found in Neotropical archipelagos suggests that the equilibrium between colonization and extinction has not been reached, though this can also be explained by incomplete sampling. Evidence indicates that ecological saturation of vertebrate species on islands is rarely achieved (Walter 2004), and this could also apply to haemoparasites. A better understanding of haemosporidian lineage diversity on islands will require a better knowledge of their vectors, hosts, and ecological factors (e.g., habitat, migratory species; Stracey and Pimm 2009; Lasky et al. 2017). Proper corroboration of taxon cycles remains a task for future studies once standardized and geographically widespread sampling of parasites, hosts, and vectors has been accomplished. We concur with fellow researchers urging for studies, comprehensive and in a wider geographical range, to fill voids of knowledge that will allow to explain identified island biogeographical patterns in general and, specifically, for parasitic organisms (Steadman 2006; Whittaker and Fernández-Palacios 2007; Losos and Ricklefs 2009; Borges et al. 2016; Santos et al. 2016; Whittaker et al. 2017).

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Chapter 9
A Macroecological Perspective on Antagonistic Interactions Through the Lens of Ecological Networks

Erick J. Corro, Wesley Dáttilo, and Fabricio Villalobos

Abstract Several biotic and abiotic factors change across time and space, which may directly affect not only the distribution and abundance of species but also other dimensions of biodiversity such as ecological functions and biotic interactions. Over such spatial and temporal gradients, interactions among species form complex ecological networks with emergent properties that cannot be observed at the species or community level. The properties of species interaction networks can be associated with geographical as well as climatic gradients and be shaped by different ecological and evolutionary processes such as dispersal capacity and evolutionary history of the interacting species. In this chapter, we first introduce the reader to the macroecological and ecological network approaches and then review how these approaches have been applied to study antagonistic interactions. In doing so, we highlight how these two approaches have advanced our understanding of broad-scale patterns of antagonistic interactions. Finally, we discuss future perspectives for the combined application of macroecology and ecological networks to the study of antagonistic interactions.

Keywords Biodiversity gradients · Correlative approach · Distance-decay · Ecological interactions · Interaction networks · Macroecology · Mechanistic approach

E. J. Corro · W. Dáttilo (✉) · F. Villalobos (✉)
Red de Ecoetología, Instituto de Ecología A.C., Xalapa, Veracruz, Mexico

Red de Biología Evolutiva, Instituto de Ecología A.C., Xalapa, Veracruz, Mexico
e-mail: wesley.dattilo@inecol.mx; fabricio.villalobos@inecol.mx

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9.1 Macroecology

Macroecology is a research programme that can be considered recent within the ecological disciplines. This programme is focused on studying the emergent statistical properties of ecological systems, answering integrative questions related mainly to species diversity, such as richness, abundance, and geographic distributions (Brown and Maurer 1989; Marquet 2009). The primary information used in macroecology comes from datasets where the occurrence and abundance of species are reported, which have in turn been compiled over time from standardized samples and censuses in the field (Brown 1995; Beck et al. 2012). In general, macroecology is concerned with how ecological traits (e.g., body size, population density, geographical range of species) affect or structure biological diversity patterns (Brown and Maurer 1989). Among other topics, macroecology also addresses the study of geographical biodiversity patterns at different spatial scales, for example, by analyzing correlations between climate and historical factors (Currie et al. 2004; Marquet 2009; Beck et al. 2012). Therefore, macroecology studies the structure and function of communities by using different mathematical and computational tools to describe and identify statistical regularities among species traits (Brown 1995; Marquet 2009). In other words, macroecology helps us to understand both patterns (e.g., latitudinal diversity gradient) and processes (e.g., energy, productivity) of biodiversity across time and space.

9.1.1 Correlative Approach

The first intuitive approach to study the patterns of geographical variation in biodiversity is to analyze the association between biodiversity aspects (e.g., species richness) and geographically variable extrinsic factors. For example, the classic latitudinal diversity gradient (LDG) of species richness that describes an inverse relationship between species richness and latitude (Pianka 1966; Willig et al. 2003; Hillebrand 2004), is usually explained by the association between species richness and environmental factors that vary with latitude (Currie et al. 2004). These "environmental hypotheses" are based on the assumption that species are in equilibrium with the environment, directly responding to the current climate conditions (Currie et al. 1999) mainly by constraining their geographical distributions (Gaston 2003). Among the most important environmental factors associated with the geographical variation in species richness are solar radiation, temperature, precipitation, and productivity (Rohde 1992; Currie et al. 2004; Hawkins et al. 2003; Mittelbach et al. 2001). In general, all these factors increase toward equatorial latitudes where species richness also increases (Gaston 2000). This association between geographical (e.g., latitude) and environmental factors (e.g., temperature) with species richness has been observed in most terrestrial taxa studied so far (from microorganisms to

plants and animals) (Willig et al. 2003; Hillebrand 2004; Andam et al. 2016; Willig and Presley 2018; but see Prieto-Torres et al. 2019).

Based on this correlative approach, macroecologists have studied different dimensions of biodiversity beyond species richness. Biodiversity dimensions considered important components of macroecological studies include species turnover or beta diversity, functional diversity, phylogenetic diversity, and biotic interactions (Schemske et al. 2009; Oliveira et al. 2016; Voskamp et al. 2017; Dallas and Poisot 2018). All these components of biodiversity can be associated with geographical factors such as latitude, and potentially associated with environmental factors such as temperature and precipitation (Melo et al. 2009; Safi et al. 2011; Schleuning et al. 2012; Dallas and Poisot 2018; see also Chap. 7). Additionally, another important research topic in macroecology is the analysis of causal patterns and processes that generate the spatial distribution of species traits (Brown et al. 2003). Examples include abundance, body and geographical distribution sizes, and species-area relationships (Marquet 2009). It has been shown that the spatial and statistical distributions of these traits are similar among most organisms, from bacteria to plants and animals, thereby earning to be entitled as "Ecogeographical rules" (Gaston et al. 2008). Some examples of these rules are as follows: (i) Bergmann's rule, which postulates that organisms tend to have larger body sizes in cold than in warm environments (Blackburn et al. 1999); (ii) Taylor's law, which posits that the variance of species abundances follows a power law function of the mean abundance of the species (Taylor 1961); and (iii) Rapoport's rule, which postulates that the size of distributional area of species is positively related to latitude (Rapoport 1982; Stevens 1989; see Stephens et al. 2016 for a review of macroecological patterns on parasite communities). As with the correlative approach, these ecogeographical rules describe the spatial patterns of species traits, but do not identify or specify the causal processes generating these patterns.

Regarding antagonistic interactions (those in which at least one of the interacting species is negatively affected), it has been observed that species richness patterns of some parasites and herbivores can be either positively (e.g., Andrew and Hughes 2004; Nunn et al. 2005) or negatively associated with latitude (e.g., the diversity pattern exhibited by parasitic wasps of the Ichneumonidae family; Owen and Owen 1974). Likewise, it has been observed that not only species richness but also interaction frequency can be associated with latitude (McArthur 1972; Merino et al. 2008; Zamora-Vilchis et al. 2012; Roslin et al. 2017). For example, a higher level of host specificity on Lepidoptera larval stages has been reported toward the tropics (Dyer et al. 2007). Moreover, a higher prevalence of parasites of the genera *Haemoproteus* and *Plasmodium* on birds has been reported at equatorial compared to temperate latitudes (Merino et al. 2008; Clark et al. 2016), as well as on locations in low elevations and with high mean annual temperature (Zamora-Vilchis et al. 2012). Recently, with a series of standardized experiments around the globe using artificial caterpillars, a higher predation rate at tropical latitudes compared to that at temperate latitudes was reported (Roslin et al. 2017). The geographic pattern of predation rate seems to be related to a higher diversity and abundance of species from both predators and caterpillars at lower altitude locations and close to the equator, which

generates a higher parasite intensity and predation pressure at equatorial latitudes (Roslin et al. 2017). Nonetheless, the frequency of interactions is not always associated with the latitudinal gradient. For example, seed dispersion patterns and herbivory by beetles on a species of *Acacia* do not seem to increase at equatorial latitudes (Andrew and Hughes 2004, 2005; Chen et al. 2017). Indeed, empirical evidence on patterns of antagonistic interactions associated with geographical and environmental gradients is still underrepresented in the literature (see Chap. 7 for a perspective from ecological niche modeling and Chap. 10 for a summary of effects of environmental gradients on avian haemosporidians).

Despite progress in macroecological theory, the correlative approach does not provide direct information about the causal process and factors that generate the observed biodiversity patterns (Ricklefs 2004; Beck et al. 2012; but see Shipley 2016 for a framework on how observational correlative data can be used to infer causation). Therefore, to evaluate potential causal processes of biodiversity patterns, macroecologists have developed new approaches based mainly on computer simulations where experiments are more feasible. The aim of these mechanistic approaches is to uncover the actual mechanisms driving biodiversity patterns across different spatial and temporal scales.

9.1.2 Mechanistic Approach

Owing to the nature of the primary data used in macroecology, it is challenging to develop experiments isolating and testing different macroecological hypothesis empirically (Pontarp et al. 2019). In order to analyze and search for the mechanisms driving any biodiversity pattern and ecogeographical rules, macroecologists now rely on sophisticated statistical and computational methods such as mathematical modeling, null models, and computer simulations (Villalobos and Rangel 2014; Cabral et al. 2017; Pontarp et al. 2019). Computer simulations and mathematical modeling allow evaluating the relative effect of hypothesized physical or biological processes on biodiversity patterns, mainly by contrasting expected patterns under such processes vs those observed in nature (Cabral et al. 2017). Under this approach and considering ecological interaction patterns, it has been possible to evaluate how biological neutral processes such as dispersal and evolutionary rates drive some of the properties of interactions such as organization patterns and phylogenetic structure (Coelho et al. 2017; Coelho and Rangel 2018). For instance, using stochastic simulation models, it has been reported that sites with higher speciation rates and species with lower dispersal rates present networks with higher values of phylogenetic structure (i.e., species that are phylogenetically close tend to share the same partners from another trophic level) than sites with lower speciation rates and species with higher dispersal rates (Coelho et al. 2017). These simulations highlight the importance of modeling processes that are considered relevant for ecological structure and that evaluate their relative effect in driving the organization of interaction patterns (e.g., Ritchie 2010).

9.2 The Complexity of Antagonistic Networks

The central study unit of macroecology are the species. However, no single species is completely isolated in nature; instead, they interact with other organisms from the same or different species in order to carry out their life cycles, either for reproduction, development or survival (Andersen et al. 2018). Therefore, species interaction patterns have become highly relevant to macroecologists. The properties that emerge from ecological interactions can be described and studied by applying graph theory through the construction of complex interaction networks (Dáttilo and Rico-Gray 2018), where species are represented as nodes and their interactions as links (Bascompte et al. 2003). This approach allows investigating and analyzing emergent properties that cannot be studied or observed by considering each community or interaction pair independently (Pellissier et al. 2018).

For example, if we wish to study antagonistic interactions such as vector-borne diseases, studying the main vector species of a disease may not be sufficient, given that several diseases can be transmitted by many different vector species. Also, it is important to consider that throughout the life cycle of a pathogen, the reservoir species are highly important for the spreading of the disease. Therefore, to fully understand the ecological patterns predicting disease presence, it is necessary to study the whole community or assemblage of both trophic levels (reservoir and vector species) and to determine how these species interact with each other. The interaction dynamics of both trophic levels form a complex system with properties that cannot be observed if we study each community (reservoir or vector) in isolation. Thus, the dynamics of interactions between species can be analysed as complex interaction networks with emergent properties that vary through time and space (Fig. 9.1).

Owing to the methodological difficulty of recording all interactions across all trophic levels in an ecosystem, ecological studies of interactions usually work with

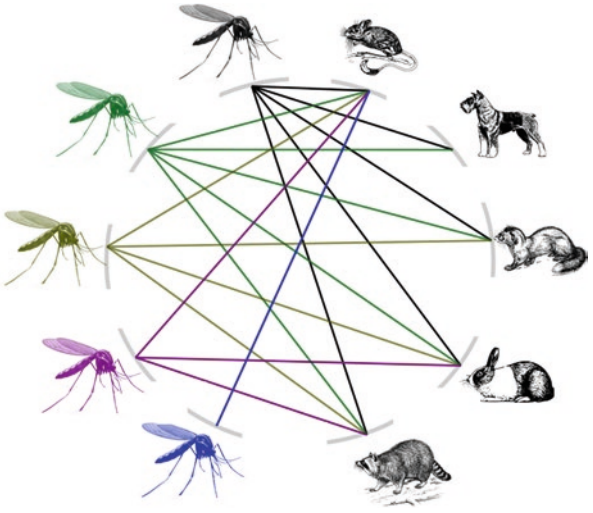

Fig. 9.1 Antagonistic interaction network between a community of mosquitoes that are vectors of a pathogen and a community of reservoir mammals. Lines represent the interactions between the species of mosquitoes and mammals

only two trophic levels; those networks are called “bipartite networks” (Bascompte et al. 2003). To generate bipartite networks, it is necessary to record the interactions on a matrix where rows represent species of one trophic level (i.e., mosquitoes/vectors) and columns represent the species of the other trophic level (i.e., mammals/reservoirs). The interaction matrix is filled with the observed occurrences or the frequencies of the interactions between species pairs of one trophic level and the other (Fig. 9.2).

There are different approaches for studying interaction networks (see: Campião & Dáttilo 2020; Dáttilo et al. 2020). For instance, if the interest of the study is to analyze the importance of each species within the network, then it is important to identify central (i.e., species with many network connections) or peripheral species (i.e., species with few network connections). Alternatively, if the focus of the study is to describe interaction dynamics of the whole system, then it is necessary to analyze the properties and structure of the complete network. There are several network descriptors at both species and network level that describe interaction networks (reviewed recently by Antoniazzi et al. 2018). In the following Table 9.1, we show some of the more studied network descriptors used in the literature.

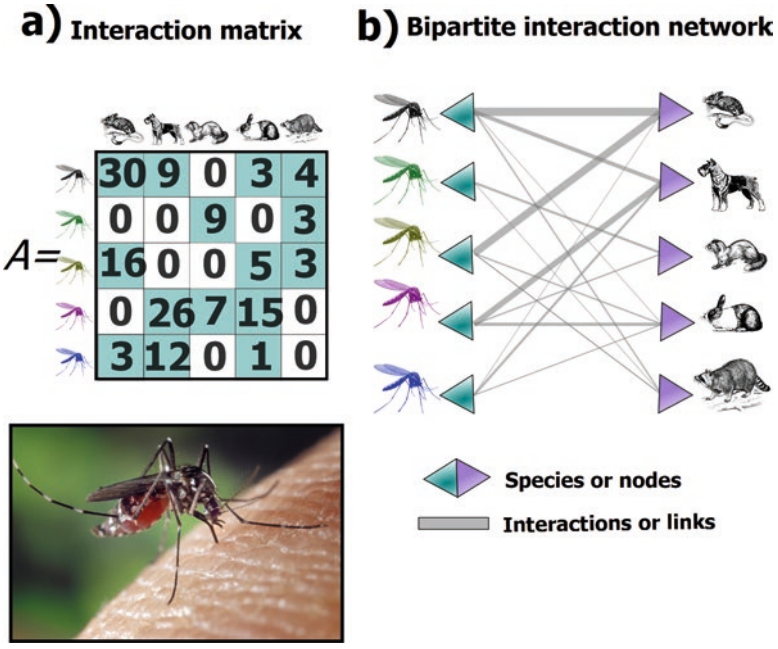

Fig. 9.2 Antagonistic interactions represented as (**a**) Interaction matrix, where rows represent the species of one trophic level and columns represent the species of the other trophic level. The matrix is filled with 0 and 1 for the absence or presence of the interaction, or with the observed frequency of each interaction. (**b**) Interaction network, where species are represented as nodes and interactions as links. Link width represents the observed frequency of each interaction

Table 9.1 Major network properties or descriptors used in the literature to describe interaction patterns

Level	Descriptor	Description
Network	Network size	Number of species or interactions within the network (Delmas et al. 2017)
	Connectance	Proportion of the realized interactions relative to all possible interactions (Jordano 1987)
	Nestedness	Degree to which specialist species (engaged in few interactions) interact with generalists (engaged in many interactions) (Bascompte et al. 2003)
	Modularity	Degree to which a network is divided into distinct sub-groups (Olesen et al. 2007)
	Vulnerability	Mean number of links of the lower trophic level (Bersier et al. 2002)
	Generality	Mean number of links of the higher trophic level (Bersier et al. 2002)
	Specialization (H_2')	Measures how evenly distributed the weighted interactions are in a network (Blüthgen et al. 2006)
Species	Specialization (d')	Describes the degree of specialization of each specie (Blüthgen et al. 2006)
	Nestedness contribution	Contribution of each specie to the nested structure (Saavedra et al. 2011)
	Among-module connectivity (c_i)	Degree of individual species' connection to all modules (Olesen et al. 2007)
	Within-module connectivity (z_i)	Degree of individual species' connection within its module (Olesen et al. 2007)
	Core-peripheral	Classify each species as a core or peripheral component of the network depending on its number of links (Dáttilo et al. 2013)

Network structure analysis has been used to study the stability and fidelity of the interactions between species. A common structure pattern observed on interaction networks is nestedness. Nestedness is a non-random topological pattern of ecological networks where species tend to interact with subsets of species with high number of interactions (commonly called generalists or core species), which also interact among themselves (Bascompte et al. 2003). This pattern generates a high level of network asymmetry, with a core of highly connected generalist species and a subset of peripheral species mostly interacting with the core set (Fig. 9.3). The structure of nested networks is highly stable to stochastic phenomena, which minimizes competition between species and increases biodiversity (Bastolla et al. 2009), probably owing to the presence of the core species that can sustain most of the interactions even if some peripheral species disappear from the network (Rohr et al. 2014).

The degree of association, or intimacy, between the interacting species is one of the properties that explain most of the antagonistic networks structure. On the one hand, antagonistic interactions with low intimacy such as predation and/or some ectoparasites, where the interacting species have high dispersal capacity and a wide number of species to interact with during their life cycle, exhibit the higher

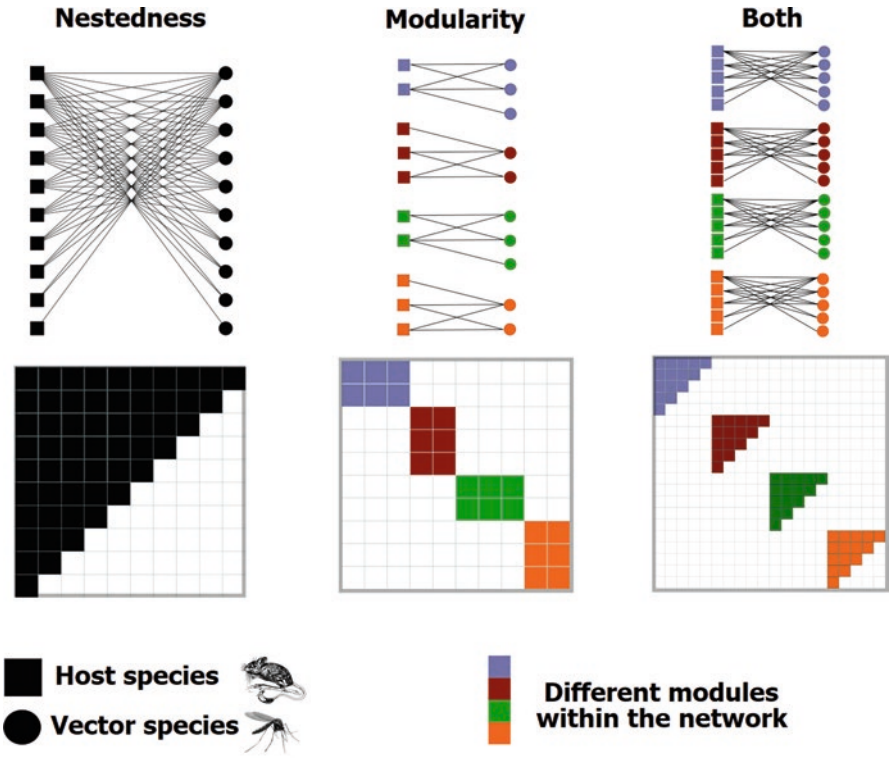

Fig. 9.3 An illustration of interaction patterns reported in antagonistic networks. Top row presents the network structure of the interactions and the bottom row depicts the matrix structure of the interactions

nestedness among antagonistic networks (Pires and Guimarães 2013). On the other hand, antagonistic interactions with higher intimacy such as endoparasitism or herbivory, where the species have a restricted number of hosts or low probabilities to change hosts during their life cycles (Graham et al. 2009), can present a network structure called "modularity" (Pires and Guimarães 2013). Network modularity arises when subsets of highly interacting species (modules) interact more amongst themselves than with other species in the network (Fig. 9.3). Furthermore, it is possible for networks to simultaneously exhibit nestedness and modularity, whereby within each module there are species with higher number of interactions and a subset of species with lower number of interactions, as reported for some host-parasite interactions (e.g., flea-mammal; Felix et al. 2017) (Fig. 9.3).

Several studies have reported that antagonistic interaction networks are highly specialized and phylogenetically structured (Cagnolo et al. 2011). Phylogenetic structure in antagonistic interaction arises when closely related species also have similar traits, thus interacting with similar species of the other trophic level in phylogenetic clusters. This pattern, also called "phylogenetic signal" of the interaction network, seems to be caused by co-evolutionary processes between interacting species (Cagnolo et al. 2011). Depending on how strong the phylogenetic signal is (i.e.,

how closely related species of one trophic level interact with similar species of a second trophic level) (Krasnov et al. 2012) one may expect a trade-off between the performance and the number of partner species within the network (generality) (Pinheiro et al. 2016; Felix et al. 2017). Evidence for this is mixed, even within the same system. Whereas Hellgren et al. (2009) failed to find such a trade-off in avian malaria, Pinheiro et al. (2016) did find support for this trade-off, but only after taking phylogenetic clusters into account and using a network approach. Using a network approach with flea-mammal interactions. it has been reported that the relation between performance and generality vary from positive to negative depending on the network scale studied (Felix et al. 2017). If the whole network is analyzed then the relation between performance and generality is negative but within each module of the network the relation is positive (Felix et al. 2017).

Despite the importance of the information generated by interaction network analysis to describe the emergent properties of antagonistic interactions, there are some biases in the data used in such analyses that must be bear in mind. First, depending on the study's goal, researchers must consider that there is species turnover across space (i.e., associated with spatial heterogeneity) and time (e.g., the optimal hour of the day for foraging of a mosquito community varies from species to species, or the turnover associated to the seasonality of the environment; e.g., Abella-Medrano et al. 2015). In addition, mismatches in temporal and spatial distributions of species can result in absences of theoretically possible interactions. These so-called "forbidden links" illustrate that not only biology and chemistry between species must be compatible, but that time and space ultimately dictate whether links have an opportunity to form (Olesen et al. 2010; see Chap. 7 for an explanation of biotic, abiotic, and dispersal capacity factors affecting the realization of a host-parasite interaction). If these changes in the occurrence and abundance of the studied species are ignored, the data will not be a representative sample of the system dynamics and networks will be biased to the site, region, hours, or season sampled. In order to know if the sampled data are representative to answer our research questions, the use of accumulation curves of interactions, similarly to those of species richness, allows researchers to evaluate if the sampling effort has captured enough interactions to be considered representative (Falcão et al. 2016).

Second, one has to consider the total number of species on the network from both trophic levels and the overall network size (Luna et al. 2017). It has been reported that small networks or networks with a very low number of species on some trophic level can generate a high variation of the calculated network descriptors when small changes occur on the presence and abundance of the interactions (Luna et al. 2017). In general, if the interaction patterns studied form very small networks, then it is better not to use the interaction network approach. A potential solution for this richness/size bias is to use standardized network descriptors (Luna et al. 2017), which are obtained by generating a stochastic distribution of the data using null models. This approach has been used to evaluate how some property of the networks (e.g., modularity, nestedness) varies across a gradient or factor controlling the differences on the number of species or network size and connectance, both for binary and quantitative networks (Bascompte et al. 2003; Dormann et al. 2009).

9.3 Macroecology of Antagonistic Interaction Networks

The increasing number of data available on interaction networks or interaction patterns around the globe has allowed researchers to generate macroecological studies, mostly focused on the correlative approach where different network properties are related to geographical or environmental gradients (Schleuning et al. 2014; Martín-González et al. 2015; Dalsgaard et al. 2017). For example, it has been observed that the nestedness and modularity of interaction networks can be positively or negatively correlated with geographic and climatic factors, such as latitude, temperature and precipitation on mutualistic networks (Trøjelsgaard and Olesen 2013; Schleuning et al. 2014; Dalsgaard et al. 2017). In these cases, networks in sites with higher current climatic stability and energy (e.g., higher and stable temperature and/or precipitation) have higher modularity and lower nestedness, probably owing to a higher specialization and diversity of species from both trophic levels (Trøjelsgaard and Olesen 2013; Schleuning et al. 2014; Dalsgaard et al. 2017). In addition to current (monthly or yearly) climate stability, higher historical (over longer time scales; e.g., millennia) climatic stability also favors the presence of networks with higher modularity and low nestedness (Dalsgaard et al. 2013; Schleuning et al. 2014).

Regarding antagonistic interactions, it has been reported that network specialization does not change toward temperate or tropical forests (Svensson-Coelho et al. 2014). Equally, herbivore-plant, flea-mammal, and bird-malaria interaction networks are not associated with geographical gradients such as latitude, even when species richness from both trophic levels is high. This pattern may be explained because antagonistic interactions are highly specialized, with such specialization being related to the invariance of the interaction networks along the studied geographical gradients (Morris et al. 2014; Guilhaumon et al. 2012). Until now, only a few studies have been conducted on this topic and there is certainly a need to perform more correlative studies across geographical and environmental gradients to better understand how antagonistic interaction networks vary through space. It is also necessary to apply mechanistic approaches to evaluate which processes are driving the observed antagonistic interaction patterns across time and space (Coelho et al. 2017; Coelho and Rangel 2018).

9.3.1 The Distance Decay of Similarity in Antagonistic Networks

A commonly observed pattern on the study of ecological communities and one of the main macroecological patterns is the distance decay of similarity (Nekola and White 1999). This pattern is observed after applying a regression model between community similarity and geographic distance, in which community composition decreases as a function of geographic or climatic distance (Buckley and Jetz 2008). Distance decay of similarity shows how climatic conditions can constrain the

presence and abundance of species across geographical and climatic gradients (Buckley and Jetz 2008; see Chap. 7). The same approach has been adopted to study antagonistic interactions, where the analysis focuses on evaluating the distance decay pattern of interaction pair's dissimilarity instead of species dissimilarity (Trøjelsgaard et al. 2015; Dallas and Poisot 2018). In this case, parasite and host species composition follow the distance decay of similarity pattern whereas the interaction composition (beta diversity of interactions) does not follow such a pattern (Dallas and Poisot 2018). On the one hand, a potential reason for these results is that the high specialization and modularity of the antagonistic networks provide species with redundant roles despite compositional changes (Dallas and Poisot 2018). On the other hand, given that the distance decay of similarity in interactions has been mainly based on geography (spatial distance), it is possible that compositional changes of interactions on antagonistic networks are more associated with climatic factors than with geography (Dallas and Poisot 2018; Rodríguez-Hernández et al. unpublished).

Such dissimilarity of species interactions, also called β diversity of interactions (β_{wn}), can be partitioned into two additive elements (Poisot et al. 2012) (Fig. 9.4). One additive element of the β diversity of interactions is generated by the species turnover between communities (β_{st}) (Poisot et al. 2012). The second additive element, called interaction rewiring (β_{rw}), is composed by the rearrangement of species

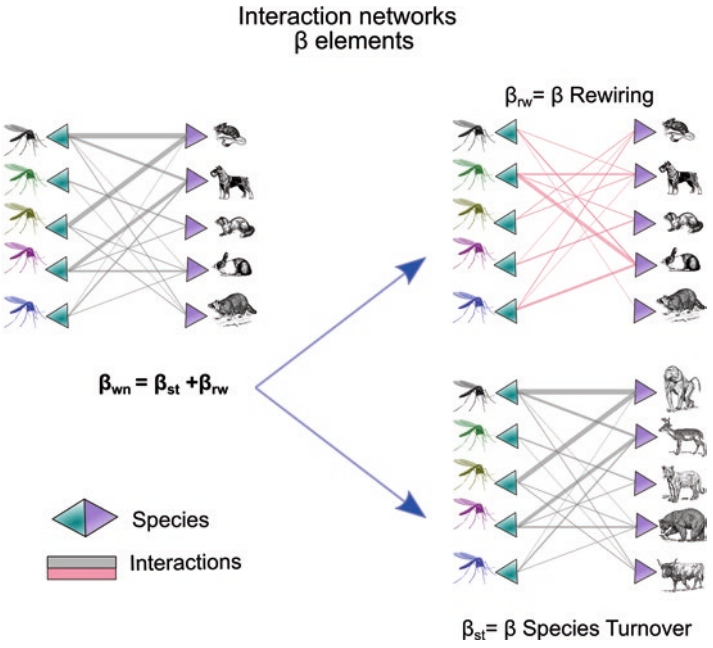

Fig. 9.4 An illustration of the β diversity of interactions (β_{wn}) and its components: species turnover (β_{st}) and interaction rewiring (β_{rw})

interactions among communities (Poisot et al. 2012). In mutualistic networks, it has been observed that the importance of the two elements of the β diversity of interactions vary depending on the geographical distance among networks (Trøjelsgaard et al. 2015). If the geographical distance between two sampled networks were close, it is expected that species communities would be similar (low β_{st}) and then the rearrangement of the interactions is important (high β_{rw}) (Trøjelsgaard et al. 2015). However, if the geographical distance between two sampled networks were higher, then it is expected that species communities would be different (high β_{st}) and the rearrangement of interactions lower (Trøjelsgaard et al. 2015).

Another approach for studying the distance decay pattern is to create a metaweb of a region – built with the complete set of interactions recorded on the local networks – and calculate the dissimilarity of this metaweb with each local network sampled. Such dissimilarity value can be used to identify hotspots of interactions (i.e., localities with low dissimilarity of interactions within the metaweb) or coldspots of interactions (i.e., localities with high dissimilarity of interactions within the metaweb) (Poisot et al. 2012). This information can be associated, for example, with geographical and climatic gradients in order to evaluate if species and interactions are filtered by the same factors (Poisot et al. 2012, 2017; Rodríguez-Hernández et al. unpublished).

9.4 Conclusion and Future Perspectives

The joint application of both research areas, macroecology and interaction networks, can help researchers elucidate how antagonistic interactions vary over geographic and climatic gradients. Nonetheless, there is still a lack of information of most antagonistic systems. For instance, despite the progress on theoretical and empirical studies of antagonistic interaction networks, one of the biggest challenges of antagonistic interaction studies is to obtain the exact identity of species from both trophic levels, since parasite species delineation is a whole research field on its own. This makes interactions from especially highly diverse environments, such as the tropics where there are several cryptic and unknown species, difficult to characterize. Recently, it has been suggested the use of DNA barcoding tools to facilitate species identification within interaction networks (Kress et al. 2015; see Chaps. 2 and 4). However, tropical areas remain a hurdle for this approach because there are no taxonomically determined voucher specimens that can serve as reference material for DNA databases.

In order to extract some generalities as well as causes of antagonistic interaction patterns, it is necessary to perform more studies of interaction networks across the world considering broad spatial and environmental gradients. Adopting tools that facilitate the recording of interactions can aid in such an effort, or at least the potential description of these by means of species distribution or niche modeling (Kissling et al. 2012; Morueta-Holme et al. 2016; see Chaps. 4, 6, and 7). Indeed, based on this latter approach, it is possible to use the co-occurrences of species from both

trophic levels as a proxy of their potential interactions (Morueta-Holme et al. 2016). Moreover, if the geographical distributions (observed and potential) of species are used in future interaction studies, it can be possible to evaluate the impact of future environmental changes such as the current climate changes on the properties of antagonistic networks (Kissling et al. 2012; see also Chap. 7 for an in-depth discussion of ecological niche and species distribution models).

In conclusion, from a macroecological perspective, it has been observed that some antagonistic network properties vary across geographical and climatic gradients. More specifically, so far, it seems that the structure of antagonistic networks does not change with latitude or some associated climatic gradients. Nonetheless, beta diversity of interactions of antagonistic networks does seem to be associated with geographic and climatic gradients, and, more importantly, these interaction patterns are not associated with the same factors as species richness within communities. Finally, we call for more studies of ecological networks at the local level in order to provide further primary data to analyze how antagonistic interactions change along spatial and temporal gradients. We also call for a more integrative approach on interaction studies, mainly through the combination of new mathematical, computational (e.g., niche modeling), and methodological (e.g., barcoding) tools to improve our understanding on the components and processes driving the dynamics of antagonistic interactions across space and time.

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Chapter 10
Effects of Ecological Gradients on Tropical Avian Hemoparasites

Leonardo Chapa-Vargas, Nubia E. Matta, and Santiago Merino

Abstract This chapter provides a brief overview of how natural gradients (e.g., latitude, altitude, and landscape gradients) affect host–parasite interactions involving blood parasites in wildlife and how biotic and abiotic factors act as disruptors. These gradients have a direct impact on prevalence, parasitemia, and the observed relationships between parasites and hosts. In the tropical zone, altitudinal gradients imitate the behavior of the latitudinal gradient, since low temperatures are common at both higher altitudes and higher latitudes. Temperature is one of the determining factors of the diversity of vectors, hosts, and vegetation that affect parasite transmission cycles. Furthermore, within landscapes, there may be many types of elements producing gradients. For instance, increasing distance from water sources, anthropogenic degradation, and even sequential stages of succession and interspersion of vegetation communities would affect host–parasite–vector interactions. However, such effects do not always operate in the same direction because responses are context sensitive. We also discuss the importance of an integrative diagnosis, using microscopic and molecular approaches, which allow better approximations and analyses at the parasite species level, thus producing stronger conclusions. The same detail is recommended for studies on the hematophagous fauna of potential vectors. The life cycle of different parasite species has its own set of characteristics, and it corresponds to the researchers to unravel the puzzle and to avoid unwarranted generalizations.

L. Chapa-Vargas (✉)
División de Ciencias Ambientales, Instituto Potosino de Investigación Científca y Tecnológica A.C, San Luis Potosí, S.L.P., Mexico

N. E. Matta
Departamento de Biología, Grupo de Investigación Caracterización Genética e Inmunología, Sede Bogotá-Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia

S. Merino
Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain

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10.1 Introduction

Gradients in ecological systems may be defined as gradual changes in some characteristic of interest that may influence some ecological process (Stevens 1992; Rohde 1992). There are many types of gradients in nature, and they may operate at different spatial scales. In addition, the effects of different types of gradients on ecological processes may interact, thus either enhancing or disrupting the response of the process involved. Some examples of gradients are those originating from latitude (i.e., position within continents in relation to the equator), elevation, temperature, distance from water sources, and distance from different types of anthropogenic degradation or natural disturbances to name a few (Fig. 10.1).

Latitude is, perhaps, the type of gradient generating the largest variation in biological diversity. Species richness for most taxonomical groups increases from the poles toward the equator (Turner et al. 2001). Although haemosporidians have been recorded throughout most of the planet and are only absent from polar regions (Merino et al. 1997a; Valkiūnas 2005; Martínez et al. 2018), they also follow the

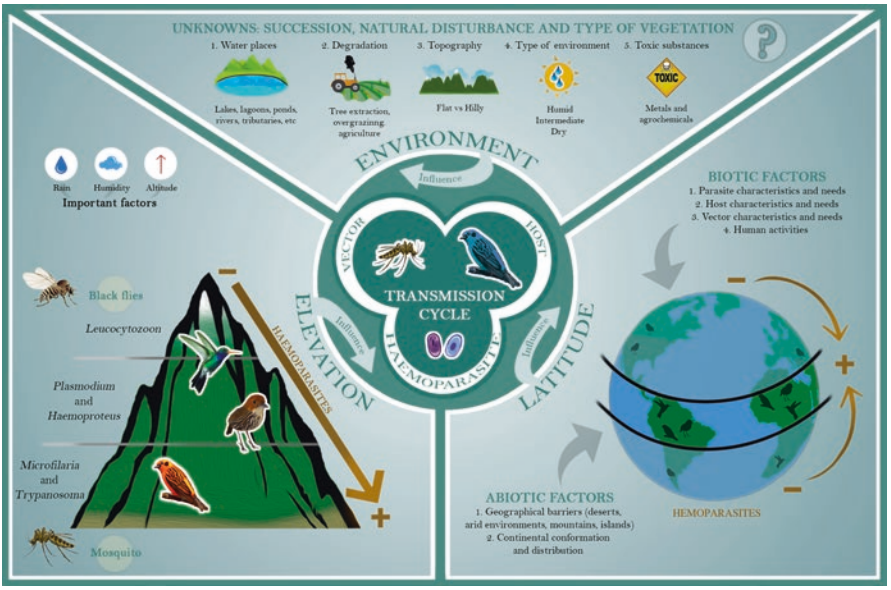

Fig. 10.1 Summary of factors influencing host–vector–parasite relationships involving hemoparasites

latitudinal gradient (Durrant et al. 2006; Merino et al. 2008; Szöllősi et al. 2011; Oakgrove et al. 2014; but see Fecchio et al. 2019a). Indeed, diversity of haemosporidian morphospecies and lineages is highest near the equator (see Chap. 9 for a thorough introduction to macroecology and ecological networks). Patterns of prevalence may also change throughout latitudinal gradients (Doussang et al. 2019) and in many cases, the variable analyzed in parasite studies of gradients is prevalence and not species richness, probably due to difficulties to assign species to blood parasites (see, e.g., Nilsson et al. 2016). The gradient in species richness imposed by latitude is further modified by distance from coastal lines. In general, within continents, species richness is usually highest near the coastline and decreases toward inland areas due to the change in humidity (Turner et al. 2001). Because Diptera vectors have higher reproductive rates associated to high humidity, haemosporidian transmission and diversity are likely higher in moist environments in comparison to dry lands (Lacorte et al. 2013). However, dry environments such as deserts also provide opportunities for vector reproduction such as the presence of water sources (e.g., springs, rivers and tributaries, and cacti which hold water; see Chaps. 5 and 6 for an in-depth introduction to Diptera families involved in avian haemosporidian transmission, as well as on an ecological synthesis of vector research in avian haemosporidians).

Altitude is an important driver of variation in climate and in vegetation; in general, higher altitudes have cooler conditions, and mountains tend to receive more precipitation in comparison to valleys. However, due to water runoff, foothills receive some of the highest amount of humidity and are crossed by rivers and tributaries in areas that are complex in topography, usually carrying running water with high oxygen contents (Butler and Hogsette 1998; Turner et al. 2001). Within some climatic regions such as those in which slopes face coastlines, where humidity tends to be high, vegetation varies partially as a response to the temperature gradient associated to altitude, but also as a response to other factors such as slope and aspect (Swanson et al. 1988). At finer scales, vegetation is further influenced by soil types and by the large variety of microclimates that originate by the complex patterns that topography produces (Swanson et al. 1988). Haemosporidians, their hosts, and vectors respond to these sources of environmental variation. Consequently, fine-scale patterns in hemoparasite diversity and in prevalence are likely associated to these complex patterns (e.g., Renner et al. 2016). In general, diversity of hemoparasites and their vectors, as well as prevalence, decreases with decreasing temperature; however, each haemosporidian genus may respond differently to the same environmental features (e.g., Santiago-Alarcon et al. 2019). A recent meta-analysis revealed that haemosporidian diversity is closely related to patterns of host endemism in the Andes ecosystems, peaking at mid-elevations (i.e., 2000–2500 m asl); furthermore, the spatial distribution of avian haemosporidians was closely associated with areas of avian endemism across large geographical scales in the tropical Andes (Gil-Vargas and Sedano-Cruz 2019).

Natural and anthropogenic disturbances and habitat degradation dramatically modify structure and composition of natural vegetation communities, thus creating additional environmental gradients (e.g., Hernández-Lara et al. 2017). Trends in

parasite–vector–host interactions frequently respond to these disturbances and to habitat degradation. Responses to degradation depend on the type of impact, whereas some types of degradation may influence haemoparsites positively, other sources may have negative effects (e.g., Sehgal et al. 2011; Renner et al. 2016; see Chap. 13 for an introduction to urban and landscape ecology, and Chap. 14 for a synthesis of anthropic impacts on avian haemosporidian ecology). In the following sections, we provide a more detailed review of current knowledge regarding some of the above-mentioned types of gradients, and how these gradients may influence blood parasite–vector–host interactions within the Neotropics (Fig. 10.1).

10.2 Latitudinal Gradients in Bird Blood Parasite Richness

The existence of latitudinal gradients in species richness of free-living organisms has been reported in many studies (Pianka 1966; Rohde 1992; Stevens 1992; Huston 1999; Chown and Gaston 2000; see Chap. 9). However, there are very few cases reported for pathogenic organisms (see Hillebrand et al. 2001; Curtis et al. 2002; Nee 2003; Guernier et al. 2004; Guégan et al. 2005) and even less if we look at avian blood parasites (Durrant et al. 2006; Merino et al. 2008; Szöllősi et al. 2011; Oakgrove et al. 2014). Based on these data, the initial hypothesis is that there will exist a maximum of diversity or richness of parasites in tropical areas (i.e., low latitude) and that these values will decrease as we move toward the poles where climatic conditions will limit the growth of vector populations and transmission of blood parasites. For example, several studies report low incidence of blood parasites in birds from polar areas as compared with prevalence at other latitudes (Greiner et al. 1975; Merino et al. 1997a; Loiseau et al. 2012; Martínez et al. 2018; but see Meixell et al. 2016).

Probably, the first insight on the prevalence of blood parasites of birds and latitude was the report by White et al. (1978) indicating that the Neotropics showed much lower parasite prevalence and a near absence of *Leucocytozoon* infections (this is partly explained by lack of research in tropical areas; see Lutz et al. 2015; Outlaw et al. 2017 for reviews) in comparison with a similar review of Nearctic avian hematozoan distribution reported by Greiner et al. (1975) (see Chap. 1 for a thorough review of avian haemosporidian research conducted during the twentieth century). Among the papers reporting blood parasite differences between latitudes, we can also cite the work by Durrant et al. (2006), who found a higher prevalence of *Haemoproteus* and *Plasmodium* in birds from Guyana as compared to prevalence in bird species from Uruguay. They also observed higher prevalence of infection by *Plasmodium* than by *Haemoproteus*. These results are in contrast with those from Chile (found at similar latitudes of Uruguay) where Merino et al. (2008) found higher prevalence of *Haemoproteus* and lower prevalence by *Plasmodium*. These facts were attributed to different vector abundance or activity in Chile compared to Uruguay. Differences could also be related to dissimilarities in habitat and climate conditions between the Atlantic and the Pacific sides of the continent, even at the

same latitude (Merino et al. 2008). Studying the distribution of several genera of blood parasites infecting birds along a latitudinal gradient that includes the world's southernmost forest ecosystems, Merino et al. (2008) found a positive significant relationship between prevalence and latitude for *Leucocytozoon* lineages and a negative relationship for *Haemoproteus*, *Plasmodium,* and coinfections. However, they did not find a significant relationship between parasite diversity and latitude, suggesting that some parasite lineages may evolve locally in isolation within some host species (see also Clark 2018). By using a global database of lineage distributions of *Plasmodium* and *Haemoproteus*, Clark et al. (2014) tested the relationship between diversity among those parasites and their avian hosts across 13 geographic regions. They found that geographic distributions of parasite genera differed, with *Haemoproteus* spp. being absent from the majority of oceanic regions while *Plasmodium* spp. being cosmopolitan and especially diverse in the Neotropics. Overall, these authors found a biogeographic pattern of higher diversity of blood parasites in low-latitude tropical areas. They suggested that there are differences in the way avian haemosporidian diverge and colonize new communities, but also that better estimates of avian haemosporidian diversity patterns are needed. Clark (2018) failed to find a latitudinal gradient in global variation in avian haemosporidian phylogenetic diversity, pointing toward an effect of differences in hosts-switching tendencies or the timing of avian evolutionary radiations to explain biogeography of these blood parasite infections. However, his results could be affected by biases in sampling effort that concentrates in higher latitudes in North America and Europe (Clark et al. 2014).

Szöllősi et al. (2011) found that prevalence of *Plasmodium* and *Haemoproteus* differed consistently between parasite lineages and host populations of blue tits (*Cyanistes caeruleus*) across Europe, indicating that the transmission success of parasites is lineage specific but also dependent on locality. They also found that parasites with high prevalence were widely distributed among blue tit populations and were found to infect more host species. Thus, they suggested that parasites with high local prevalence can have a wide distribution at a global scale and that neighboring host populations shared more parasite lineages compared to geographically more distant locations.

In another work, Oakgrove et al. (2014) studied resident and migratory bird species across a latitudinal gradient in Alaska and looked for associations between bioclimatic conditions and the distribution of parasite prevalence. They found an effect of latitude on *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* prevalence, which decreases toward the north, but they detect other factors affecting these parasites. For example, *Leucocytozoon* was the genus with the highest diversity, prevalence, and coinfection prevalence; temperature seasonality, precipitation, and tree cover were the environmental factors affecting the prevalence of coinfection probability, which indicates the need for a minimum of temperature, water, and tree cover for development and successful transmission of these parasites.

It is difficult to conclude and generalize what specific factors affect parasite latitudinal gradients. Hence, we next discuss some of the factors that we consider most relevant that could affect the existence of latitudinal gradients in diversity and

prevalence of avian blood parasites. We group them by their origin in two sets, biotic and abiotic disruptors of ecological gradients, but in some cases, one disruptor could be included in both categories.

10.2.1 Disruptors of Biotic Origin

There exist a large taxonomic diversity of blood parasites infecting birds (see Chaps. 1 and 2). These taxa have different life cycles and require different conditions to complete their cycles (Valkiūnas 2005). Therefore, ideal conditions for one parasite may not be optimal for another and vice versa. For example, *Leucocytozoon* appears to be better adapted to complete their cycle in cold and mountainous climates that may not be suitable for other parasite genera (Merino et al. 1997b; Merino et al. 2008; Oakgrove et al. 2014; but see Chap. 1 where studies have shown that *Leucocytozoon* spp. are widely distributed across Africa). This could be, at least partially, by the fact that black flies, the main vectors of *Leucocytozoon*, need running water for their development and reproduction (see Chaps. 5 and 6). Such water conditions are common in mountainous areas after the melt of snow. Thus, a potential disrupting factor when looking for latitudinal gradients of blood parasites infecting birds is the inclusion of different study areas, which will be more or less suitable for different vector taxa, as well as the parasites they transmit. For example, Merino et al. (2008) found a positive significant relationship between prevalence and latitude for *Leucocytozoon* lineages, but a negative relationship for *Haemoproteus*, *Plasmodium*, and coinfections. The different pattern for *Leucocytozoon* could be attributable to more suitable habitats for simuliid vectors at high latitudes in Chile. This relationship between leucocytozoids and high latitudes has been reported previously in other regions (see Greiner et al. 1975).

There are some host species and families that appear to be less susceptible to infections by blood parasites, and their inclusion in studies of latitudinal gradients of blood parasites may yield results that complicate the identification of the gradient. For example, Psittaciformes and many sea birds are not frequently infected with blood parasites (Quillfeldt et al. 2011). The explanation for this fact is not clear and many hypotheses have been suggested; among them, the lack of favorable habitat for at least some vectors, the resistance of the taxa involved, or even eating habits (see Mendes et al. 2005; Quillfeldt et al. 2014; Masello et al. 2018). In this respect, Quillfeldt et al. (2010) showed that latitude appears to have an effect on the prevalence of infection on seabirds, with polar species showing lower prevalence than those from milder climates. Although the lack of suitable vectors could be part of the explanation in this case, the presence of vectors that may adapt to cold conditions could change this conclusion, but this most likely differs among bird and parasite taxa. For example, birds in polar and marine environments where (liquid) fresh water is scarce could be infected by parasites transmitted by mites and ticks instead of mosquitoes and other dipterans. This is the case of *Babesia* in gulls and penguins (Merino 1998; Montero et al. 2016), *Hepatozoon* in boobies (Merino et al. 2014), or

Lankesterella in snow buntings (Martínez et al. 2018). Conversely, other groups of birds tend to be infected frequently by various genera of blood parasites and, therefore, seem very susceptible to infections, as is the case of thrushes and finches (e.g., Greiner et al. 1975; White et al. 1978; see also Chap. 1). Reasons for this are also unclear, but habitat, body mass, abundance, nest type and plumage brightness, and foraging and social behavior among other factors could be part of the explanation (e.g., Scheuerlein and Ricklefs 2004; Fecchio et al. 2011; González et al. 2014; Lutz et al. 2015; Santiago-Alarcon et al. 2016). In order to avoid bird taxonomic and phylogenetic noise in susceptibility to infection, it would be ideal to look for latitudinal gradients using the same bird species or a well-defined set of bird species and using a homogeneous sampling design (i.e., same or similar number of samples of each species at each location). Age and sex of the sampled birds could also affect the results because the incidence of infection is frequently related to these factors (Valkiūnas 2005). The season of sampling can also affect parasite detection and, therefore, the results of the analysis on the presence of latitudinal gradients. It is known that, in general, many blood parasite infections are chronic and suffer relapses during the bird-breeding season, probably triggered by hormonal changes during breeding season or other type of stressors (Valkiūnas 2005; Santiago-Alarcon et al. 2018; but see Pérez-Rodríguez et al. 2015). The reproductive season of birds also coincides with mild climate that also favors the development of vectors and allows for successful parasite transmission. In particular, latitudinal migratory birds are subjected to parasite transmission both during their summer and winter quarters, and they must, therefore, increase their defenses to cope with a larger array of parasites year-round in two completely different areas (Møller and Erritzøe 1998; Møller and Szép 2011; see Chap. 16 for a review of avian haemosporidian research in relation to migratory behavior). Migratory birds can act as vectors spreading diseases by carrying more or less generalist parasites through their migration routes and exposing them to the populations of sedentary birds along these routes. For example, Merino et al. (2008) reported that specificity at the host-family level was only found for *Haemoproteus* lineages infecting birds in the family Emberizidae and that individuals of a long-distance migrant, the white-crested elaenia (*Elaenia albiceps*), were found infected by the same parasite lineages in localities separated by 20° of latitude. Infections by these lineages were detected in other sedentary birds, especially in juveniles and nestlings of different bird species. They concluded that long-distance migrants are able to distort the presence of latitudinal gradients of diseases due to their potential role in spreading infections (see Chap. 16).

Another potential barrier to the spread of parasites is the requirement of competent hosts and vectors. In this sense, generalist parasites capable of developing in different hosts are more likely to spread than those with more specific requirements. Generalist species of parasites are also more likely to remain in places where the density of each host species is low, because they can complete their transmission among different host species. This is an expected situation in the tropics where we find a great diversity of potential host species, but with low population density (see Moens and Pérez-Tris 2016; Fecchio et al. 2018a, b). The same also applies to the invertebrate hosts that act as vectors of blood parasites. Those blood parasites that

are able to infect vectors successfully, that is, able to complete their cycle in different vectors and produce infective stages, will have more chances of reaching their vertebrate host than those with more specific vector needs (see Chap. 6). Moreover, parasites with highly specific host requirements could have higher risk of extinction if their hosts also go extinct (Windsor 1995).

The current scenario of climate change can also potentially act as a disruptor of latitudinal gradients (Garamszegi 2011). Climate change may cause the movement of species including their expansion with the arrival of more benign weather conditions (Merino 2019). Parasites and pathogens typically follow hosts in their expansion, although there is usually a lag in timing. Low density in host invasion fronts could produce serial founder events leading to local extinctions and absence of parasites from frontal host populations (e.g., Coon and Martin 2014). However, the parasites eventually catch up with their hosts in the recently colonized areas (Phillips et al. 2010). Invasive species may also play an interesting role in this kind of studies. For example, it is know that house sparrows (*Passer domesticus*) have aided the geographical expansion of a detrimental lineage of *Plasmodium* across their new range (Marzal et al. 2011), and the introduction of blood parasites and their vectors in some areas have produced important impacts on the native avifauna (van Riper et al. 1986; Tompkins and Gleeson 2006; see Chap. 15 for a review of invasion biology in relation to parasites).

Finally, we can mention different types of disruptors associated with human activity that can affect latitudinal gradients in birds' blood parasites. Humans can create or eliminate reproductive areas for disease vectors, as it occurs when drying up wetlands to eliminate the incidence of malaria in humans (Naranjo-Ramírez et al. 2016), or the creation of swamps and water reserves that can be used by vectors for reproduction (Martínez and Merino 2011; Sehgal 2015). Several studies also focus on the effect of forest fragmentation on both birds and their parasites. Although deforestation could negatively affect the relationship between vectors, hosts, and parasites, the effect between prevalence and deforestations is not always straightforward (Sehgal 2015). Urbanization in relation to latitudinal gradients can also affect the results obtained in terms of the incidence, lineage richness, and assemblage abundance structure of blood parasites (e.g., Carbó-Ramírez et al. 2017). Infections can be reduced in cities by effects of pollution, urbanization, or augmented by the presence of locations with water (e.g., ponds, stagnant water), which are appropriate for vector reproduction (Sehgal 2015; Martínez de la Puente et al. 2016; Abella-Medrano et al. 2018; see Chaps. 13 and 14 for an introduction and synthesis of urban ecology and its relationship with avian parasites).

10.2.2 Disruptors of Abiotic Origin

Apart from the biological characteristics of the involved organisms, other factors exist that may affect the existence of latitudinal patterns in the distribution and incidence of blood parasites. The existence of certain geographical barriers can isolate

populations of vectors, birds, or parasites making it harder to spread diseases (e.g., Prieto-Torres et al. 2018 for birds isolated in neotropical seasonally dry forests). For example, the existence of deserts around tropical areas can operate as barriers to the extension of vectors and parasites since, in many cases, conditions for disease transmission are confined to the few oases and streams present in desert areas (e.g., Ayadi et al. 2017). It is common to find low prevalence of blood parasites in birds living in desert or semidesert environments (Barrientos et al. 2014; Martínez et al. 2016).

Great mountain barriers (e.g., the Andes) can also serve as impediment for several diseases due to the difficulty for some vectors to reproduce and spread successfully at high altitudes (see section of altitudinal gradients). In this case, the effect of the altitude is the same as that of the latitude where high altitudes correspond with environmental conditions found at high latitudes. Another factor that can influence the existence or act as a disruptor of latitudinal gradients is the different conformation of continents in both hemispheres. This can cause differences in biodiversity distribution as pointed by Chown et al. (2004). The proportion of oceanic to land surface in the Northern hemisphere is approximately 1:1, whereas in the Southern hemisphere, it is about 16:1 for latitudes between 30 and 60°. This implies a marked influence of oceans in landmasses of the Southern hemisphere generating mild climates as compared to the northern landmasses across the same latitude. Obviously, this affects the distribution and diversity of species at all ecological levels including microorganisms (Merino and Potti 1996; Bonan 2002; Harvell et al. 2002). The higher extension of landmasses in the east–west direction in Eurasia compared with other continents with a predominant landmass distribution in north–south direction has been also suggested as a potential factor facilitating expansion of diseases across Eurasia, given that conditions did not vary considerably for landmasses across the same latitude (i.e., Eurasia) than in continents where the latitude effect is more marked (Diamond 1997). For example, in the case of southern South America, the height and extension of landmass are reduced as we move toward the south. This implies an oceanic influence on the south of the continent that tends to ameliorate the climate favoring the existence and development of vectors and the possibility of diseases transmission, even at high latitudes (Merino et al. 2008). Finally, islands also have a clear effect of oceans on their climate, but usually infections are less frequent due to the lack of appropriate vectors (Scheuerlein and Ricklefs 2004; Barrientos et al. 2014; Gangoso et al. 2019). However, the naïve native avifauna on islands could be severely affected when a new parasite is introduced (van Riper 1991; Parker 2018; see Chap. 8 for a synthesis on island biogeography of avian haemosporidians in the Neotropics).

10.2.3 Recommendations

There are many factors that can influence the finding of latitudinal gradients of bird blood parasites. We should keep these factors in mind when determining if a gradient exists or does not. In this sense, it is necessary to try to homogenize the samples;

for example, by habitats and species composition, or preferably by comparing common species. It is also necessary to try to prevent or, at least, control for the effect of factors, such as urbanization, deforestation, the presence of dammed areas, host phylogeny, and the proportion of sampling from different host sexes and ages. Geographical gradients in prevalence of avian hematozoa may differ between parasite genera and hemispheres, probably in relation to the existence of appropriate vector–parasite–host interactions. In this respect, there is much work to be done especially in terms of the distribution of vectors (biting midges, blackflies, ticks, mosquitoes, mites, etc.) and the diseases they spread. In addition, the majority of studies have focused on Europe and North America, and it would be of interest to include other geographical areas, particularly tropical ones. It is also recommendable to always include both microscopic and molecular techniques for detection of parasites (Valkiūnas et al. 2006; see Chap. 2 for an introduction to avian haemosporidian life cycles and study methods).

10.3 Altitudinal Gradients

Several authors propose that the richness of mammals, reptiles, amphibians, birds, and insects decreases with altitude (Stevens 1992; Ya'Cob et al. 2016). Nevertheless, there is evidence that this rule is only valid for specific taxonomic groups (Rohde 1996). Mountains can be considered as continental Islands due to their isolation from surrounding vegetation, and given their distinctive climatic characteristics, some species have evolved *in situ* (i.e., endemics) and species living there need particular adaptations (e.g., hematological changes for high altitudes; Ishtiaq and Barve 2018). In this context, it is generally accepted that the prevalence of parasites decreases with increasing elevation (van Rooyen et al. 2013). However, there are few well-documented reports on hemoparasite population parameters and diversity in tropical areas in relation to highlands and altitudinal effects. Elevational gradients provide an excellent means of understanding the distribution, prevalence, and richness of species and to test what climatic factors influence them the most. Temperature is closely related to elevation, and in only short geographic ranges, significant differences in temperature can be observed. Temperature is a critical environmental factor determining vector presence, host distribution and abundance, and consequently the prevalence of parasites (Jones et al. 2013; Harrigan et al. 2014).

The richness of hosts and the potential vectors that are present in an area are responsible for the prevalence of parasites detected there (Pulgarín-R et al. 2018; Gil-Vargas and Sedano-Cruz 2019). In fact, Poulin (2014) stated that there exists a strong covariance between host and parasite species richness. The primary drivers of the current geographic distribution of parasites are the result of an interaction among ecological and environmental characteristics, physiological features, evolutionary processes, and anthropogenic changes that affect hosts and vectors (Ricklefs 1987, 2004; Jones et al. 2018). To explore this in more detail, we discuss some studies analyzing two of the most important biotic and abiotic factors (i.e., vectors and

temperature) that influence parasite prevalence along elevational gradients in the tropics, as well as some study cases.

10.3.1 Vectors

The natural distributional patterns of arthropods, some of them vectors, are largely determined by altitude, temperature, rainfall, and relative humidity (Tanga et al. 2010). The prevalence of hemoparasites also is influenced by arthropod vector abundance, host species, and susceptibility of the host to infection (Atkinson et al. 2001; see Chaps. 5 and 6 for an in-depth review of Diptera vectors and their role as vectors of avian haemosporidians and other diseases; see Chap. 11 for a synthesis on avian haemosporidian host specificity; see Chap. 17 for an in-depth analysis of experimental parasitology).

Recently, in the Andean Mountains of Colombia, Mantilla et al. (2018) conducted a study analyzing how the ENSO (El Niño-Southern Oscillation) period affects the prevalence of *Leucocytozoon* and their possible vectors along an elevational gradient from 1800 to 4750 m asl in three different ecoregions: Andean, sub-Andean forest, and Paramo. There was a negative association between Simuliidae species richness and elevation, which agrees with findings by Ya'Cob et al. (2016). Moreover, Simuliidae species composition changes across ecoregions, and during the ENSO periods, there were marked changes in the altitudinal distribution and occurrence of some Simuliid species. The most dramatic changes in simuliid population were observed in the Paramo ecosystem, during El Niño. In this period, warm water is pushed toward the eastern Pacific coast of South America, causing atypical climate changes, and affecting the abundance and distribution of organisms at large scales (Larkin and Harrison 2005). During El Niño, it was not possible to find some *Gigantodax* species, previously detected during La Niña phase (La Niña refers to periods in which surface temperatures at the eastern Pacific coast of South America are lower than average). Besides, the prevalence of *Leucocytozoon* also was different between two ENSO periods, being higher during La Niña. It means that changes in the prevalence of *Leucocytozoon* were directly associated with the dynamic of the observed simuliid communities (Mantilla et al. 2018; Illera et al. 2017).

It is worth noting that in South America, the Andean mountains are the only known Neotropical ecosystem where *Leucocytozoon* transmission has been confirmed by both microscopic and molecular tools. This narrowly confined transmission of *Leucocytozoon* contrasts with that observed in the Old World, where transmission occurs in both the lowlands and highlands (Illera et al. 2017; Valkiūnas 2005; Sehgal et al. 2006; see Chap. 1). Even though in the South American lowlands, *Leucocytozoon* is found in migratory birds, and the family of their main vector, blackflies (Simuliidae), is also present and highly diverse (Adler 2019), transmission is not completely demonstrated, and only a single report of *Leucocytozoon* detected by molecular tools was recently published (Fecchio et al. 2018c). Lotta et al. (2016) hypothesized that a specific genus of Simuliidae such as

Gigantodax spp. that is distributed only in the Andean ecosystems might be the vector of *Leucocytozoon* spp. which might explain such restricted distribution. Further investigations are needed throughout lowland areas in the Neotropics in order to rule out the local transmission of parasites of the genus *Leucocytozoon*.

Other important variables, which affect the dynamic of transmission, were studied by Atkinson et al. (2014), which compared changes in avian malaria prevalence through a period of 20 years and the occurrence of mosquito larvae of *Culex quinquefasciatus* along an elevational gradient in the Hawaiian Islands. During that period, the prevalence of infection increased significantly at the three sampled sites across the altitudinal range and was more marked at 1100 m asl. At the same time, the detection of *Culex* larvae and the mean air temperature also increased, and those changes were accompanied with declining precipitation and changes in streams flow across the area. Overall, it is important to mention that each vector species can be affected in different ways by the same environmental conditions.

10.3.2 Temperature

Temperature is one of the primary abiotic variables driving elevational distribution of species in different taxa. In this case, it is a very important variable that regulates the development of parasites (Santiago-Alarcon et al. 2012; see Chap. 2). Hemoparasites undergo several developmental stages in vertebrate hosts, which represent a shelter for the parasites from external abiotic conditions. However, haemosporidians need ectothermic hosts (hematophagous invertebrates) where with few exceptions the sexual cycle occurs and produces the infective parasite stage (sporozoites; see Chaps. 5 and 6). These vectors are affected by climatic conditions (Morand 2015) that positively or negatively impact the life cycle of the parasites they transmit (Lapointe et al. 2010). In this way, it is logical to conclude that variations on parasites' geographic distribution are highly dependent on vectors life history traits and adaptations to abiotic environments such as temperature and humidity, which also affect the parasites speed of development inside the vector.

One of the most dramatic avian hemoparasite impacts is the case of avian malaria in Hawaii caused by *Plasmodium relictum* and transmitted by *Culex quinquefasciatus*; as a result of this exotic pathogen, in addition to habitat loss, fragmentation, and destruction, and introduced predators, half of the Hawaiian honeycreepers (Drepanidinae) became extinct (van Riper et al. 1986). Some bird species are altitudinal migrants on the islands, which helped them to escape avian malaria transmission. Researchers hypothesized that those birds escaped infection because the mosquito vector was not able to reproduce at those altitudes. However, an elegant study by Lapointe et al. (2010) demonstrated that low temperatures and not the distribution of the vector *Cx. quinquefasciatus* were responsible for preventing the development of sporozoites, and consequently, *Plasmodium* transmission did not occur in highlands.

Under warmer temperatures, the time for the completion of the parasite's cycle in the vectors is shorter (Santiago-Alarcon et al. 2012). The opposite is expected in the highlands where low temperatures predominate. This is one of the explanations that Zamora-Vilchis et al. (2012) provided for the finding of low prevalence of hemoparasites in highlands of Queensland, Australia. However, in the Neotropical region, parasites of the genus *Leucocytozoon* are distributed only in the highlands at altitudes above 2100 m asl, and the peak of prevalence occurs in the Paramo ecosystem (>3100 m asl). Furthermore, *Leucocytozoon* is the only genus found at 4000 m asl in the Colombian Andes where on average the temperature is low (−4 °C with a maximum at 6 °C), indicating that *Leucocytozoon* parasites and their vectors are well adapted to these environmental conditions (Lotta et al. 2016).

There is growing concern about the behavior of vector-borne diseases under the scenario of climate change. Given the previously reported effects of ENSO, it is possible that under a scenario of global warming, we could also face the extinction of specialists and the expansion of generalist parasites (Carlson et al. 2017). Along with the expansion of generalist parasites, there would be a gradual change in vegetation composition and structure, and new adaptations of vectors to be able to overcome these novel environmental conditions (Mantilla 2016). Overall, the research findings indicate that host populations at high altitudes in a scenario of climate warming will face population declines and extinctions because of a possible distribution of new vectors and host species migrating from lower elevations, which would represent potential competitors, pathogens, or predators for naïve locally adapted species.

Despite the lack of marked seasons in the tropics (implying favorable conditions occurring year-round for parasite transmission), there are dry and wet seasons, which also govern the presence and distribution of avian hemoparasites. For instance, Zamora-Vilchis et al. (2012) reported in birds from the Australian wet tropics that parasite prevalence was positively associated with annual temperature, and it was higher than expected during the warm season independently of elevation. The seasonality of rainfall can also directly impact haemosporidian host specificity in the tropics (Fecchio et al. 2019b).

10.3.3 Study Cases

Illera et al. (2017) evaluated the prevalence and richness of avian haemosporidians in both Mediterranean and Atlantic temperate mountain ranges, and the main conclusion was that each genus of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* (Table 10.1) parasites shows different biotic and abiotic preferences, and one of the most critical factors affecting prevalence was host richness. In this study, in agreement with other studies in the old World, parasites of the genus *Leucocytozoon* were found both in lowlands and highlands, in contrast to the current situation observed in the New World.

Table 10.1 Studies investigating avian Haemosporidian prevalence across different elevational gradients

Species or order (n)	Elevation, m asl	Prevalence	Sampling sites	Additional information	Author
Zonotrichia capensis (184)	4105	18%	Peruvian Andes		Jones et al. (2013)
	3700	42%			
	435	15%			
	80	0%			
Zonotrichia capensis (13) (26) (15) (20)	3300	8%	Ecuadorian Andes	There is no relationship between levels of testosterone (an immunosuppressor) and elevation	Escallón et al. (2016)
	2100	35%			
	1500	73%			
	600	31%			
Zonotrichia[a] *capensis* (28)/ (125)	1850	3.6%/10.5%[a]	Colombian Andes	Prevalence of hemoparasites varies depending on host species	Mantilla et al. (2016)
(60)/ (244)	2400	3.3%/26.63			
(76)/(427)	2560	25/10.5			
Zonotrichia capensis (23)	3077	60.9%	Perú	Prevalence differed markedly between genera: *Haemoproteus* was most prevalent at higher altitudes up to approximately 2200 m asl, and *Plasmodium* was most prevalent at lower altitudes	Doussang et al. (2019)
(6)	3849	100%	Argentina		
(12)	1666	58.3%	Chile		
(35)	1643	35%	Chile		
(68)	588	89.7	Chile		
(16)	104	6.3%	Uruguay		
Troglodytes aedon (140)	129–4454	30%	Peruvian Andes	*Leucocytozoon* parasites strongly associated with montane habitats	Galen and Witt (2014)
Passeriformes (1445)	28–2090		Iberian mountains	*Plasmodium* prevalence and richness were inversely associated to altitude, while *Leucocytozoon* and *Haemoproteus* were positively related to altitude	Illera et al. (2017)
Passeriformes and Apodiformes (428)	3227	50%	Ecuadorian Andes	Higher prevalence was found at highlands (with lower mean annual temperatures)	Harrigan et al. (2014)
	2182	60%			
	1235	12%			
	119	8%			
Passeriformes and Falconiformes (415)	1650	15%	Chichibu mountains, Japan	The most prevalent parasite was *Leucocytozoon*	Imura et al. (2012)

(continued)

Table 10.1 (continued)

Species or order (n)	Elevation, m asl	Prevalence	Sampling sites	Additional information	Author
Parus major (55)			Western Switzerland	Altitude did not affect overall prevalence. However, it influences the genera prevalence: *Leucocytozoon* parasites are more prevalent at high altitudes, *Plasmodium* spp. are more prevalent at lower altitudes, and the prevalence of *Haemoproteus* spp. increases with altitude	Van Rooyen et al. (2013)

Numbers in parenthesis represent sample sizes

[a]It shows the comparison between avian hemoparasite prevalence obtained only in *Zonotrichia capensis* compared to that of all Passeriformes sampled

In South America, the Andes ecosystem is an interesting natural system for the evaluation of the importance of latitudinal and altitudinal gradients in the transmission of avian hemoparasites. In this ecosystem, researchers have investigated *Zonotrichia capensis* (rufous-collared sparrow), which has a broad geographic distribution from southern Mexico to Patagonia in Argentina and which occupies open spaces from sea level to 4600 m asl (Cheviron et al. 2008; Doussang et al. 2019; Cadena-Ortiz et al. 2018; Mantilla et al. 2016; Escallón et al. 2016). Doussang et al. (2019) evaluated the distribution of avian hemoparasite prevalence in both latitudinal and altitudinal gradients from Central and South America, and they found that in this bird species, the most frequent infections are caused by parasites of the genus *Haemoproteus*. However, *Plasmodium* spp. lineage diversity is higher in comparison to *Haemoproteus*. Several studies compared the prevalence of infection in the rufous-collared sparrow across elevational gradients in South America, which concluded that despite the fact that at high elevations the overall prevalence of hemoparasites is lower, the elevation at which these phenomena occurs is variable, depending mainly on the landscape features and the ecosystem type (Table 10.1; see Sect. 10.4 and Chap. 14 for anthropogenic impacts on hosts and parasites).

González et al. (2014) found that in Colombia, the prevalence of the genus *Leucocytozoon* increased with elevation in agreement with results from other studies in Switzerland (van Rooyen et al. 2013) and Peru (Galen and Witt 2014), whereas the prevalence of *Trypanosoma* and microfilariae decreased with elevation (Fig. 10.2). The overall prevalence of the genera *Plasmodium* and *Haemoproteus* did not vary significantly with elevation; however, elevational changes in prevalence were evident when the analyses were conducted at the species or lineage level (Mantilla 2016).

González et al. (2015) and Mantilla et al. (2016) demonstrated that there is a change in the parasite species distribution and their lineages along elevational gradients in the Andean Mountains in Colombia. This is also related to changes in the

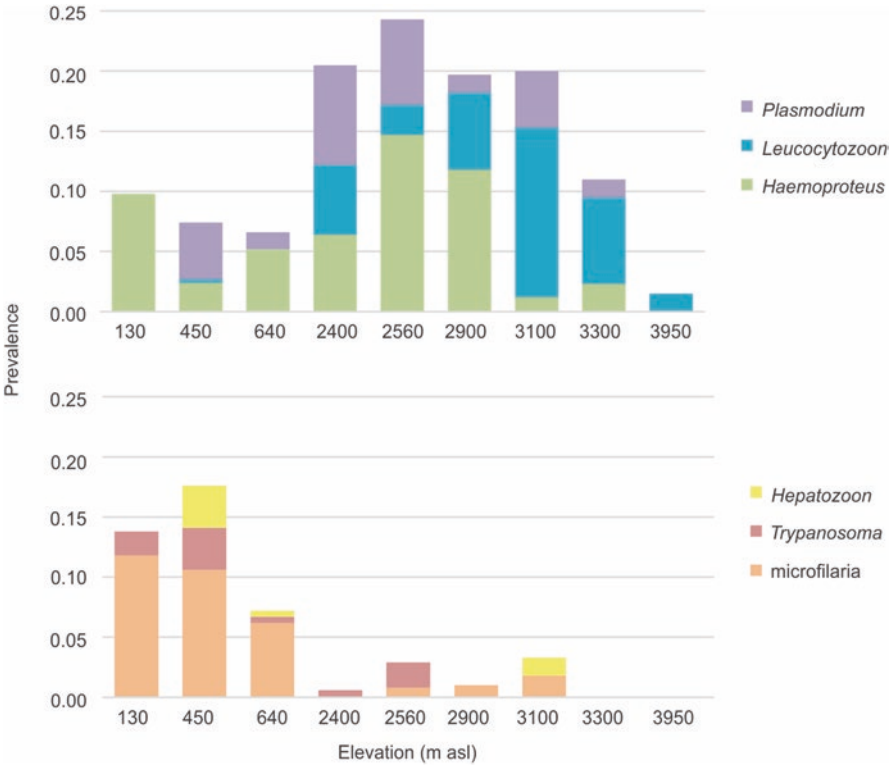

Fig. 10.2 Distribution of the prevalence of six parasite taxa by elevation (m asl). *PLoS ONE* 9: doi: https://doi.org/10.1371/journal.pone.0100695

vegetation, running water, and temperature that changed avian and vector composition. This pattern agrees with the study by Galen and Witt (2014) in the Peruvian Andes, where the elevational distribution of avian Haemosporida lineages of the same genus did not overlap, implying a high parasite lineage turnover as altitude changes, which might actually be related to the change of ecosystem types at different altitudes. Zamora-Vilchis et al. (2012) found that avian hemoparasite species richness declined with elevation. Bearing in mind the evidence, it is critical not to make generalizations, since parasite species distributed at high elevations usually are not shared with those found in lowland areas, even *Haemoproteus* or *Plasmodium* lineages, which are widespread along different altitudes (see also Table 10.1).

10.3.4 Recommendations

Avian hemoparasite species and lineages vary along altitudinal gradients depending on the hosts, landscape, and environmental conditions. Future research must consider the species or lineages of the parasite, rather than only considering genera and

infection status. Likewise, we emphasize the importance of examining vector communities at the species level, in order to detect subtle changes in their distribution and occurrence as a function of environmental changes.

Under the current climate change, vector-borne diseases can reach higher elevations and infect naïve hosts. Management plans should be carefully designed to anticipate and to avoid biodiversity losses, and even avoid extinctions of threatened and endemic bird species, particularly in highlands. Understanding how different sources of heterogeneity at the landscape level may influence parasite–vector–host interactions could help with the design of strategies aimed at compensating for the effects of climate change at larger latitudinal and altitudinal scales.

More surveys in tropical regions are needed to be able to make accurate conclusions about avian hematozoa biodiversity, richness, and distribution because current knowledge in the tropics and particularly the Neotropics is still fragmentary and restricted mainly to a few countries (e.g., Colombia, Brazil, Mexico; see also Chap. 1 for a synthesis of the research conducted in tropical areas during the last century, and Chap. 6 for current knowledge on avian haemosporidian vectors of tropical areas).

10.4 Within-Landscape Gradients

Ecological landscapes are geographical areas that are spatially heterogeneous in some features of interest (e.g., anthropogenic impacts) (Turner et al. 2001). This heterogeneity creates environmental gradients that may influence ecological processes such as parasite–vector–host interactions. There are many different types of within-landscape gradients, and they may function as disruptors of other gradients, such as the ones that have been discussed in the previous sections. Some examples of within-landscape gradients that may influence interspecific interactions involving hemoparasites include distance from water sources, levels of habitat degradation due to human activities, urbanization intensity, vegetation successional stages, interspersion of vegetation types such as those existing in altitudinal gradients and on relatively large geographical areas, and incremental levels of exposure to pollutants (Wood et al. 2007; Knowles et al. 2014; Lachish et al. 2011, 2013; Renner et al. 2016; Hernández-Lara et al. 2017). Thus, the focus of this section is at finer scales.

10.4.1 Effect of Water

Water sources within landscapes may include lakes, lagoons, natural and artificial ponds, rivers and tributaries, and finer-scale elements that hold small volumes of water or humidity such as rocks, soil, and live or dead vegetation. Because dipteran vectors require water or at least some moisture for the viability of some stages of their life cycles (Brokent 2004; see Chaps. 5 and 6), these landscape elements facilitate haemosporidian dispersal (Lapointe et al. 2012), and it is likely that some of the

most diverse and abundant vector populations for either Haemosporida or other types of hemoparasites are often those inhabiting humid ecosystems (Lachish et al. 2013). Indeed, reported prevalence and richness of haemosporidian assemblages tend to be higher in tropical moist ecosystems in comparison with dry environments (Lacorte et al. 2013). Due to the high humidity in these environments, vector community attributes and prevalence may be high and constant within entire landscapes. It has been reported that mosquito communities in humid landscapes of Central Veracruz, Mexico, are very similar in species richness and composition regardless of land-use type, changing only in abundance structure (Abella-Medrano et al. 2015). Similarly, temperature was a significantly better predictor of *Plasmodium* spp. prevalence in comparison to moisture in central Africa (Sehgal et al. 2011). However, in the same study areas, prevalence of *Trypanosoma* spp. was better predicted by moisture and secondarily by temperature and elevation. Therefore, an alternative is that even in these moist landscapes, there may be some variations in both vector abundances and hemoparasite prevalence, resulting from varying distances to water sources, degree of habitat degradation, within-landscape connectivity, or other landscape features that may act as disruptors (e.g., Hernández-Lara et al. 2017; Abella-Medrano et al. 2018).

In environments where moisture varies as a function of vegetation types (e.g., altitudinal gradients), topography determines availability and spatial distribution of water sources such as lakes ponds, rivers, and tributaries (e.g., Swanson et al. 1988). Rivers and tributaries in these landscapes provide well-oxygenated running water, which is necessary for the successful development of the larval phase of dipterans from the Simuliidae family, also known as black flies, the main vectors of leucocytozoids (Butler and Hogsette 1998). *Leucocytozoon* spp. infections are usually recorded at high altitudes (González et al. 2014; Harrigan et al. 2014), and in these mountainous landscapes, prevalence by this genus is often high (e.g., Rodríguez et al. 2009; González-Quevedo et al. 2016). Distance to rivers and tributaries in these conditions may influence black fly abundance, causing *Leucocytozoon* spp. prevalence to vary according to topographic features in environments having high and intermediate humidity values. However, to our knowledge, the effect of distance from such rivers and tributaries on black fly diversity and abundance and on *Leucocytozoon* prevalence is currently understudied in tropical regions. One recent study from northern South America reported that diversity and prevalence of *Plasmodium* and *Haemoproteus* responded to host species and to host by climate interactions, but not to water availability (Pulgarín-R et al. 2018). In contrast, in drier environments that are topographically less complex, recorded prevalence of *Leucocytozoon* is usually extremely low (e.g., Belo et al. 2012; Reinoso-Pérez et al. 2016; Ham-Dueñas et al. 2017).

Finally, in dry lands, infection may respond to water availability and seasonal rainfall patterns, but the responses may vary among avian and hemoparasite species. In the United Kingdom, for instance, transmission rates of *Plasmodium circumflexum* in blue tits and in great tits (*Parus major*) strongly varied among years and decreased with distance from the Thames river. The effect of the river, however, was not recorded for *P. relictum*, and differences among years in transmission rates were

strong (Lachish et al. 2011). Similarly, it has been suggested that temperature and humidity in arid ecosystems may influence vector distribution and abundance (Belo et al. 2012). Therefore, whenever there is water available, as in proximity to ponds, springs, or other water reservoirs, or during periods of intense rain, prevalence may be high, and infection rates could decrease with distance from these landscape elements (Belo et al. 2012). Therefore, in some dry landscapes such as in Arizona, the absence of haemosporidians may result from very limited water availability (Deviche and McGrawK 2005). In other cases, such as in dry scrublands of central Mexico, where local inhabitants build water ponds for livestock management and where the density of these ponds is high, haemosporidian assemblages are species rich and both prevalence and parasitemia decrease with increasing distance from such water sources, but again, the magnitude of response varies among bird and parasite species (Reinoso-Pérez et al. 2016; Ham-Dueñas et al. 2017). Moreover, in this region, the effect of distance from water ponds may be confounded with habitat degradation (see sect. 10.4.2 below). Therefore, studies in dry lands where the density of water sources is low and some water sources are away from human settlements may help to disentangle the effect of water sources from that of other fine-scale factors such as small human settlements (e.g., Tinajero-Hernández et al. 2019).

10.4.2 Human Activities

Human activities have dramatically altered the structure and composition of natural landscapes at a massive scale (Primack 1993). These changes may have strong influences on parasite–host–vector interactions (see Chap. 14 for an in-depth synthesis of Anthropic effects on host–parasite interactions). Some examples of human degradation which may alter such relations include land-use changes that convert areas covered by natural vegetation to agricultural fields, where avian, vector, and parasite communities may be different from the original ones (e.g., Patz et al. 2000; Abella-Medrano et al. 2015; Hernández-Lara et al. 2017; MacGregor-Fors et al. 2018). Introduction of livestock may promote increased abundance of vector populations (Patz et al. 2000). Establishment of urban areas may modify vector and parasite incidence which may be higher or lower, and in such urban areas their communities may contain novel species and lineages (Patz et al. 2000; Carbó-Ramírez et al. 2017; Abella-Medrano et al. 2018). Finally, introduction of toxic substances such as agrochemicals, metals, and metalloids dumped into the environment by mining activities may affect hosts and/or vectors (Valkiūnas 2005; Ruiz-García 2017; Santiago-Alarcon and Delgado 2017; Monzalvo-Santos 2017). All these types of degradation may create local environmental gradients and alter parasite–vector–host interactions.

Although hemoparasite prevalence seems to be low in urban in comparison to rural areas (Geue and Partecke 2008; Santiago-Alarcon et al. 2018; see Chap. 14), small human settlements, by modifying the surrounding landscape, may have a

different type of effect on interspecific relationships involving hemoparasites (e.g., Tinajero-Hernández et al. 2019). In semiarid scrublands of central Mexico, for instance, numerous small villages (< 500 inhabitants) were established during the late nineteenth and early twentieth centuries, and for nearly 100 years, tree extraction and overgrazing by goats have been the most common land uses surrounding these villages. In these areas, the tree layer has been almost eliminated, and due to the browsing by goats, shrubs occur in low densities and are short in height, creating a particularly open habitat. Contrastingly, at relatively large distances from the villages (>10 km), scrublands have been subject only to moderate disturbance. In these habitats, trees and shrubs occur at higher densities, and shrubs have higher heights. Within this gradient, the highest and lowest haemosporidian parasitemia values in two bird species, the house finch (*Haemorhous mexicanus)* and the canyon towhee (*Melozone fusca*), were found in villages and in relatively undisturbed scrublands, respectively (Reinoso-Pérez et al. 2016). It is possible that ecological stress for the House finch and the canyon towhee is high at the suboptimal habitat (i.e., villages and surrounding degraded scrublands), and thus, birds' immunological capacity to fight off infections may be impaired. Most water ponds present in these landscapes have been built by humans and are located at or close to the villages. Hence, habitat degradation may be correlated with the presence of water ponds, which provides suitable breeding habitat for vectors. Therefore, additional research is needed to disentangle the potential simultaneous effects of water sources and habitat degradation (e.g., Santiago-Alarcon et al. 2019). Some additional information supporting the potential effect of habitat degradation pertains to the black-throated sparrow (*Amphispiza bilineata*) and the american kestrel (*Falco sparverius*). These species are well adapted to open habitats (Pidgeon et al. 2003; Smallwood and Bird 2002). A recent study reported significant lower prevalence in black-throated sparrows at degraded scrublands adjacent to the villages in comparison to the relatively undisturbed scrublands (Ham-Dueñas et al. 2017). Moreover, ecological stress, measured through the heterophil/lymphocyte ratio (Ellis et al. 2012), was lower in the open preferred habitat. The degraded scrublands resemble the preferred, open habitat, for the black-throated sparrow; therefore, in spite of being a degraded habitat, this is an almost optimal habitat for the sparrow, where feeding resources are abundant, and competitors as well as predators may occur in low numbers. Regarding the american kestrel, lower prevalence and parasitemia values have been recorded in agricultural fields and undisturbed scrublands in comparison to villages (Tinajero-Hernández et al. 2019). Agricultural fields are open habitats in which some power poles and isolated trees are present; this type of habitat facilitates visibility and provides perches that kestrels use while foraging for mice and small arthropods (Smallwood and Bird 2002). These conditions consisting of abundant feeding resources and presence of habitat elements which facilitate prey acquisition may guarantee an environment where stress is moderate, and thus, kestrels may allocate a considerable amount of energy to cope with infections. Moreover, the potential presence of agrochemicals in these agricultural fields may negatively affect vectors and decrease infection rates, thus indirectly providing a benefit to Kestrels.

A study in Venezuela did not detect differences in haemosporidian prevalence and lineage richness between distant sites (>500 km) (Belo et al. 2012) possibly because habitat disturbance in this landscape occurs at a very local scale. There are many other examples of effects of habitat degradation on parasite prevalence in different habitat types, and these effects sometimes increase infection rate in some African rainforest birds (Chasar et al. 2009), but they may also decrease prevalence such as in tundra habitats of North America (e.g., Bennett et al. 1992) and in Neotropical montane forests of Veracruz, Mexico (Hernández-Lara et al. 2017), or be neutral (Fokidis et al. 2008; see Chap. 14). The presence and direction of effects of these types of gradients may thus be context sensitive.

10.4.3 Toxic Substances

Human-generated substances that may be toxic to organisms, also known as novel entities (*sensu* Steffen et al. 2015), may strongly influence parasite–vector–host interactions (Santiago-Alarcon and Delgado 2017). Through anthropogenic activities, ecosystems have been exposed to thousands of these pollutants; some of the most common types of toxic substances include agrochemicals, metals that are dumped in the environment by the mining industry, hydrocarbons, and colorants. The purpose of some of these pollutants, such as organochlorine agrochemicals, is to control populations of arthropods that attack crops (Landis et al. 2011). Other pollutants, such as metals, hydrocarbons, and colorants, are not intended to control arthropod pests. However, many of these novel entities have negative effects not only on arthropods but also on other organisms, including vertebrates (Santiago-Alarcon and Delgado 2017). Because novel entities have the capacity to kill arthropods (Landis et al. 2011), abundance of vector populations is likely to be low in exposed areas, but increase with distance from polluted sites. Consequently, hemoparasite prevalence in hosts may increase with distance from such polluted areas or may at least be significantly higher in unexposed than in exposed sites (reviewed in Valkiūnas 2005). On the other hand, pollutants may also negatively affect vertebrate hosts through various mechanisms, including damage to the DNA, the endocrine and the immunological system, physiological pathways, morphological changes, and by inducing changes in reproduction and survival rates (Landis et al. 2011). When vertebrates are exposed to such negative factors, their ability to cope with infections may be impaired. Consequently, the probability of acquiring an infection and the intensity of the infection may be higher. Due to the simultaneous effects of pollutants on both vectors and hosts, the resulting effect is difficult to predict and may vary among bird and vector species depending on the physiological ability to fight infection and to resist exposure to a specific substance. An additional factor to be considered is the combination of different types of pollutants in the environment and the interacting effects that they may have (Chapa-Vargas et al. 2010; Monzalvo-Santos et al. 2016). The number of studies investigating effects of pollutants on

prevalence and parasitemia is very low (reviewed in Valkiūnas 2005), particularly in tropical areas (see Santiago-Alarcon and Delgado 2017). However, results of ongoing research suggest that prevalence decreases with increasing amounts of heavy metals (antimony, arsenic, and lead) in feathers of birds from central Mexico, possibly due to the negative effect of these metals on vectors (e.g., Raymis-Keller et al. 1998; Migliorini et al. 2004). In addition, it was estimated that bird physiological stress (estimated through the heterophil/lymphocyte ratio) was greater in sites exposed to metals than in unexposed sites (Monzalvo-Santos 2017). Similarly, it was found that haemosporidian prevalence and parasitemia decreased with increasing amounts of organochlorines (endosulfide) in bird feathers (Ruiz-García 2017). In order to better understand the effects of different types of pollutants on parasite–vector–host interactions, investigations involving many different host species in different types of environments and exposed to different types of pollutants should be implemented. In addition, studies should evaluate how exposure to pollutants influences vector abundances and prevalence of haemosporidians in birds and vectors, and the concentration of pollutants in vectors themselves should be evaluated.

10.4.4 Recommendations

Field studies should be designed so that within-landscape local heterogeneity (i.e., local environmental gradients such as distance to water sources, pollutants, and vegetation structure) can be evaluated in such a way that individual factor effects on host–vector–parasite dynamics can be separated and determined. Within landscapes, there are many other potential factors that may generate environmental gradients; some examples include natural disturbances (e.g., fire, tree fall gaps, and floods) and vegetation succession. The effects of these local-scale gradients on parasite–vector–host relationships have not been studied, but these effects may be relevant because they can disrupt or act in synergy with the effects of other gradients at both local and regional scales (e.g., van Hoesel et al. 2019).

10.5 Conclusion

There is a vast body of evidence favoring the existence of effects of different types of environmental gradients on parasite–vector–host interactions involving hemoparasites in wild vertebrates. Understanding the fine details related to these gradients is a very complex issue. Often times, the effects are highly dependent on context, and some gradients may interact with the effects of others. Latitudinal gradients, for instance, may not be evident if a disruptor such as an altitudinal gradient is present. Moreover, some environmental gradients, such as those imposed by pollution sources, may act simultaneously on more than one organism involved in the parasite–vector–host interaction, and the type and direction of response may be

difficult to predict. In spite of this complexity, current knowledge indicates that some general trends include higher prevalence, parasitemia, and parasite lineage richness toward the equator, at lower and intermediate altitudes, at shorter distances from water sources, in degraded habitats, and in areas that are not exposed to pollutants. However, these patterns may not always hold; therefore, context and idiosyncratic responses of individual bird, vector, and parasite species are very important (e.g., Schmitz 2010).

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Chapter 11
Host Specialization and Dispersal in Avian Haemosporidians

Marcos Robalinho Lima and Javier Pérez-Tris

Abstract In order to be able to understand the ecological and evolutionary processes involved in the emergence of infectious diseases, one needs to comprehend how parasites arrive at new geographical areas and how they manage to maintain viable populations and even expand their ranges. We discuss host specificity in avian haemosporidians and how encounter and compatibility filters affect the dispersal of avian haemosporidians, and how these filters affect avian haemosporidian assemblages at different spatial and evolutionary scales. There are at least three important barriers to the dispersal of avian haemosporidians: (i) geographic barriers, (ii) environmental barriers, and (iii) interspecies barriers. In this chapter, we discuss the factors involved in these barriers and their effects on the structure of avian haemosporidian assemblages. Host specificity plays an important role in parasite dispersal, and in the case of avian haemosporidians that are vector-borne parasites, it needs to be evaluated both at the vector and bird host levels. Understanding the effects of these factors on host–vector–parasite dynamics is important to unravel the dispersal and diversification mechanisms of avian haemosporidians. We end this chapter reviewing host specialization in avian haemosporidians of tropical regions, discussing the mechanisms involved in the dispersal and specialization of these parasites and point out important research gaps that need attention.

Keywords Compatibility filter · Encounter filter · Environmental barriers · Host shifting · Host specificity · Parasite community assembly · Phylogenetic barriers

M. R. Lima (✉)
Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Londrina, Brazil

J. Pérez-Tris (✉)
Department of Biodiversity, Ecology and Evolution, Complutense University of Madrid, Madrid, Spain

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11.1 Introduction

How do parasites arrive and establish themselves in new geographic locations? This is a very important question to be answered because of the increase in the emergence of infectious diseases (Jones et al. 2008). In order to answer this question, one needs to identify the barriers involved in the dispersal and establishment of parasites, how these barriers affect parasite community assembly and how these barriers affect *host shifts* (Combes 1991; Clark et al. 2018; see Glossary for definition of terms in Italic font). More importantly, one needs to understand how these barriers work at different scales. For example, how does *host specificity* affect the establishment of novel parasite populations in newly colonized areas? Or how do hosts' life history strategies affect the maintenance of a parasite population within a given area?

Clark et al. (2018) identified three important barriers for avian haemosporidian dispersal: (i) *geographic barriers* (e.g., distance between regions, presence of mountain ranges, presence of water barriers or even scarcity of water between regions); (ii) *environmental barriers* (e.g., temperature and precipitation gradients, habitat differences between regions); and (iii) *interspecies barriers* (e.g., ecological similarity of hosts, immunological similarity of hosts, phylogenetic relatedness of hosts, specificity of vertebrate host–vector relationships). These barriers work at different scales, with geographic and environmental barriers working at larger scales, while interspecies barriers will act at both local and regional scales (Fig. 11.1). At the local scale, diverse ecological communities could reduce the spread of avian haemosporidians due to a *dilution effect* (Civitello et al. 2015), a hypothesis that has yet to be formally tested for avian haemosporidian parasites. For example, if vectors are not host specific, then encounter rates with the bird host could be reduced because of the large presence of other bird species that are incompatible hosts (Fig. 11.1c), a circumstance that may be particularly promoted by competition in highly diverse avian communities, where the abundance of compatible bird hosts could be reduced leading to a reduction in parasite abundance (Keesing et al. 2006; Civitello et al. 2015). However, if avian haemosporidians are capable of infecting a broad range of bird species (i.e., host generalists), then the dilution effect will be reduced (Fig. 11.1b), and depending on the avian assemblage abundance structure an *amplification effect* is possible. Therefore, a specialized parasite lineage dispersing to a new location with a diverse host community might be less likely to encounter its host and persist than a generalist parasite (e.g., metacommunity dynamics; Suzán et al. 2015).

Host specificity measures the degree of specialization of a parasite, and is inversely proportional to the number and diversity of host species it can infect (Poulin 2007). In the case of avian haemosporidians (i.e., vector-borne parasites), host specificity applies to both birds and dipteran vectors. According to Combes (1991), two filters should determine the distribution of parasites: (i) an *encounter filter* and (ii) a *compatibility filter*. Vectors will determine the bird hosts that avian haemosporidians will encounter among those available in the local parasite community (Fig. 11.2a). In general, there seems to be a vector-family specificity in

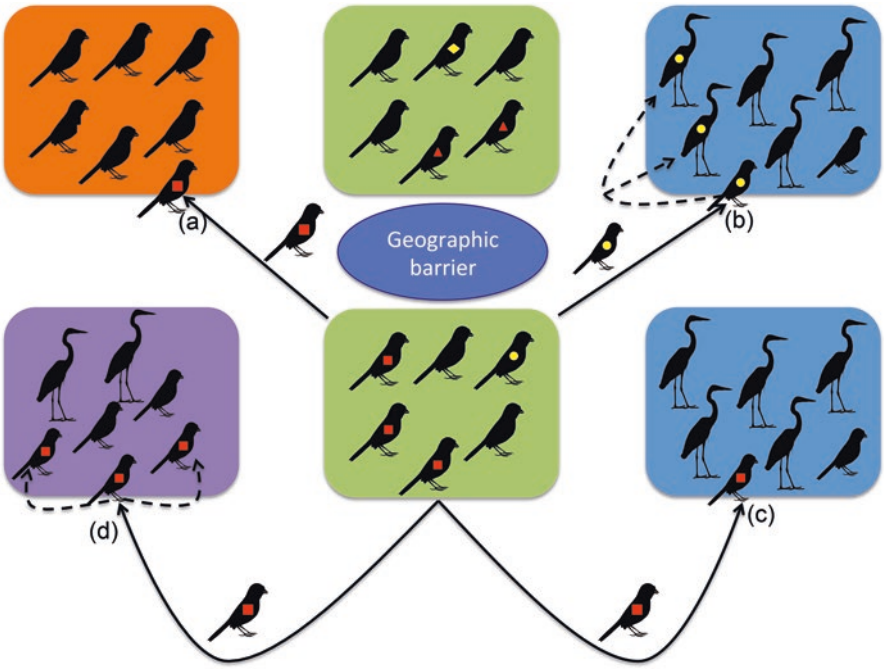

Fig. 11.1 The green communities are phylogenetically similar. However, because of a geographic barrier (e.g., water barrier), each community has its own avian haemosporidian lineages (represented by red squares, yellow circles, red triangles and yellow diamond). The lines show dispersal of infected birds into novel communities and dashed lines when avian haemosporidians establish themselves in the novel community. Colored rectangles represent communities that are environmentally similar. Avian haemosporidians can fail to colonize a novel community because of environmental barriers, such as lower precipitation in the novel community (**a**). Avian haemosporidians will also fail to colonize a novel community due to the interspecies barrier and a potential dilution effect because there will be a higher abundance of non-host species (**c**). However, avian haemosporidians are capable of shifting hosts when lineages are host generalist or re-encounter previously compatible host species (**b**). Colonization of novel communities by avian haemosporidians will be more likely to occur when the novel community presents phylogenetically similar hosts (i.e., ecological fitting) reducing the species barrier (**d**). (Figure is adapted from Clark et al. (2018). Gabriel L. M. Rosa designed bird silhouettes)

avian haemosporidians (Valkiūnas 2005; Santiago-Alarcon et al. 2012a). However, blood-sucking dipterans can present plastic feeding behavior that extends across different vertebrate groups (Santiago-Alarcon et al. 2012a,b; Santiago-Alarcon et al. 2013; Pettersson et al. 2013; see Chaps. 5 and 6 for a thorough description of blood sucking dipteran vectors and a synthesis of research on vectors involved in the *transmission* of avian haemosporidians across tropical regions). This behavior can lead to host shifts in birds when avian haemosporidians encounter other suitable hosts, influencing the distribution of parasites in a local community (Santiago-Alarcon et al. 2013). Conversely, vectors will influence the structure of parasite–host associations if they feed on a limited range of host species, which will restrict

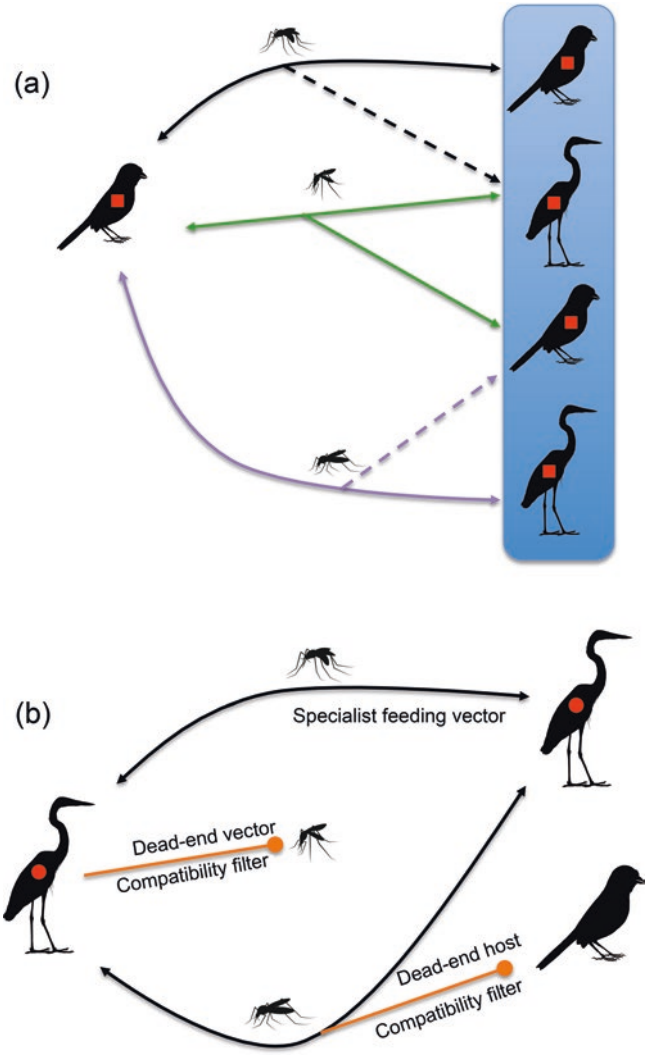

Fig. 11.2 Encounter (**a**) and compatibility (**b**) filters. In (**a**), each colored line represents an infection pathway provided by a specific vector species. The blue rectangle represents the encounter filter, and dashed lines the avian hosts the vectors did not encounter. In (**b**), the compatibility filter (represented by the orange line), showing the possibility of a vector or avian host acting as incompatible (dead-end) hosts (see *host compatibility* in the glossary) because of their resistance to the avian haemosporidian. Freepik designed Mosquito silhouettes, and Gabriel L. M. Rosa designed bird silhouettes

transmission of parasites to potentially suitable hosts (Malmqvist et al. 2004; Hellgren et al. 2008). Therefore, vectors may serve as ecological barriers to transmission because vectors will determine the routes of infection (i.e., network of hosts that vectors feed on; see Chap. 9 for an introduction to network and macroecological theory applied to antagonistic interactions).

The compatibility filter is influenced by both vector and bird hosts (Valkiūnas 2011; Medeiros et al. 2013; Gutiérrez-López et al. 2016; Clark et al. 2018). Dipterans can present resistance toward avian haemosporidians (Fig. 11.2b), which restricts the parasite lineages that are capable of completing their life cycle within a vector (Santiago-Alarcon et al. 2012a; Valkiūnas et al. 2013). Avian hosts can also show resistance toward avian haemosporidian lineages (Westerdahl et al. 2012, 2013, Sarquis-Adamson and MacDougall-Shackleton 2016), which can be qualitative (i.e., prevents parasite infection) or quantitative (i.e., reduces infection effects). This compatibility filter at the avian hosts will also structure parasite–host associations (Fig. 11.2b). For example, if a vector feeds on a resistant avian host, the parasite may not be able to be further transmitted, especially if the avian host is capable of clearing the infection (Westerdahl et al. 2012). The complex life cycle of avian haemosporidians suggests that these parasites should be host generalist (Noble et al. 1989; Poulin 2007) because high host and vector specificity could lead to local parasite extinction when parasites face low population sizes of their specific vector or avian host species (for evidence of the evolutionary stability of host specific vs generalist parasite strategies, see Pérez-Rodríguez et al. 2015; Ellis and Bensch 2018). Understanding these compatibility filters is important because host shifting is common in avian haemosporidians with little evidence of co-speciation (Ricklefs et al. 2014; Ellis et al. 2015; Nylin et al. 2018).

11.2 Host Specificity in Avian Haemosporidians

Highly specialized parasites are those that are restricted to a single host species, while generalist parasites are capable of infecting multiple taxa. However, categorizing a parasite as either specialist or generalist can be a difficult task. For example, if a parasite infects more than one host species within a genus, would it be considered a generalist? An alternative would be to quantify how phylogenetically distant hosts are (Poulin and Mouillot 2003; Hellgren et al. 2009; Clark and Clegg 2017), where generalist parasites would be those capable of infecting host species that are phylogenetically distant (e.g., infecting bird species from different orders; Santiago-Alarcon et al. 2014). Also, it is important to consider how infection levels vary among the different host species being used. For example, parasites may have a main host species while infecting other host species at lower intensities or prevalence (Moens et al. 2016; Huang et al. 2018). Another important aspect to consider is the time scale of the processes involved in the host–parasite interaction. For example, recent adaptation to specific host attributes (such as its habitat niche) may be responsible for an increase in parasite compatibility (Clark and Clegg 2017; see Chap. 7 for an introduction to ecological niche modeling and its applications to host–parasite interactions). Conversely, ancient processes in the evolutionary history of a host–parasite interaction would cause ancestral habitat filtering. In the latter case, parasite success or failure in mechanisms such as adaptation to the host immune system and host dispersal could lead to the maintenance or loss of the parasite (Clark and Clegg 2017). Therefore, host specificity is best seen on a gradient of

host specialization that considers infection levels (i.e., parasitemia) and prevalence (Moens et al. 2016; Huang et al. 2018).

The intensity of infection (or *parasitemia*) is difficult to measure (see below and Chap. 2 for a review of haemosporidian study methods), but it is an important attribute of the parasite that could lead to higher mosquito infection and an increase in parasite transmission (Cornet et al. 2014). It has been shown for *Plasmodium relictum* that mosquitos feeding during the acute phase of infection will have higher infection (Pigeault et al. 2015), which could lead to a higher transmission of this malaria parasite to other hosts (but there may be higher vector mortality during this acute phase, so there must be a trade-off between parasitemia and transmission potential). In accordance with this idea, it has also been shown that *Plasmodium relictum* will have higher within host parasitemia in the evening, which is when its vector *Culex pipiens* is most active (Pigeault et al. 2018). Moreover, *Plasmodium relictum* can react to the biting of uninfected vectors by increasing their replication within the host (i.e., higher parasitemia), which will lead to a better timing of replication with the presence of vectors, an important attribute in regions that have seasonal vector activity (Cornet et al. 2014; Pigeault et al. 2018). This is important because it shows that *Plasmodium relictum* during chronic infections can present flexibility regarding its replications that can lead to higher transmission of the parasite during the season of higher vector availability. Therefore, not only the prevalence of infections in a population will affect parasite dispersal, but also parasitemia within hosts can affect the dispersal of avian haemosporidians by the potential increase in the infection rate of vectors that could boost parasite transmission to other hosts.

Specialist parasites need to cope with fewer defence mechanisms (i.e., host species with different immune systems), which could make them more efficient at evading the hosts' immune system and allow them to efficiently replicate and transmit to other hosts (Poulin 1998). This more efficient performance should result in a greater infection rate (proportion of infected individuals – prevalence) and higher parasite intensity (number of parasites found in an infected individual) within its host's population due to the evolutionary tolerance to infection developed by the avian host. Conversely, generalist parasites have to surpass different immune systems potentially reducing their replication and transmission rate (Hellgren et al. 2009). These ecological differences regarding host use can explain differences in parasite abundance and occupancy in local communities. According to the niche breadth hypothesis, generalist parasites will be more effective at colonizing different host communities allowing them to occupy larger distributional ranges when compared to specialist parasites (Drovetski et al. 2014). However, the trade-off hypothesis suggests that specialist parasites will trade *host range* for increased prevalence across the geographic range of their specific hosts. If these hosts have large distributional ranges, then specialist parasites will not only be locally abundant (i.e., highly prevalent), but will also be able to attain large distributional ranges themselves (Drovetski et al. 2014; Lima and Bensch 2014).

In vector-borne parasites, transmission to suboptimal or incompatible hosts may occur often, particularly in highly diverse bird communities such as in the

Neotropical region (Civitello et al. 2015). In this case, generalist parasites might have a competitive edge because they will manage to increase their host encounter rate despite infecting suboptimal hosts (Keesing et al. 2006). For example, in a megadiverse forest in Ecuador, *Haemoproteus* lineages were considered to be more host generalist than *Haemoproteus* parasites of temperate forests (Moens and Pérez-Tris 2016). This is an interesting result because *Haemoproteus* lineages are usually considered more host specific (Beadell et al. 2009; Hellgren et al. 2009; Olsson-Pons et al. 2015; but see Ellis et al. 2015), but in the Neotropical region of South America, they show a more generalist pattern of host use (Belo et al. 2011; Svensson-Coelho et al. 2013; see Chap. 1 for a synthesis on avian haemosporidian research of the twentieth century demonstrating that *Haemoproteus* are widespread and common across tropical avian assemblages). However, the Neotropical region is notorious for a large number of single lineage recoveries within communities (Lacorte et al. 2013; Fecchio et al. 2018a), which is indicative of suboptimal sampling of parasite diversity. Further research with increased sampling effort is needed to clarify whether these parasites are specific to single host species (which would somewhat contradict the expected low specialization of parasites in regions of high avian diversity), or whether they are rare but host generalist parasites.

At a local scale, the prevalence of avian haemosporidians can be associated with host abundance (at least at the parasite community level; Ellis et al. 2017), and both specialist and generalist avian haemosporidians can infect a similar number of individual hosts (Medeiros et al. 2014). Although a reduction in the number of compatible host species should reduce transmission and overall parasite prevalence – because the encounter of uninfected vectors with infected individuals will be reduced (Keesing et al. 2006) – specialist avian haemosporidians could reach higher prevalence on their specific hosts when compared with generalist avian haemosporidians on those same hosts (Medeiros et al. 2014). Also, recent data suggests that even generalist parasites will fare better in a set of main host species (Huang et al. 2018), indicating that prevalence and parasitemia will vary on a host-by-host basis, further contributing to the continuous nature of parasite specificity. However, one of the difficulties in understanding these trade-offs is determining the parasitemia (i.e., the number of individual parasites infecting a host), which is usually done using either microscopy or qPCR (Asghar et al. 2011; Ishtiaq et al. 2017; Huang et al. 2018). The use of microscopy is rather challenging because blood smears need to be of high quality and evaluated by experienced personnel (Valkiūnas et al. 2008; see Chap. 2 for a review of haemosporidian study methods). Also, wild birds will usually have low blood infection intensities lacking full-grown gametocytes, making it more difficult to detect and identify infections (Moens et al. 2016). Although qPCR methods can quantify infection intensity without the need to evaluate blood smears (Ciloglu et al. 2019), determining the standard curves using DNA samples of known infection intensity that is accessed by microscopic analysis (e.g., Ishtiaq et al. 2017; Asghar et al. 2011; Moens et al. 2016) is required to generate comparable estimates of parasitemia. In addition, the direct observation of mature gametocytes in peripheral blood is the best standard for scoring a host species as a competent reservoir for the parasite (Moens et al. 2016). Therefore, the combination of microscopy and

molecular techniques (for identification of lineages based on DNA sequence and quantification of parasite intensities) is vital to better understand the trade-offs of being a specialist or generalist parasite. For example, a parasite well adapted at exploiting its host should show a greater capacity of infection (i.e., have high prevalence) along with a greater capacity for reproduction (parasitemia) within its host (Huang et al. 2018). Moreover, adaptation to hosts can vary, and this variation should be reflected in the prevalence and intensity of infection (i.e., parasitemia). Therefore, in order to determine if an avian haemosporidian lineage is a generalist or specialist parasite, one needs to consider both the prevalence and the parasitemia in each host species (Moens et al. 2016; Huang et al. 2018). With these data, one can answer questions such as: 1) Does the same avian haemosporidian lineage have similar prevalence and parasitemia in the same host species independent of locality? 2) Are there particular host species that are more susceptible to avian haemosporidians? 3) How does parasitemia and prevalence vary when coinfections of avian haemosporidians occur? (e.g., Palinauskas et al. 2018). Answering these questions will greatly contribute to our understanding of parasite dispersal and host ranges.

Studies on the association between avian haemosporidian lineages and vectors are scarce (see Chap. 6 for a synthesis of the current knowledge on dipteran vectors of avian haemosporidian parasites). The difficulty resides in identifying the vector species (particularly in tropical regions; see Chap. 5 for a thorough introduction to blood sucking Diptera families across tropical regions), which lineages are capable of infecting vectors and also discovering the vertebrate species that vectors prefer feeding on. The development of molecular markers has allowed great advances in the study of avian malaria (Bensch et al. 2009; see Chap. 4 for a presentation and discussion of molecular methods used in avian haemosporidian research). However, the identification of avian haemosporidian lineages within insect vectors does not necessarily mean that they are capable of completing sporogony (see Chaps. 2 and 6; Njabo et al. 2011; Valkiūnas 2011). Current data on molecular markers suggest that most *Plasmodium* lineages are capable of infecting several vector species (Kimura et al. 2010; Martínez-de la Puente et al. 2011; Njabo et al. 2011; but see Gager et al. 2008). For example, *Plasmodium relictum,* can infect over 20 different mosquito species belonging to at least four different genera (Santiago-Alarcon et al. 2012a). Moreover, different vector species can share similar or identical *Plasmodium* lineages (Ferraguti et al. 2013). Data on vector competence of other avian haemosporidians also show several cases of large vector range for several *Haemoproteus* and *Leucocytozoon* lineages, as well as the same vector species being capable of harbouring several lineages (Santiago-Alarcon et al. 2012a). Data also suggest that the encounter filter might not be a strong driver in the structuring of *Plasmodium* assemblages (Medeiros et al. 2013). However, for *Leucocytozoon*, evidence suggests that vectors can work as encounter filters because blackflies feed on a subset of vertebrate hosts (Hellgren et al. 2008). Transmission success will depend on both the host and vector range of avian haemosporidians, wherein one extreme there will be lineages that are both vector and host generalist, and in the other extreme lineages that are both vector and host specialists. Understanding how transmission varies is important because it has direct consequences on the dispersal and

transmission of diseases in the wild. For example, avian malaria parasites that are transmitted by a wide range of vectors and capable of infecting a wide range of birds should have high transmission and dispersal success (Martínez-de la Puente et al. 2011).

11.3 Dispersal and Colonization of Communities

Dispersal of avian haemosporidians relies mainly on the bird hosts because vector dispersal is usually limited (Ejiri et al. 2011; but see discussion in Ellis et al. 2019). Moreover, bird hosts are endotherms and have longer lifespans, which guarantees a more constant environment when compared to vectors that are ectotherm and have very short lifespans (Sehgal 2015; Fecchio et al. 2019a, b; see Chap. 5). Vector specificity seems to play a minor role in the dispersal and structuring of avian haemosporidian communities (Gager et al. 2008; Njabo et al. 2011; Medeiros et al. 2013), probably because avian haemosporidian lineages are less selective regarding vector use (Kimura et al. 2010; Njabo et al. 2011) and vectors are less selective regarding which bird hosts they feed on (Santiago-Alarcon et al. 2012a,b; Santiago-Alarcon et al. 2013; Medeiros et al. 2013). However, some avian haemosporidian lineages can be vector specialists (Gager et al. 2008) and more studies are needed on vector use by avian haemosporidians, particularly experimental studies in order to determine vector specificity and other factors that can affect the transmission of these parasites (Valkiūnas et al. 2013; Palinauskas et al. 2015).

If the bird hosts indeed are the main drivers in avian haemosporidian distribution and dispersal, then one can expect lineage turnover to follow bird host turnover (with a role for vectors as parasite–vector and bird–vector relationships probably following similar patterns). Indeed, bird community similarity can predict parasite similarity (Ellis et al. 2015; Clark et al. 2018; Fecchio et al. 2018a; Álvarez-Mendizabal et al. unpublished). Therefore, the overlap of host species across different communities will connect these communities and allow the dispersal of avian haemosporidians (Clark et al. 2018). For example, in the Amazonian region, areas of bird endemism also constrained the distribution of *Plasmodium* lineages despite them presenting low host specificity and lack of specific phylogenetic constraint (Fecchio et al. 2018a). Also, generalist avian haemosporidians should be able to disperse more easily because more than one host species could provide a link between communities. However, dispersal will be limited if communities present interspecies barriers (Fig. 11.1). Indeed, phylogenetic host relatedness may to a variable extent determine avian haemosporidian turnover (Ellis et al. 2015; Clark and Clegg 2017; Clark et al. 2018), which suggests that dispersal will be more likely to occur between phylogenetically similar host communities (controlling for geographic distance, as phylogenetically similar host communities tend to be geographically close), a pattern that occurs for both generalist and specialist avian haemosporidians. Moreover, recalling what was discussed above, dispersal of avian haemosporidians between different populations of bird hosts from different bird

communities is more likely to occur for avian haemosporidian lineages that have a large geographic range (Drovetski et al. 2014; Mata et al. 2015). For example, specialist avian haemosporidian lineages will only occur in more than one bird community or be able to cross biogeographic barriers if their specific bird host is abundant in both communities (Mata et al. 2015; Clark et al. 2018). Conversely, generalist lineages should be less limited geographically because of their capability of infecting several host species, which could increase their connectivity with different host communities (Ewen et al. 2012).

Environmental and ecological conditions can also affect the dispersal and distribution of avian haemosporidians (Fecchio et al. 2019a, b; Clark et al. 2016, 2018). Climate will affect the distribution of host species and vectors (Fig. 11.3), but could also affect the development of avian haemosporidians directly when they are infecting their ectothermic vectors (Paaijmans et al. 2010). For example, *Plasmodium relictum* requires a temperature of over 13°C to complete its development within the vector *Culex quinquefasciatus* (LaPointe et al. 2010). Moreover, evidence suggests that host specificity may be a labile trait that varies biogeographically, potentially as

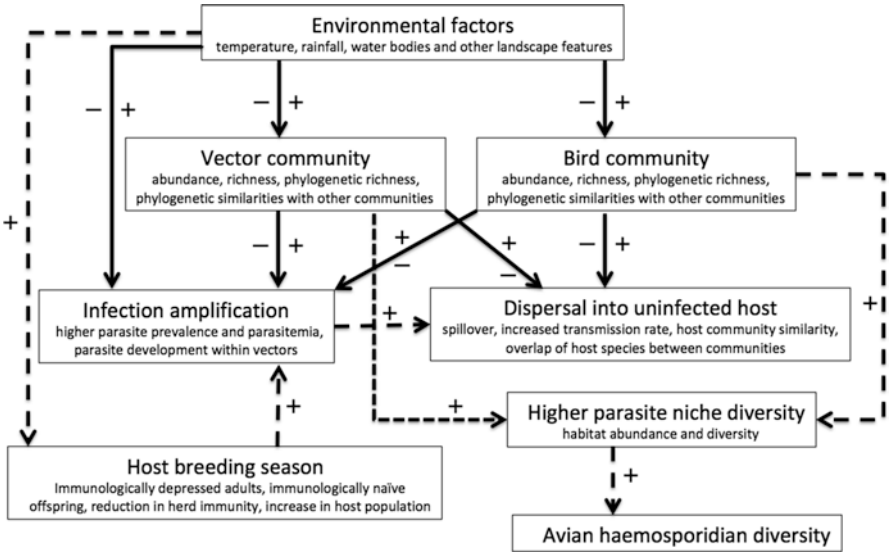

Fig. 11.3 Hypotheses on how environmental and biological factors can affect the incidence, transmission, and dispersal of avian haemosporidians. Understanding the effects of these factors on host–parasite dynamics should help unravel the dispersal and diversification mechanisms of avian haemosporidians, which in turn can help the understanding of the emergence of infectious diseases in wildlife and humans. Lines show positive and negative effects, while dashed lines show potential positive effects when there is an increase in one of the parameters inside the box. For example, vector communities that are richer in species and/or have higher abundance of vectors should have higher parasite niche diversity, which in turn should lead to an increase in avian haemosporidian diversity. The same idea applies for the bird community box. Conversely, host community similarity (i.e., high phylogenetic similarity between bird communities) should increase connectivity for many parasites species, which in turn could lead to more similar avian haemosporidian communities

a response to climate variation (Fecchio et al. 2019a, b). For example, regions that have marked rainfall seasonality or wetter dry seasons have more specialized avian haemosporidian lineages (Fecchio et al. 2019a, b). Interestingly, regions of high bird diversity such as the Amazonia and the Andes, which have high rainfall, tend to have less specialized lineages with host switching as the main diversification mechanism (Galen and Witt 2014; Moens and Pérez-Tris 2016; Fecchio et al. 2018b). However, water availability is an important environmental predictor of vector abundance (Smith et al. 2004; Okanga et al. 2013; Sehgal 2015; see Chaps. 5 and 6), which can lead to higher incidence of avian haemosporidians in wild birds (Wood et al. 2007; Krama et al. 2015; Ferraguti et al. 2018; Santiago-Alarcon et al. 2019). Landscape features and other environmental variables seem to be important in determining the distribution of avian haemosporidian lineages (Pérez-Rodríguez et al. 2013a; Sehgal 2015; Ferraguti et al. 2018; see Chaps. 10 and 14). For example, temperature and distance to artificial water reservoirs can be important predictors of avian haemosporidian prevalence (Pérez-Rodríguez et al. 2013a; Sehgal et al. 2011; González-Quevedo et al. 2014). It is possible that at a more local scale, landscape features will have a stronger effect on vector distribution and abundance, which in turn will structure avian haemosporidian assemblages locally by increasing the incidence of infection and potential dispersal (i.e., spillover) to uninfected bird hosts (e.g., Renner et al. 2016; Santiago-Alarcon et al. 2019; Fig. 11.3).

Understanding host–parasite dynamics is crucial for understanding parasite dispersal and how different ecological factors will act on different scales. For example, increased vector abundance should lead to the increase in local parasite prevalence (Fig. 11.3). Even on a local scale, avian haemosporidian prevalence can vary substantially because of local environmental features such as distance to water, variation in vegetation cover, and temperature (Wood et al. 2007; Sehgal et al. 2011; González-Quevedo et al. 2014; Renner et al. 2016; Ferraguti et al. 2018). Also, prevalence can vary seasonally due to climatic differences and the entering of new recruits (after the breeding season) that are expected to be immunologically naïve (Cosgrove et al. 2008), which in turn can increase the incidence of infection (Fig. 11.3). Therefore, dispersal of avian haemosporidians relies on several factors: (i) host and vector specificity; (ii) environmental factors that affect the distribution and abundance of vectors and bird hosts; (iii) breeding season of bird hosts (increase in immunologically naïve hosts); (iv) vector community composition (abundance, richness, phylogenetic diversity, phylogenetic similarities between different communities); and (v) bird community composition (abundance, richness, phylogenetic diversity, phylogenetic similarities between different communities). For example, bird communities that have high phylogenetic diversity and bird richness provide a more diverse array of available niches for parasites (Lacorte et al. 2013; Clark and Clegg 2017; Clark et al. 2018), which should lead to higher avian haemosporidian diversity (Fig. 11.3; Poulin 2014). Testing the effects of the factors presented in Fig. 11.3 is important to determine how avian haemosporidians dispersal is affected, and it should help in the understanding of how avian haemosporidian communities are structured and connected locally and regionally (see Chap. 0 for an ecological niche modeling perspective).

11.4 Dispersal of Avian Haemosporidians on Islands

Colonization of islands by vector-borne parasites such as avian haemosporidians is a complex process. Avian haemosporidian must first manage to arrive at an island, which can occur via host migration or dispersal or even due to the arrival of vagrant hosts or windblown vectors (Clark and Clegg 2015; see Parker 2018 for a review of research on the Galapagos Islands). However, several factors are expected to affect the persistence of avian haemosporidians on islands (see Chap. 8 for research on avian haemosporidian island biogeography in the Neotropics). For example, host specificity can play a significant role in determining the persistence of avian haemosporidians on islands, where generalist avian haemosporidian lineages are expected to have a higher probability of persistence (Ewen et al. 2012; Pérez-Rodríguez et al. 2013a; Clark et al. 2014) and also a higher probability of becoming invasive (Mack et al. 2000; see Chap. 15 for an in-depth treatment of the role of parasites on invasion biology). Generalist parasites should be better colonizers because they could switch to other more abundant host species present on the invaded island, allowing them to persist despite having a low prevalence in the arriving host species (Ewen et al. 2012; Pérez-Rodríguez et al. 2013a). The presence of suitable vectors on islands is also important for the maintenance of arriving avian haemosporidians, which might have a greater difficulty of arrival (particularly for more isolated islands) and persistence. It is known that temperature and water availability are important elements for the development of vectors (Lapointe et al. 2010; Okanga et al. 2013; see Chaps. 5 and 6) and will also affect the prevalence of avian haemosporidians (Sehgal et al. 2011; González-Quevedo et al. 2014; Ferraguti et al. 2018). Therefore, depending on the environmental conditions of the islands, vector abundance and diversity may be limited.

The diversity of haemosporidians on oceanic islands is lower than for continental areas (Clark et al. 2014), which is expected. Also, avian haemosporidian lineages that have large geographical range sizes or that are more common on the mainland will usually be more common on islands (Santiago-Alarcon et al. 2010; Ewen et al. 2012; Perez-Rodríguez et al. 2013a), and islands closer to the mainland tend to have higher avian haemosporidian diversity (Fallon et al. 2005; Ricklefs et al. 2011; Perez-Rodríguez et al. 2013a). Thus, dispersal of avian haemosporidians to islands is facilitated if islands are close to the continent or have been connected to mainland in the past (Fallon et al. 2005; Perez-Rodríguez et al. 2013a; Soares et al. 2017). Moreover, the prevalence on islands can be lower when compared to the mainland and can have lower temporal stability (Perez-Rodríguez et al. 2013a), which can lead to increase lineage turnover due to colonization and extinction events (Fallon et al. 2004).

Biogeographic patterns and dispersal can vary regarding *Plasmodium* and *Haemoproteus*. For example, *Haemoproteus* distribution in southern Melanesia was more associated with the presence and availability of bird hosts, while *Plasmodium* distribution was more associated with geography and local island conditions instead of host availability (Olsson-Pons et al. 2015). Moreover, generalist lineages are

usually more common on islands (Ishtiaq et al. 2008; Clark and Clegg 2015). Evidence suggests that *Haemoproteus* lineages are less frequent on islands at the global scale (Clark et al. 2014), which could be explained by the fact that *Haemoproteus* parasites tend to be more specific regarding bird hosts (Beadell et al. 2004, 2009; but see Moens and Pérez-Tris 2016; Ellis et al. 2015) and vector species (Martínez-de la Puente et al. 2011). More research is needed, however, given the current island geographical sampling bias (i.e., Hawaii, Galapagos, Caribbean Antilles), where on some archipelagos *Haemoproteus* is the dominant avian haemosporidian genus (Parker 2018). It is also possible that there is a paucity of suitable vectors (Diptera: Ceratopogonidae) for *Haemoproteus* parasites on islands or a lack of suitable vector habitat. Vector specificity would explain the lower colonization success of *Haemoproteus* compared to *Plasmodium*, a parasite genus that has been a protagonist of notorious cases of invasion of island bird communities (Beadell et al. 2006; Ewen et al. 2012).

11.5 Host Specialization in Tropical Avian Malaria

Understanding parasite diversity and specificity in tropical habitats is important for public health and the conservation of biodiversity. Tropical bird communities might be reservoirs of parasites that could cause tremendous problems if moved away from their native range, either by their impact on poultry or their effects on native wildlife (Vanstreels et al. 2014; Moens and Pérez-Tris 2016; see Chap. 15). Conversely, the valuable biodiversity of tropical habitats may be threatened by invasive parasites (Marzal et al. 2015). The degree of specificity of host–parasite interactions plays an undoubtedly prominent role in parasite exchange, both by determining the capability of tropical parasites to infect a wide range of host species (and therefore cause problems if moved outside their native range [Ewen et al. 2012; Moens and Pérez-Tris 2016]), or the compatibility of tropical bird species as hosts of generalist parasites that might be imported into the tropics, causing disease (Marzal et al. 2015; Ferreira-Junior et al. 2018; Ortiz-Catedral et al. 2019).

Most research on avian malaria parasites has traditionally been done in temperate regions of the world (Bensch et al. 2009; see Chap. 1 for a review of avian haemosporidian research in tropical regions of the world during the twentieth century). Studies in tropical regions are scarce and are concentrated in a few areas (Clark et al. 2014). As a consequence, the current knowledge of the diversity and host specificity of avian malaria parasites in the tropics remains incomplete (Outlaw et al. 2017), making it difficult to establish general patterns for tropical parasites. Various studies have tried to point out the singularity of tropical habitats from this perspective, aiming at identifying different factors that may affect diversity and specificity of parasites in the tropics (e.g., Svensson-Coelho et al. 2014). A general conclusion is that tropical and temperate communities are formed by different parasite species, with a minority of elements of parasite diversity being shared between regions (Svensson-Coelho et al. 2013; Moens and Pérez-Tris 2016). This is a

remarkable observation, given the fact that migratory birds usually carry parasites acquired in temperate areas to the tropics and vice versa, yet the parasites typically fail to thrive in different regions (Hellgren et al. 2007; Pérez-Rodríguez et al. 2013b; Ricklefs et al. 2017; see Chap. 16 for a synthesis of current research on avian migration and its role on avian haemosporidian latitudinal dispersal). Knowing what keeps parasites confined to different transmission areas (from phylogenetic similarity of host communities to variable availability of competent vectors; see, e.g., Ricklefs et al. 2017) is central to our understanding of the evolution of parasite specificity in the tropics.

The most immediate elements invoked to explain the structure of tropical parasite assemblages are climate and vector diversity. Environmental factors and most notably temperature may affect parasite life cycles, even blocking their development in vectors in climatically unsuitable habitat (LaPointe et al. 2010). This could explain why the transmission of avian haemosporidian lineages that are brought over by migratory birds in their peripheral blood is interrupted during the reproductive season (and reciprocally, temperate avian haemosporidian lineages fail to establish themselves in tropical non-breeding habitats when birds return; Hellgren et al. 2007; Ricklefs et al. 2017). Parasites may be therefore constrained to exploit the subset of compatible host species that are encountered by their vectors during periods of favorable environmental conditions, increasing specificity as a consequence. In fact, climate has recently been identified as a relevant factor explaining geographical variation in specificity of avian haemosporidian parasites (Fecchio et al. 2019a, b).

However, climate is too variable in the tropics to make it possible to define a common scenario for the evolution of parasite specificity in the region, where sharp environmental gradients take place at small geographic scales compared with temperate regions (Malhi et al. 2010; see Chap. 10 for a review of avian haemosporidian studies conducted on environmental gradients). For example, tropical mountain ranges such as the Andes create broad geographic areas characterized by sharp elevational gradients from the lowland rainforest at the sea level to the open Paramo ecosystem well above 3000 m asl. These gradients may contribute to the structuring of parasite assemblages among the bird species that occur at different elevations, increasing parasite diversity and specificity at the regional scale (Galen and Witt 2014). On the other hand, these gradients might favor environmentally generalist parasites that are capable of thriving across the broad range of environmental conditions that their hosts may encounter within short distances (i.e., from tens to couple of hundred kilometers). Conversely, climate may constrain parasite geographic distributions if parasites are unable to thrive in unsuitable climatic regions (e.g., colder habitats at higher elevations, drier habitats such a tropical seasonally dry forests), although such climate constraints may attenuate due to global change, which is of major concern for tropical birds inhabiting high-elevation and geographically restricted habitats (Prieto-Torres et al. 2016; Liao et al. 2017). Whether regional climatic gradients promote or reduce parasite–host specificity remains an open question, on which research in tropical habitats may shed much light.

In combination with climatic effects, the specificity of parasite–vector associations may contribute to shaping the network of bird–parasite interactions, increasing or lowering the specificity of their relationship in different regions (Santiago-Alarcon et al. 2012a). As it was discussed above, the diversity of vectors changes across geographic regions, both qualitatively and quantitatively, and this may affect the patterns of parasite distribution among bird hosts (see Chap. 6). Only a few studies have analyzed the role of dipteran vectors in structuring bird–parasite relationships, showing that compatibility filters (Fig. 11.2) are more likely to be operating at the bird host level (Medeiros et al. 2013). However, we know too little on the diversity of vectors and the structure of parasite–vector interactions to confidently interpret the role of vectors in shaping bird–parasite relationships. This lack of knowledge is probably more limiting in the tropics (Santiago-Alarcon et al. 2012a; Chap. 6), where an increased diversity of dipteran species capable of transmitting parasites is probably increasing the complexity of bird–vector–parasite interactions, obscuring the role of vectors in the structuring of bird–parasite relationships (Svensson-Coelho et al. 2016; see Chap. 9).

If the diversity of vectors is an important factor explaining bird–parasite relationships in the tropics, the diversity of bird hosts may also be relevant in this regard. Tropical habitats are renowned by their great bird diversity, which may promote dilution effects and select for generalist strategies of host exploitation among avian malaria parasites (this may lead to amplification effects on other geographical areas with less host diversity, if such generalist parasites are able to reach them). Supporting this idea, the megadiverse rainforests of the Amazonian slopes of the Andes are home to the most generalist members of the genus *Haemoproteus* reported so far (Moens and Pérez-Tris 2016). However, this pattern is far from general: a comparison of two bird–parasite interaction networks (one in temperate and one in tropical habitat) failed to detect any difference in the degree of host specialization of both parasite communities. In fact, parasites showed high host specialization in both cases, meaning that a generalist strategy is not necessarily general among the parasites that occur in bird diverse regions (Svensson-Coelho et al. 2014). There is also evidence of environmental influences contributing to the variation in host specificity of avian malaria parasites. For example, in tropical Africa, parasite diversity and specialization was found to be higher in lowland rainforests compared to the more climatically variable highland forests (Loiseau et al. 2012). In addition, the diversity of host life histories may also contribute to increase the variation in parasite host ranges. For example, long-lived or abundant host species favor parasites that specialize in their exploitation (e.g., Santiago-Alarcon et al. 2016; Svensson-Coelho et al. 2016). These processes may increase modularity in the network of bird–parasite interactions, which in turn may increase variance in the success of specialists and generalist parasites among bird communities (Pinheiro et al. 2016; Chap. 9). To sum up, we still know very little about the relative influence of habitat features and bird communities in shaping avian malaria relationships. This knowledge gap may be of conservation concern, because the above influences may prove critical for the understanding of human impacts on the ecology of wildlife

diseases, such as those associated with habitat modification and hunting (Chasar et al. 2009; see Chap. 14).

To further complicate our understanding of bird–parasite relationships in the tropics, local variation in the intensity of infection (i.e., parasitemia) among the many hosts infected by generalist parasites, indicates that even the most generalist parasites seem to be more specialized than they appear based solely on their list of infected species. For example, *Haemoproteus witti*, a parasite first discovered in hummingbirds, scores the greatest host range among parasites of its genus in various bird communities in the Neotropics, where it infects birds of several orders. However, this parasite is only found to produce gametocytes detectable in peripheral blood in hummingbirds, supporting the idea that *H. witti* is more specific than its reported list of infected hosts may suggest (Moens et al. 2016).

In summary, our knowledge of the patterns of host specialization and dispersal of avian malaria parasites is in its infancy. We are well aware of the importance of these attributes of parasites for understanding their patterns of global distribution, both in space and among host species, which ultimately determine (or are the result of) the capability of parasites to colonize new hosts or geographic regions (Ewen et al. 2012). In order to anticipate future problems associated with disease emergence, we must disentangle the network of bird–vector–parasite interactions, and understand how its architecture promotes or constraints parasite transmission across species or geographic areas. Research in the tropics may prove influential in this field because of the existence of sharp environmental gradients associated with tropical mountains, which may facilitate the analysis of environmental influences on bird–parasite relationships at more local scales compared to other regions. From a conservation perspective, we also need to improve the current understanding of the impact of native and introduced parasites on tropical avifauna, in order to design management plans to avoid and/or contain parasite spread.

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Glossary of Technical Terms

Amplification effect A positive correlation between host diversity and parasite transmission success. In avian haemosporidians, an amplification effect may be expected when the parasite increases host range (i.e., becomes more generalist) in diverse ecological communities.

Compatibility filters The different biological barriers that prevent certain elements in the network of host–vector–parasite relationships from establishing an interaction.

Dilution effect A negative correlation between host diversity and parasite transmission success. In avian haemosporidians, a dilution effect may be expected when the proportion of incompatible or suboptimal hosts increases in diverse ecological communities.

Encounter filters The ecological barriers that prevent the parasite to encounter a potential compatible host.

Environmental barrier Environmental features that potentially affect the distribution of species (parasite, host, and vector). For example, regions may have different habitats, macroclimates, topography, and landscape (i.e., variation in the land surface regarding the distribution of habitats).

Geographic barriers Natural geographic features that act as physical barriers to the dispersal of avian haemosporidians between different regions, such as the presence of a mountain range or a water body.

Host compatibility The capacity of a bird species to act as a suitable host for a parasite. Incompatible hosts, also known as dead-end hosts, cannot be successfully exploited by the parasite, either because the host clears the infection before the parasite can be transmitted by vectors (i.e., the host is resistant to the infection) or because the parasite fails to develop transmissible blood stages. Host compatibility can also be applied to vector species.

Host range Also known as host breath. It represents the diversity of host species that a parasite can exploit, which can be measured by the richness or phylogenetic diversity of compatible host species.

Host shift The event that leads to the colonization of a new host species in the evolutionary history of interactions of a parasite.

Host specificity The degree to which a parasite is specialized in a subset of the vertebrate hosts or vector species.

Host susceptibility The degree to which a host may be exploited by a parasite. In avian haemosporidians, this can be measured with prevalence and infection intensity or parasitemia. When hosts are not susceptible at all, they are termed incompatible or non-competent hosts.

Interspecies barrier The variation in the attributes of host and vector species that prevent the transmission/spread of parasites into different host and vector species. These attributes can include ecological traits, such as nesting and foraging behavior of hosts, vector feeding behavior, and microhabitat use of both hosts and vectors. Immunological similarities of hosts and vectors (i.e., phylogenetic relatedness) should also have an important role.

Parasitemia An index of infection intensity. When applied to avian haemosporidian parasites, it is measured as the proportion of circulating red blood cells that are infected by parasites (usually researchers calculate such proportion based on the observation of 10,000 red blood cells).

Transmission The passage of a parasite from one vertebrate host to the next assisted by an insect vector.

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Chapter 12
Cophylogenetic Patterns and Speciation in Avian Haemosporidians

M. Andreína Pacheco and Ananias A. Escalante

Abstract An extraordinary surge in the number and quality of avian haemosporidian studies in the Neotropics is unveiling the complex ecology and evolution of a successful group of parasites that have a global distribution and staggering diversity. However, despite avian haemosporidian parasites being ubiquitous, many factors still limit our understanding of their diversity. First, traditional taxonomy demands information that is relatively challenging to scale up, so several molecular lineages that are likely new species remain as "dark taxa". Second, there exists only a limited characterization of how parasites inhabit multiple hosts from a handful of censuses. Third, an understanding of the temporal and spatial scales of speciation in this group is limited by a framework built on associations and general patterns. These factors will be discussed by explaining how species are described and delimited, how the available evidence provides insight into possible mechanisms that may elucidate the staggering diversity of haemosporidian parasites, and finally, how the available tools allow us to make preliminary inferences about the time scale of such speciation processes. Although broad in scope, this chapter highlights the need for understanding community-level processes to explain the origins and speciation in this parasite group.

Keywords Allopatric and Sympatric Speciation · Cospeciation · Host–parasite interactions · Host switches · Molecular Clock · Phylogenetic Species

12.1 Introduction

Developing a species concept has been a challenge in evolutionary biology (Zachos 2016). Furthermore, despite being commonly treated as the same problem, the idea of a species concept differs from the tasks of how to discover, delimit, and identify them (Hey 2006; De Queiroz 2007). Indeed, there are several shared premises

M. A. Pacheco (✉) · A. A. Escalante (✉)
Biology Department/Institute of Genomics and Evolutionary Medicine (iGEM), Temple University, Philadelphia, PA, USA
e-mail: maria.pacheco@temple.edu; ananias.escalante@temple.edu

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(implicit or explicit) among the many proposed species concepts (De Queiroz 2007; Zachos 2016). First, the species is an organizational level comprised of lineages descending from a common ancestor. Second, individuals belonging to one species share biological characteristics that emerge and are further shaped by similar evolutionary processes. Third, these biological characteristics differ sufficiently from those observed in other species, allowing for generalizations about a specific species and its populations. Finally, species are transient at the macroevolution level, but stable at ecological-microevolutionary time scales so that they can be discovered, delimited, and characterized. Despite these shared premises, the ongoing discussion focuses on how to delimit and describe species in ways that are operational, reproducible, and objective (Hey 2006; Zachos 2016).

The discovery and delimitation of species are essential to the description and understanding of biodiversity. Furthermore, species discovery and delimitation enable the integration of heterogeneous and, usually incomplete, types of data from diverse sources. These data are critical for understanding evolution, and characterizing processes at both population and community levels (Hey 2006; Zachos 2016; see Chap. 11 for a thorough revision of host specialization and dispersal in avian haemosporidians). In the case of parasites, identifying or describing species has practical implications in terms of health and policy (Garnham 1966; Stentiford et al. 2014). The importance of this topic translates into multiple criteria for differentiating and describing species, a discussion that is not new in avian haemosporidians (Hewitt 1940).

In this chapter, the methods used to delimit species in avian haemosporidian parasites are discussed, emphasizing that species are testable hypotheses. In such a context, the requirements of taxonomy versus those from systematics, evolutionary biology, and ecology are compared. A discussion on the current understanding of factors driving haemosporidian speciation follows. Finally, attempts to provide a time scale for the diversification of haemosporidian species are described and compared. Overall, this chapter attempts to highlight the main concepts of the field (as summarized in the glossary presented in Fig. 12.1) and the issues requiring attention.

12.2 Species Discovery and Delimitation in Avian Haemosporidians: An Overview

Characterization of haemosporidian parasites is conventionally done via morphological description of parasite blood stages through the examination of Giemsa-stained blood films using a light microscope (e.g., Valkiūnas 2005; see Chaps. 2 and 6 for a thorough introduction to haemosporidian life cycles). Parasites are then described or identified as a distinct species based on morphological characteristics (Garnham 1966; Valkiūnas 2005; Valkiūnas and Iezhova 2018). The characters used for species identification include specific parasite structures and measurements taken during the blood cells' infection of the host and sexual stage differentiation (gametocytes). This method has been called the "morphological species concept" (Martinsen et al. 2006) to emphasize that this approach discovers and delimits species based on visible characters on blood stages. Because a putative species is an

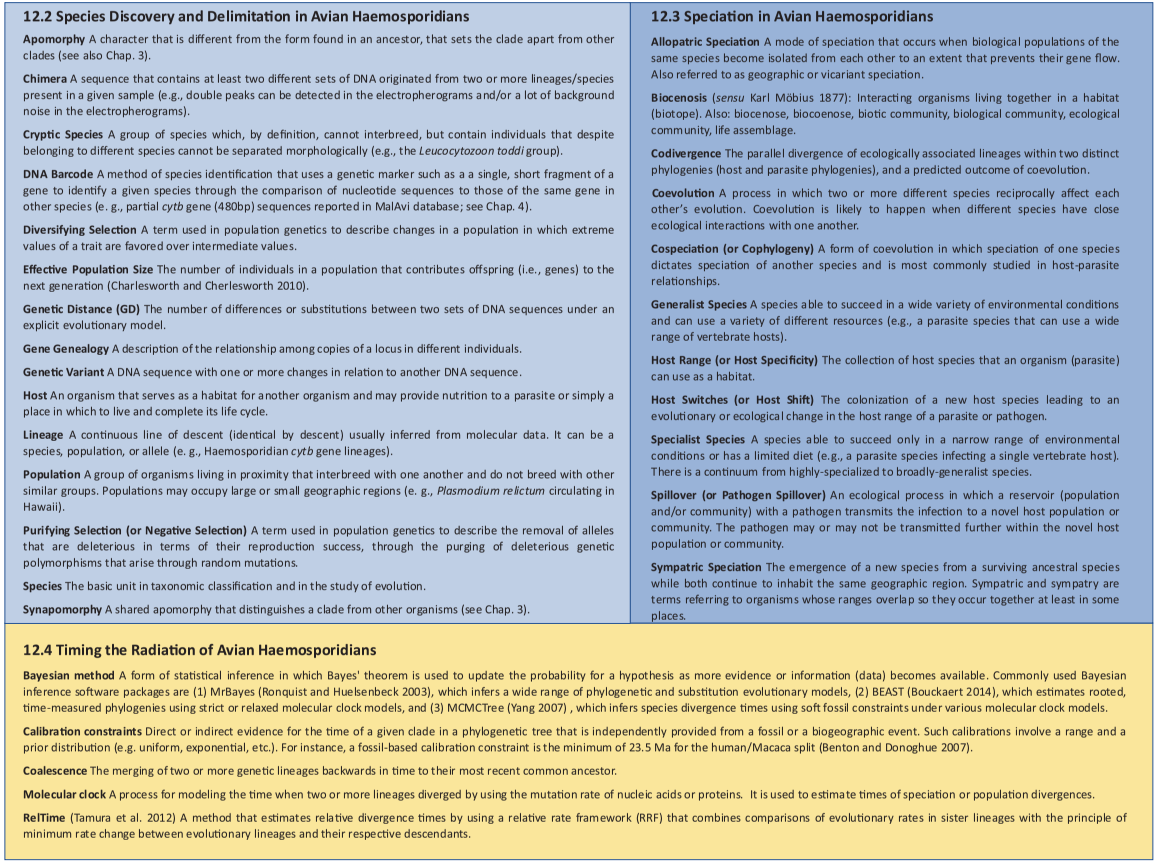

Fig. 12.1 Glossary

approximation to biological reality, it remains a hypothesis. In consequence, biological material (type and voucher preparations) must be available to evaluate and test the taxonomy (Valkiūnas and Iezhova 2018). Although the goal of this approach is to follow a rigorous and reproducible process, there are some caveats associated with reliance on morphological traits.

Morphological characteristics, for example, could be less evident or even change across hosts (Garnham 1966; Perkins 2014). They are also affected by a lack of expertise in the fixation and examination of blood films (Perkins 2014; Valkiūnas and Iezhova 2018). The situation is further complicated by submicroscopic infections (a low-density parasitemia below the limit of detection by examination of blood films but that are found by molecular methods), making the observation of parasite stages challenging in the first place. Finding all possible blood stages of a species may require examination of multiple host specimens (Valkiūnas and Iezhova 2018), something that is not common in biodiversity surveys. Finally, even morphologically distinct species that were well described long ago are not adequately delineated across their distribution in terms of hosts and geography (e.g., *Plasmodium relictum*, Valkiūnas et al. 2018). Altogether, these factors make it challenging to scale up classical taxonomic methodology to support the study of haemosporidian species diversity (Outlaw and Ricklefs 2014). Because of these limitations, morphology is not widely used in biodiversity studies. Instead, molecular information is currently the predominant approach used in detecting and identifying parasite lineages, inferring patterns in parasite diversity, and constructing parasite phylogenies.

Molecular phylogenetic analysis of haemosporidian parasites started in the early 1990s using 18 s SSU rRNA in the context of inquiring about the origins of human malarias (Waters et al. 1991; Escalante and Ayala 1994). However, the use of this locus for phylogenetic purposes was abandoned within Haemosporida due to the occurrence of nonconcerted evolution among stage-specific expressed paralogs that makes interpreting gene trees in terms of species trees challenging (Gunderson et al. 1987; Nishimoto et al. 2008). It was not until the parasite's mitochondrial cytochrome b gene (*cytb*) was used in a haemosporidian phylogeny (Escalante et al. 1998) that molecular lineages became a proxy to delimiting species in avian haemosporidian parasites (Bensch et al. 2000; Ricklefs and Fallon 2002; Fallon et al. 2003; Hellgren et al. 2004; Ricklefs et al. 2004; Bensch et al. 2009; see Chap. 3 for an introduction to the methods of phylogenetics and systematics).

Targeting the *cytb* gene to discover and delimit species has been referred to as the "genetic species concept" (Martinsen et al. 2006) because genetic material is investigated to delimit a species. However, the use of the term may not be accurate. Haemosporidian *cytb* lineages are not tested as groups of genetically compatible interbreeding natural populations that are isolated from other such groups (e.g., measuring gene flow, see Zachos 2016). Nevertheless, this method has advantages over that of morphology in the sense that information is easily generated, reproducible, quantitative, and can be unambiguously digitalized. In addition, mitochondrial genomes are well conserved among haemosporidian genera and exist as multiple copies per parasite (Pacheco et al. 2018a), allowing for suitable primers to detect diverse parasites using *cytb* with high sensitivity (Bensch et al. 2000; Ricklefs and Fallon 2002; Hellgren et al. 2004; Waldenström et al. 2004; Pacheco et al. 2018b).

The use of *cytb* gene for species delimitation also has made evident some of the limitations of the use of parasite morphology by discovering cryptic species (Sehgal et al. 2006; Clark et al. 2015; González et al. 2015; Palinauskas et al. 2015; Lotta et al. 2016). For these reasons, the use of *cytb* gene has become the de facto – DNA-based identification method or "barcoding" of haemosporidian parasites (Bensch et al. 2009; Outlaw and Ricklefs 2014; Pacheco et al. 2018a, b).

It is worth distinguishing between species identification via molecular data (DNA barcoding in the strict sense) and species discovery/delimitation using a DNA barcode (Collins and Cruickshank 2013). The first requires that molecular data be linked to described taxa, something that is still a work in progress in haemosporidian parasites (Valkiūnas and Iezhova 2018) from the Neotropics. On the other hand, species discovery and delimitation require a criteria such as a genetic distance, phylogenetic analyses, or a character (e.g., a unique insertion) (Collins and Cruickshank 2013; Outlaw and Ricklefs 2014).

There are technical problems worth mentioning related to the use of molecular methods in general, and for single-gene approaches in particular. Multiple species coinfecting a single host (usually called mixed infections) are common in avian hosts (Bernotienė et al. 2016; Pacheco et al. 2018b). Thus, there is a real possibility of creating DNA chimeras between different parasite species/lineages when a PCR amplicon is sequenced directly. This problem can be mitigated by careful examination of the electropherograms, cloning, deep-sequencing, and/or studying a series of positive specimens (not necessarily from the same host species) showing the reproducibility of the lineage. Another problem is that primers can fail to amplify a particular species or group. For example, the prevalence of *Leucocytozoon* species is underestimated by commonly used primers (Jia et al. 2018; Lotta et al. 2019). This problem can be solved by generating additional genome-level data that can support the design of new primers that can detect these taxa. Finally, the widely used fragment of the *cytb* gene has a limited number of informative sites (Pacheco et al. 2018b). This issue can be solved by using primers that can amplify a larger *cytb* fragment (Pacheco et al. 2018b). Beyond these technical aspects, the use of molecular data to delimit species has additional considerations worth mentioning (see also Chap. 4 for a review of molecular methods and good practices in the study of haemosporidians).

Defining useful criteria to separate species, such as the genetic distance between lineages, requires an understanding of existing genetic diversity and mutation rates. In the case of *cytb* and other mitochondrial genes, it seems that they do indeed evolve at a mutation rate that allows for species delimitation, at least at the scale that morphological species have been described (Pacheco et al. 2018a). A potential problem, however, is that genetic polymorphisms may be shared between closely related species, leading to incomplete lineage sorting (Fig. 12.2). Importantly, mitochondrial genes are expected to have a smaller effective population size (see Glossary, Fig. 12.1) than nuclear genes, so they likely will more rapidly coalesce to their most recent common ancestor than alleles in nuclear loci, facilitating complete lineage sorting. Still, a single-gene phylogeny, even one with excellent statistical support, does not necessarily reflect the species tree. Since inbreeding in the haemosporidian parasites is expected to be high due to their life cycle (see Chap. 2), the fixation of variants should also limit shared polymorphisms between closely related species unless there

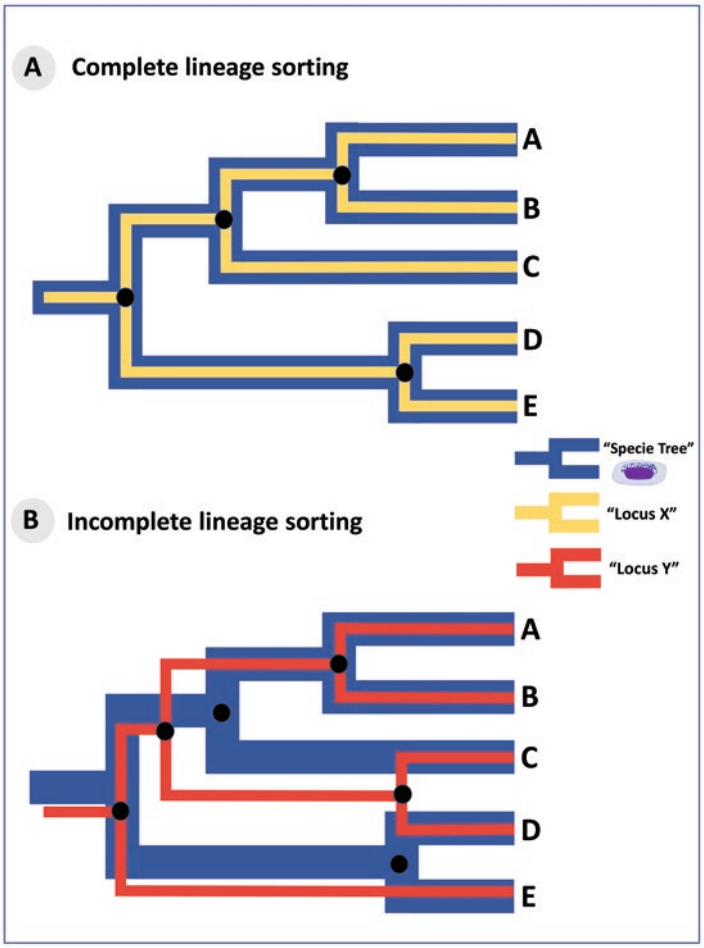

Fig. 12.2 Schematic representation of complete (**a**) and incomplete lineage sorting (**b**). In complete lineage sorting, the tree produced by a given locus "X" is the same as the population or species level tree. In contrast, in incomplete lineage sorting, the tree produced by a given locus "Y" differs from the population or species level tree, probably as a result of the fixation of an allele that was part of ancestral polymorphisms present at the moment of species divergence. As a result, species and the gene level trees could vary depending on the selected genes used for assessment. In haemosporidians, the most commonly used loci are *cytb*, *cox1* (mitochondrion genome), and *clpc* (apicoplast genome) genes and more recently, nuclear genes obtained by next-generation sequencing (NGS)

is hybridization or strong diversifying selection (Fig. 12.1), patterns that have not been detected in mitochondrial genes (Pacheco et al. 2018a). Finally, an additional point to consider is how purifying selection (Fig. 12.1) acts on coding genes between lineages. In the case of *cytb* and other mitochondrial genes, it has been found that the strength of purifying selection changes between haemosporidian clades; thus, what is a meaningful difference in *Haemoproteus* (*Parahaemoproteus*) spp. may not be the

same in *Plasmodium* spp. or *Leucocytozoon* spp. (Pacheco et al. 2018a). All these elements should be considered when a single gene, even one with a justifiable distance criterion, is proposed for species delimitation.

Regardless of these limitations, the single-gene approach based on *cytb* gene has proven to be an extraordinary tool to unveil the diversity of haemosporidian species. It is important to highlight that with the scale-up of molecular methods comes a proliferation of biodiversity data without specimens (Troudet et al. 2018). This "dark taxa" (Page 2016) problem is worsened when nonorthologous *cytb* gene regions are used (Fallon et al. 2003; Hellgren et al. 2004).

Building on this experience, there are two major multigene approaches to species delimitation that have been utilized (Bensch et al. 2004; Martinsen et al. 2008; Galen et al. 2018a; Pacheco et al. 2018a). One uses the complete mitochondrial genome (Pacheco et al. 2018a), while the other instead implements the use of various loci, including a combination of other mitochondrial genes (e.g., cytochrome c oxidase subunit 1 gene, *cox1*), some apicoplast genes (e.g., *clpc* gene, Santiago-Alarcon et al. 2010; Valkiūnas et al. 2019), and/or several genes from the nuclear genome (e.g., Bensch et al. 2016; Galen et al. 2018a, b). Thus far, both approaches have provided similar results with the exception being among those taxa that are not sampled in all estimated phylogenies, and as such, cannot be fully compared between studies. Although these methods offer additional information when compared to single-gene data, the choice of loci remains essential (see also Chap. 4).

Regarding loci choice for multigene approaches, it is worth noting that the mitochondrial genome is a single locus with multiple genes, so it has the advantage (or problem, depending on its use) that it is not expected to recombine. In addition, it also appears not to be saturated at the time scales under consideration, and has comparable codon usages across haemosporidian taxa, reducing the risk of model misspecifications during phylogenetic analyses (Pacheco et al. 2018a). However, as in the single-gene approach, use of the mitochondrial genome requires attention to coinfections. Using cloning or next-generation sequencing may be required to resolve coinfections, tools that are not widely available in the research community given their cost. The use of multilocus data that incorporate nuclear genes has some additional issues that demand attention. One possible source of error is the concatenation of loci from coinfections (more than one species coinfecting a host) in an alignment. This potential problem is worsened given short data reads that can be produced by next-generation sequencing, as DNA chimeras can be assembled due in part to the lack of reference genomes. In addition, different gene genealogies (Fig. 12.1) may have disparate phylogenetic signals; the possibility of the maintenance of ancestral shared polymorphisms in closely related species, particularly during fast speciation (incomplete lineage sorting, Fig. 12.2); and possible differences in codon usage between orthologues leading to model misspecification in phylogenetic analyses. Irrespective of these cautionary notes, the information obtained thus far from the use of mitochondrial genomes (Pacheco et al. 2018a), multiloci phylogenies (Galen et al. 2018a), and the combination of morphological and molecular data (Hernández-Lara et al. 2018; see also Chap. 3) are comparable. It is worth noting that, in general, all criteria used in contemporary studies to

describe and delimit avian haemosporidian parasites (morphological, single gene, and/or multiple loci approaches) consider species as part of a phylogenetic hypothesis (Zachos 2016). This common characteristic makes the "phylogenetic species" concept the prevailing one regardless of the method used to delimit the taxa.

How to discover and delimit species? Described species are expected to be evaluated in terms of how empirical data and analytic methods allow taxonomists to define groups of organisms that belong to a species category (or any taxa). Consensus is emerging on the merits of an integrative approach, as such a method creates consistency between different lines of evidence both at and above the species level (Lotta et al. 2016; Hernández-Lara et al. 2018; Valkiūnas and Iezhova 2018; Pacheco et al. 2018a). However, such congruence is not perfect between all approaches as evidenced by cryptic species or species with apparent morphological differences (*H. jenniae*/*H. iwa*, Levin et al. 2012) and limited mtDNA genes divergence (Levin et al. 2012; Pacheco et al. 2018a; Valkiūnas et al. 2019). Nevertheless, the discussion, for those interested in ecological and biodiversity patterns, is not on which criteria are necessary to name a species, but rather on how to compile evidence indicating that a new parasite species has been found, how potential species disperse, and their relative abundance within and among hosts in a particular geographic or temporal context. Under such circumstances, molecular methods have been the tool of choice (Outlaw and Ricklefs 2014).

The use of nonmorphological data to name species has been long deliberated on in haemosporidian parasites (Garnham 1966; Valkiūnas 2005), with early proponents considering such nonmorphological data as crucial to discovering and delimiting species (Hewitt 1940; Garnham 1966). Nevertheless, morphological traits are usually required to name a species even when such characteristics may not be sufficient and cryptic species appear to be common (Perkins 2014; Sehgal et al. 2006; Palinauskas et al. 2015; González et al. 2015; Lotta et al. 2016). Thus, many potential species discovered by using molecular data remain as "dark taxa" (Page 2016).

This "taxonomic load" has been mitigated by recording detection-based occurrences of lineages against a reference database such as MalAvi (Bensch et al. 2009) and the use of standard sets of primers targeting a specific gene region of *cytb* as a reference (Hellgren et al. 2004; Bensch et al. 2009; see Chap. 4). There are two criteria used to define avian haemosporidian lineages, the most common focusing on nucleotide differences (Bensch et al. 2009) and the second being a combination of genetic information and host/geographic distribution (Fallon et al. 2005; Outlaw and Ricklefs 2014). Unfortunately, not all lineages are fully comparable because of different *cytb* gene region targets. Regardless of its limitations, MalAvi and its implicit use of barcoding based on the *cytb* gene have enabled the recording of reproducible lineages that can be tested as species hypotheses. Further improvement of this system includes an expansion of the targeted *cytb* gene region to generate better-supported phylogenies, and allowance of the unification of commonly used systems for naming lineages by investigating archived specimens (Fallon et al. 2005; Bensch et al. 2009; Outlaw and Ricklefs 2014; Pacheco et al. 2018b). Although genomic approaches are starting to be used (Bensch et al. 2016; Galen

et al. 2018a, b; Barrow et al. 2019;), it is likely that single-gene information will remain in use for many years (Outlaw and Ricklefs 2014). Thus, it is crucial to consolidate the current system for recording detection-based occurrences. Improving such a unified system of lineages (e.g., promote the use of a larger fragment of the *cytb* gene), whether they are linked to morphological data or not, should be a priority.

12.3 Speciation in Avian Haemosporidians

Classic parasite speciation models (Johnson et al. 2003) emphasize codivergence or host switches (Figs. 12.1 and 12.3). Codivergence involves parasite speciation following their host cladogenesis, assuming that any bifurcation between host lineages is likely to result in the isolation of its associated parasites. Host switches, on the other hand, imply that parasite speciation is mediated by a change and subsequent specialization of a parasite to a new host. A new host is defined as one species that was not colonized during its demographic history by the parasite ancestral population or its immediate common ancestor. Parasites may fail to speciate (e.g., the same parasite species sharing different hosts and maintaining gene flow) or become extinct in a host species as part of these dynamics (Johnson et al. 2003). Geographic contexts also underlie these processes. This has been traditionally summarized under the concepts of allopatric and sympatric speciation (Figs. 12.1 and 12.4) (Huyse et al. 2005). Although the use of geographic speciation and codivergence/host-switching models is common, transforming such models into hypotheses that can be tested by spatial phylogenetic patterns of avian parasites and their hosts require definitions that can be generalized and compared across different settings.

Even though defining a geographic scale is essential in understanding avian haemosporidian parasite diversity patterns, the use of "allopatric" and "sympatric" speciation models is challenging. In particular, sympatric speciation could be defined as parasite speciation in the same host (Fig. 12.4; e.g., Pérez-Tris et al. 2007) rather than parasite speciation in different host species in the same area (Brooks and McLennan 1993). Parasite speciation driven by different hosts in the same area (geographically sympatric) could be equated to a host switch if one of the hosts is new or some form of ecological isolation in different hosts leads to parasite speciation (Fig. 12.4). Thus, being "geographically sympatric" does little to inform the underlying host–parasite dynamics. The issue of defining a geographic scale for parasite speciation processes (allopatric) is complicated by the fact that haemosporidian parasite dispersion depends on suitable vectors across the avian hosts' home-ranges, making it difficult to delimit areas smaller than entire biogeographic regions or outside of islands. In the end, there are many ways for parasites to be considered allopatric by defining a barrier. Thus, the term "allopatric" does not inform about the host–parasite dynamics.

On the other hand, the importance of a host switch in terms of speciation needs some elaboration when we consider specialist versus generalist parasites. Biased

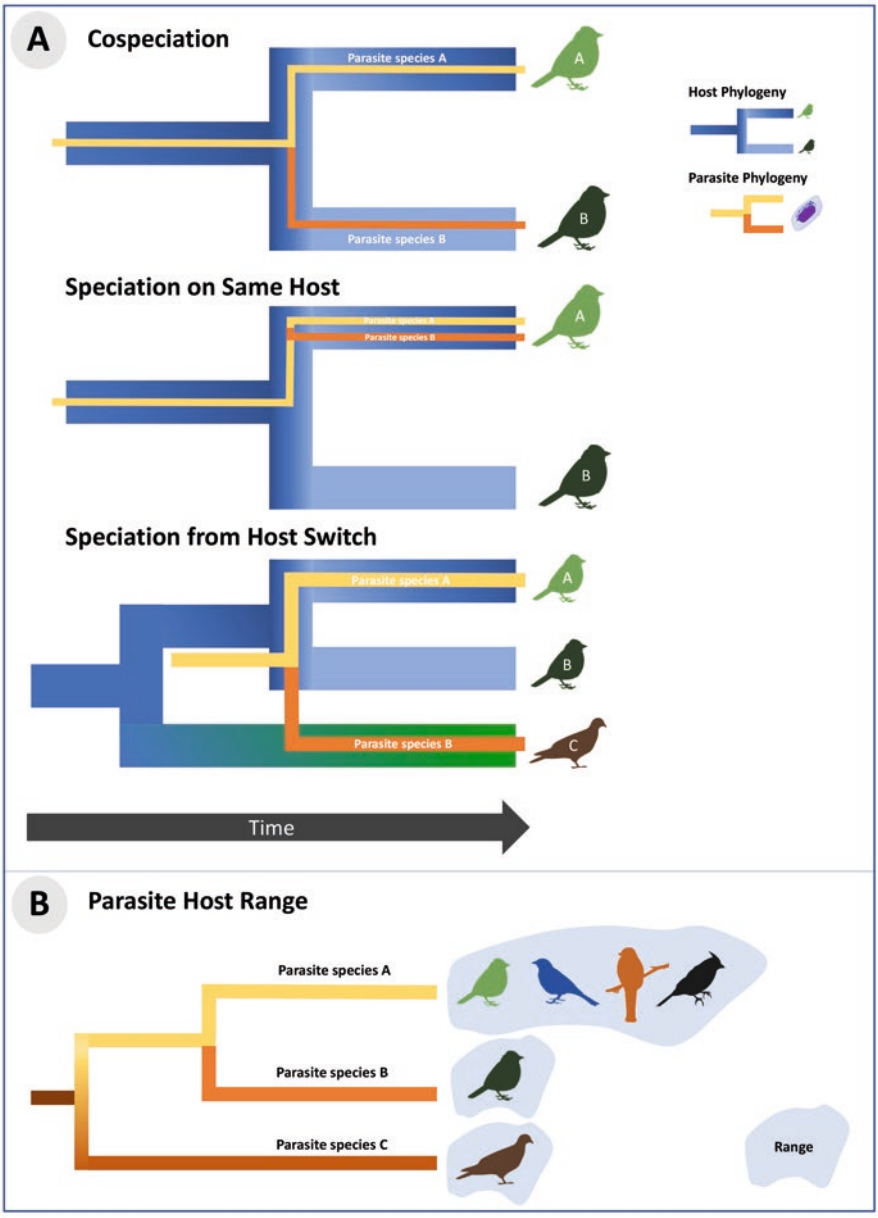

Fig. 12.3 Speciation processes (**a**) and host–parasite associations (**b**, host range). Host and parasite phylogenies are shown in different colors. Different host species are represented by avian silhouettes with different colors. Host-switching involves the movement of a small subset of a parasite species into a new host. This can be followed by speciation (**a**), or the new host will be added to the species range of the parasite (**b**)

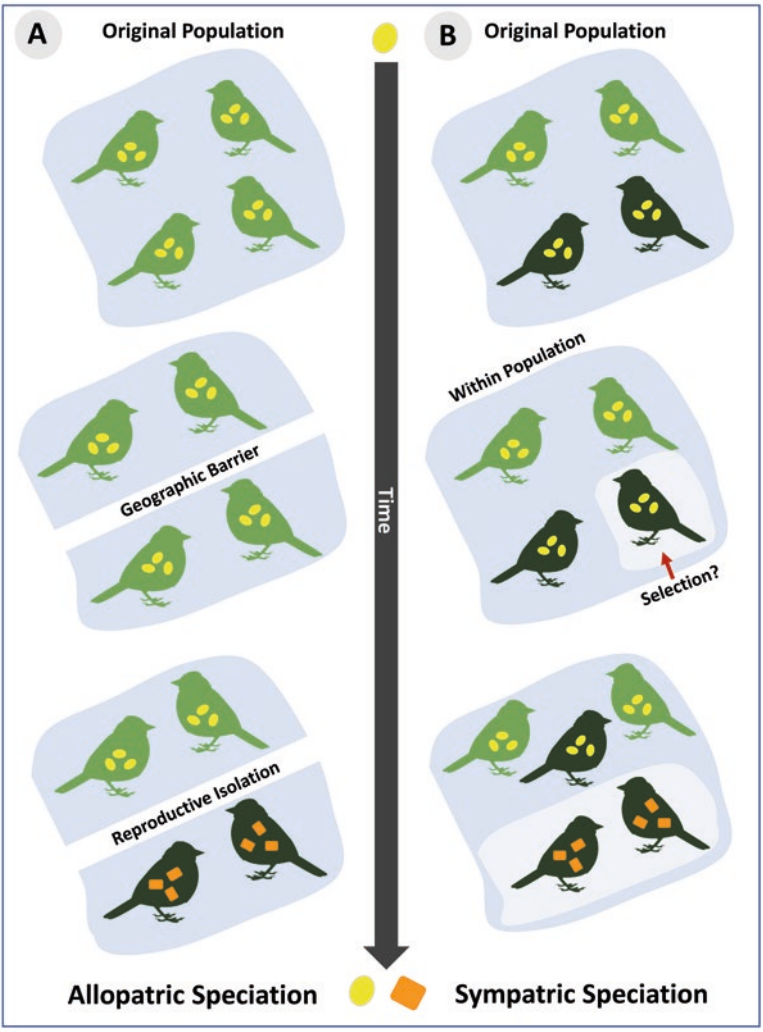

Fig. 12.4 Two major modes of geographic speciation: (**a**) allopatric versus (**b**) sympatric speciation. In allopatric speciation, an ancestral parasite species can be subdivided geographically together with its ancestral host species through time (also known as geographic speciation or vicariant speciation). Sympatric speciation might occur when species arise in the absence of a physical barrier on the same host species population

samplings of avian hosts still limit our perspective of the geographic dispersion and host range of haemosporidian parasites. Parasites are usually aggregated in and actively transmitted by only a few individuals in a population (Poulin 2007a). As such, the absence of a given parasite species in a host may be an artifact of the sampling effort (number of specimens, time, and place). In such a context, whether avian haemosporidian species are generalists or specialists (Brooks and McLennan 1993; Poulin 2007b) is difficult to deduce. Although the use of multiple hosts is the result of host switches, determining how host switches have been a factor driving

speciation requires additional evidence such as some metric of host competence (Moens et al. 2016) or an idea of the demographic histories of both hosts and parasites (Muehlenbein et al. 2015). Nevertheless, it is essential to study a host-switch event if it is known that the host is indeed new (spillover). However, this type of hard evidence has been documented solely in cases of invasive parasite species (Valkiūnas 2005; Jaramillo et al. 2017; Santiago-Alarcon and Merkel 2018; Ferreira-Junior et al. 2018).

Because of all these considerations, the value of terms such as allopatric, sympatric, host-switch, specialist, and generalist to explain avian haemosporidian speciation seems to be relative to the contexts in which they are used. Although these factors may limit our capacity in comparing studies in terms of testable hypotheses, there has been extraordinary progress in our understanding of the diversity and speciation of avian haemosporidians in the Neotropics.

Let us start by stating the obvious, avian haemosporidians require hosts in two major clades of taxa to complete their life cycles. They need an avian host in which asexual reproduction occurs and a Dipteran host (commonly referred to as the vector) in which sexual reproduction happens (see Chaps. 2 and 6). As this is shared among all haemosporidian parasites, the common practice in most research programs is to focus on ecological and evolutionary processes at the vertebrate host community level, even when in many cases the vectors are ignored. Under all parasite speciation models or diversity patterns, the level of specialization of the parasite to their vertebrate hosts is based on the known hosts (e.g., Moens et al. 2016). This seems a reproducible approach, especially if the parasite host-range can be translated into a metric (e.g., Poulin 2007b; Clark et al. 2018) and updated as new information is generated. It is worth noting that if the host is a valuable character to delimit species, it is circular to use that information as evidence of parasite specialization; this is not the case in avian haemosporidians because the host is not a valid taxonomic character (Garnham 1966; Valkiūnas 2005).

Since many avian haemosporidian species/lineages are not specialists in the sense of one parasite exploiting solely one vertebrate host, the selective impact required by sympatric parasite speciation (within a host) has been questioned (Ricklefs et al. 2014). Whereas sympatric lineages have been found in specific hosts (Pérez-Tris et al. 2007; Santiago-Alarcon et al. 2011), secondary introductions cannot be ruled out considering that most parasites have a relatively broad host range and also avian migration (local and regional) may affect the parasites found in a given host (Ricklefs et al. 2014). The selective impact of host switches in an otherwise generalist parasite also needs additional evidence supporting the hypothesized selective regime of such an event (Moens et al. 2016) if some sort of ecological speciation is considered (Schluter 2009; Nosil 2012; Stroud and Losos 2016). Alternatively, host switches in a given group of parasites may lead to isolation by the environment (Wang and Bradburd 2014) or nonecological speciation where host specialization plays a limited or no role (Czekanski-Moir and Rundell 2019). However, studying parasite diversity, where the parasite clades, the spatial scale, and host pools are defined, provides a useful approach to understand speciation in avian haemosporidian parasites (Clark et al. 2018; Fecchio et al. 2019).

The best examples of speciation in Haemosporida are found in parasites infecting nonhuman primates, where their hosts do not have the dispersion observed of those of avian haemosporidians. Of specific note, various *Plasmodium* species exhibiting an extraordinary diversity of life history (adaptive radiation) traits including dramatic changes in gene families (Rice et al. 2014; Muehlenbein et al. 2015; Castillo et al. 2017) originated from a common ancestor in Southeast Asia. Here, the evolutionary histories of the hosts, specifically their dispersion/speciation in a changing landscape, seem to have driven a parasite speciation process where multiple parasite species sharing a common ancestor can exploit overlapping host ranges, likely as a result of reintroductions into the ancestral host populations. Thus, both geography and host demographic histories played a role in this speciation process (Muehlenbein et al. 2015). This phenomenon may also be operating in avian haemosporidian parasites, albeit at a different scale due to the enhanced mobility of their hosts and where ecological or nonecological speciation could play a role in the diversification of particular parasite clades.

Patterns of local parasite diversity and dispersion have been reported, indicating that ecological/environmental isolation when exploiting multiple hosts could lead to regional parasite diversification (Fecchio et al. 2018; Jones et al. 2018; Ellis et al. 2019; Fecchio et al. 2019). Parasite diversification appears to be better explained by a model considering the effects of local host diversity and distribution/aggregation on parasite dispersion and diversity (Santiago-Alarcon et al. 2014; Clark et al. 2018), in contrast to a traditional geographic (e.g., allopatric) speciation model (Ricklefs et al. 2014). In this context, it is worth emphasizing that having a broad host range does not imply that a given parasite is equally fit and transmissible between all host species by all vectors (Martínez-de la Puente et al. 2011; Moens et al. 2016; Huang et al. 2018). Thus, understanding the dynamics of the parasite host-range in terms of processes leading to its speciation is an important aspect that requires well-designed studies. Ideally, an accurate description of the realized host ranges demands measurement of the phylogenetic diversity of the hosts as a first approximation, followed by an assessment of the demographic histories of both the parasite populations and the hosts being colonized. This may be possible if the research agenda moves from the necessary exploratory biodiversity censuses seeking associations to a more detailed sampling of particular parasite clades in their hosts (e.g., Muehlenbein et al. 2015). The potential parasite host range (the hosts where a parasite has been reported) remains likely biased by the type of sampling that favors some species (e.g., passerines) over others (Clark et al. 2014); however, such bias will be mitigated by subsequent studies supported by a database such as MalAvi (Bensch et al. 2009) where the occurrences of lineages can be compared and recorded.

Although the majority of the work conducted so far relates to the impact of avian hosts on parasite speciation, the role played by vector diversity requires attention. Emerging patterns indicate that shifting vector clades at the subfamily level may have played a significant role in haemosporidian cladogenesis, including those found in birds (Valkiūnas 2005; Martinsen et al. 2008; Pacheco et al. 2018a). In contrast, at a more recent time scale, changes in vector species (technically a host

switch but in the vectors) leading to parasite cladogenesis have not been documented. In particular, the limited information from species such as *Plasmodium relictum* and human malarias suggests that parasite species can be transmitted by multiple vector species. Shared vector species by a parasite can have diverged millions of years ago, indicating that this type of host switch (recent vector changes) seems to be not relevant in terms of parasite speciation (Pacheco et al. 2018a).

Nevertheless, like in other parasites (Poulin 2007a, b), avian haemosporidian species may experience differential fitness effects across both their avian and dipteran host ranges (Martínez-de la Puente et al. 2011; Moens et al. 2016; Huang et al. 2018). Thus, a hypothesis that could be tested is how changes in fitness in the species belonging to defined parasite clades enable ecological isolation of particular lineages (Wang and Bradburd 2014), leading to local diversification. In this context, one should recognize that the parasite fitness landscape may be driven not only by avian host and vector species but also by environmental factors. These can include, for example, temporal changes in the densities of host populations and their local capabilities to transmit specific parasites due to physiological and immunological effects as in the case of avian hosts (Gervasi et al. 2015; Wells and Clark 2019), or the abundance, age structure, and environmental factors that may affect the vector competence of Dipteran species (LaPointe et al. 2010; van Hoesel et al. 2019; see Chap. 7 for a thorough presentation of ecological niche models in the context of haemosporidians). Exploring how changes in the abundance of vector species affect parasite specificity is also an issue that needs to be investigated. In particular, vector species with different vertebrate host preferences may lead to an increase in the relative importance of a particular vertebrate host species in parasite transmission or even lead to the colonization of a new host (e.g., Abella-Medrano et al. 2018). Considering such complexities, longitudinal studies on defined hosts, their parasites, and vectors (diversity, ecology, and distribution) will provide extraordinary information.

Geography, in terms of the spatial aggregation of avian hosts and suitable vectors, has received predominant attention. However, the data (Clark et al. 2018; Jones et al. 2018; Fecchio et al. 2019) seem to indicate that more general frameworks are worth exploring, such as those described under "ecological fitting" (Agosta et al. 2010) and "host range evolutionary dynamic" (Muehlenbein et al. 2015; Pacheco et al. 2018a; see also Chaps. 7 and 9 for macroecological, interaction networks and ecological niche modeling perspectives). In these frameworks, parasites may exploit multiple hosts sharing specific characteristics that make them susceptible (ecological fitting, see Chap. 11), and are also sensitive to evolutionary interactions between hematophagous insects and vertebrate hosts, which have provided diverse and even transient biocenoses (Fig. 12.1) in specific geographic contexts affecting the diversification of haemosporidian parasites (host range evolutionary dynamic). Both proposals are compatible with each other because of their emphases on community-level processes (including both vertebrate and invertebrate hosts) and the underlying assumption that there is parasite phenotypic plasticity that should be considered in both their avian hosts and vectors at the time of interpreting patterns. In both cases, geography remains critical because the groups of hosts (avian and vectors) must

share spatial and temporal distributions (see Chap. 7). The "when" and "where" matter. For instance, one could envision that an area (or time) where (when) the avian hosts are effectively isolated because temperatures are too low to enable the parasite to develop in the vector would act as a barrier. Whether this has led to ecological or nonecological speciation still needs to be documented in avian haemosporidian parasites. The apparent difference between the two proposals, "ecological fitting" and "host range evolutionary dynamic," is that the latter emphasizes to a greater extent the evolutionary demography of the hosts (both vertebrate and dipteran) involved in the parasite life cycles in a defined space than the other.

There is evidence indicating that some parasite clades may be associated with clades of avian hosts above the species level (e.g., Matta et al. 2014; Marroquin-Flores et al. 2017; Ellis and Bensch 2018). Such apparent specialization at the family or order level requires additional scrutiny simply because we ignore its mechanisms. The bias in the avian species sampled also remains, along with the fact that sub-sequential colonization of hosts due to ecological/historical factors and extinctions could lead to apparent cophylogenetic or incongruent processes (Brooks and McLennan 1993; Poulin 2007b). Nevertheless, such codivergence may reflect ancient community-level events, so a more rigorous quantification of the pattern is needed correcting for sampling biases (e.g., across species, potential seasonal changes, and geographic areas).

12.4 Molecular Clock: Timing the Radiation of Avian Haemosporidians

Time has been considered implicitly in avian haemosporidian speciation whenever we attempt to explain the diversification of parasite lineages (e.g., Jones et al. 2018). However, more complete explorations of biogeographic patterns, and further understanding of speciation processes, necessitate explicit inferences of the time scale of such events and processes (e.g., Fecchio et al. 2019). Although a detailed discussion of molecular dating methods, which have contributed to a prolific improvement and generation of new statistical methods (dos Reis et al. 2016; Bromham 2019), exceeds the aims of this chapter, this field likely will continue to be an active research area. Instead, here, general considerations and important findings of molecular clock methodology to the field of haemosporidian speciation are discussed. Let us start by providing a brief description of molecular dating methods and their assumptions.

Technically, any phylogenetic inference implicitly considers time that is summarized in the form of branch lengths. In the case of molecular phylogenies, time is accounted for by appropriate substitution models (see Chap. 3). Thus, model misspecification can affect the topology of the tree, in addition to changing the time estimates and its variance. Unlike standard phylogenetic analyses, however, molecular dating models seek to estimate time explicitly in a given phylogeny. Thus,

molecular dating methods include not only the tree topology and its branch lengths but also information on whether there is heterogeneity in evolutionary rates between branches (Bromham 2019).

The simplest molecular dating model assumes a strict clock or constant rate of evolution, and as such assumes no heterogeneity in evolutionary rate. Under this model, divergence times for each clade are estimated by averaging the branch lengths and translating them into a time estimate by using a single substitution rate. This rate is calculated based on events called "calibration constraints", which usually involve a range, rather than a set time point. Primary calibration constraints, or direct evidence of a given event used as a time reference, are independently provided from fossil data but can alternatively also include known biogeographic events (Bromham 2019). Unfortunately, there is minimal evidence of parasites in the available fossil record, so host data are often used as a reference (Mu et al. 2005; Pacheco et al. 2011). Such secondary calibrations (indirect evidence involving additional assumptions) are a problem, and calibration constraints should be carefully described to be reproducible and comparable (Pacheco et al. 2011; Bensch et al. 2013).

The earliest attempt to estimate the time of origin of haemosporidian parasites focused on the origins of human malaria species. These early discussions assumed (commonly at the time) that there was a unique and universal rate for the evolution of 18S SSU rRNA (see Ayala and Fitch 1992). In such studies, the genus *Plasmodium*, and by extension all Haemosporida, were older than their vertebrate hosts (see Ayala and Fitch 1992). However, it was soon evident that the assumption of a universal rate could not be held due to differences based on specific sites aligned in a given set of species (Escalante and Ayala 1995). This was followed by a complete rejection of the assumption of a constant rate of evolution for the 18S SSU rRNA as a result of close examination of species alignments (Nishimoto et al. 2008).

The first modern time inferences in haemosporidian parasites also focused on the origin and demographic histories of the two major human malaria parasites, *Plasmodium vivax* and *Plasmodium falciparum* (e.g., Mu et al. 2005; Cornejo and Escalante 2006). As part of such studies, an average mutation rate was inferred for the *cytb* gene from a single calibration constraint (Mu et al. 2005), assuming codivergence of some parasite species with two clades of geographically isolated hosts in Southeast Asia (see Mu et al. 2005 and Cornejo and Escalante 2006 for a detailed explanation). These were demographic history studies rather than attempts to elucidate the origin of the genus.

Following these investigations, a formal time-tree of *Plasmodium* was proposed based on the assumption of a constant average rate of evolution of the mitochondrial genome. It is worth noting that a constant evolutionary rate was assumed even when a strict clock model was rejected. To mitigate the problem, one group of species, in which the strict clock was not statistically rejected and which also included the calibration event, was used to estimate a constant rate and then extrapolated on the other groups without the calibration (Hayakawa et al. 2008). Using this *ad hoc* approach, the time for the origin of the genus *Plasmodium* was estimated to be 22–41 Ma (million years ago) (Table 2 from Hayakawa et al. 2008, considering the standard errors).

It was thus concluded using this method that the genus was of relatively recent origin (Hayakawa et al. 2008). This phylogeny included three sole avian *Plasmodium* species representatives.

Then, using an *ad hoc* statistical method and assuming a constant rate of evolution, an average was estimated in avian haemosporidians for a fragment of the *cytb* gene based on the estimated time for a host switch as calibration (Ricklefs and Outlaw 2010). The estimated substitution rate was different from the one obtained from the complete mitochondrial genomes (Hayakawa et al. 2008). However, such a finding was expected because the calibrations and data (species included in the alignment and the number of sites) were different. Thus, the discrepancies between these two studies could be the result of a combination of rate heterogeneity (nonorthologous gene regions were sampled), sampled species, and different assumptions.

In contrast to the above-described mitochondrial studies, a more recent genomic-level attempt to estimate the origin of *Plasmodium*, which included the use of several coding protein genes (Silva et al. 2015), obtained older time estimates for the origin of *Plasmodium* in primates and rodents using relative rates (no avian haemosporidians were included). This method estimates the relative time against a reference divergence between a chosen pair of species for each locus. In particular, the divergences between *Plasmodium vivax* and *Plasmodium knowlesi* were used in the original study, but other pairs could be used. These pairwise estimates assume a constant rate of evolution for each protein across the aligned species without explicitly considering the phylogeny (not a unique rate for all proteins, but each protein has the same rate across taxa). The relationship between relative divergences among species pairs across all proteins is then inferred from the slope of a total least squares regression, a type of errors-in-variables regression. All gene-encoding divergences are then summarized as a single parameter used to estimate relative distances between pairwise comparisons of taxa. For example, the method estimated that the split between the human parasite *P. falciparum* and the rodent parasite *Plasmodium yoelii* was found to be 6.1 times older than the split of the chimpanzee parasite *Plasmodium reichenowi* from the human parasite *P. falciparum*. Absolute times were calculated by then assuming rates of silent site substitution for invertebrates and vertebrates (see Silva et al. 2015). Depending on the rate used, the time for the divergence of a given parasite species pair changes dramatically.

Whereas having a rate of evolution that can be used across any dataset seems desirable (Ricklefs and Outlaw 2010), it is not a robust approach (Graur and Martin 2004). As an example, the three modern studies described above differ in time and rates estimates. Their commonality resides in assuming constant rates of evolution at some level and performing statistical calculations in the absence of a phylogenetic model (Graur and Martin 2004). Unfortunately, given the differences in methods and assumptions, their findings are difficult to compare beyond the observation that they differ from each other (Bensch et al. 2013). Rather than looking for a common rate, what should be emphasized is the use of mainstream molecular dating approaches in avian haemosporidians. These studies involve the use of Bayesian methods for time inference and an emphasis on searching for appropriate calibration constraints.

Bayesian timing methods involve several models that differ in their assumptions (Tamura et al. 2012). Thus, comparing assumptions and priors as a way of learning from their effects on the data is the approach that will be emphasized in the paragraphs that follow. Bayesian methods incorporate prior knowledge to estimate a posterior distribution of times given the data. Data, in this case, are the aligned sequences from the sampled taxa. Priors include the values used as calibrations and the assumptions used to model the rate heterogeneity (dos Reis et al. 2016). Ideally, we expect that our data, not the priors, determine the posterior distribution (time estimates given a phylogeny). It is worth noting that adding species without calibration likely increases the variance in time estimates (dos Reis et al. 2016; Bromham 2019) especially if there is heterogeneity in evolutionary rates between clades (e.g., Outlaw and Ricklefs 2010). Thus, adding more species will not necessarily lead to a more accurate time estimate. Adding more genetic information in the form of more loci, on the other hand, may be beneficial if loci have congruent phylogenetic signal, are not saturated, share patterns of rate variation across lineages, and do not require the use of different substitution models (e.g., similar GC content).

It is also important to realize that the use of multiple calibrations in these methods does not necessarily imply that they all have the same effect. Some calibrations can seriously affect the time estimates over others (Battistuzzi et al. 2010; Tamura et al. 2012). However, this is not a problem *per se* because researchers can explore the effect of different calibrations and explain why the chosen calibrations were kept over others for a specific scenario. Finally, the distributions used around the calibration constraints as priors (to model the calibration density or the more likely times in a given interval) is particularly relevant if the calibration does not include both minimum and maximum constraints (Warnock et al. 2012).

In addition to the inclusion of calibration constraints, Bayesian methods attempt to model variation in evolutionary rates among lineages (rate heterogeneity) as autocorrelated or independent. The idea that evolutionary rates within a phylogeny are autocorrelated was proposed based on the expected similarities in genomes and niche conservation between species sharing a recent common ancestor (dos Reis et al. 2016). This autocorrelation is modeled with a single parameter. Thus, rates on descendent branches are similar to the rate of the ancestral branch. Given the methods available for model autocorrelation, a limitation is that the variance of evolutionary rates increases between lineages with the passage of time (dos Reis et al. 2016). Alternatively, independence can be assumed. Under this model, rates for branches on the tree do not correlate and are randomly sampled from the same distribution. Plainly, the rates between ancestors and their descendants come from the same distribution as the other branches in a phylogeny (dos Reis et al. 2016). We recommend those interested in this subject to first learn about the relevant limitations of these methods before inferring a particular time-tree.

A first attempt to explore alternative scenarios (more than a single evolutionary rate) in haemosporidian parasites was done in the context of a *Plasmodium* phylogeny using complete mitochondrial genomes that included a handful of nonmammalian parasites (like Hayakawa et al. 2008), but was based solely on different calibrations within primates (Pacheco et al. 2011). This study also compared two

Bayesian methods, one that assumed autocorrelation (MultiDivTime) and the other independent rates (BEAST, Bouckaert et al. 2014). The calibrations used were all secondary calibrations and based on codivergences and biogeographic events (see Pacheco et al. 2011 for a detailed explanation). The estimates with the autocorrelation model yielded slightly older times than the independent rate models in *Plasmodium*. However, given the limited species sampling of the phylogeny, the credibility intervals of the estimated times between the two methods overlapped. Furthermore, under some scenarios, there was some overlap with the Hayakawa et al. (2008) and Ricklefs and Outlaw (2010) estimated evolutionary rates. From this early work, up to three different calibrations were explored and incorporated into scenarios that yielded comparable estimations of evolutionary rates. These scenarios were further investigated considering different calibrations and assumptions in rodent malarias (Ramiro et al. 2012), producing similar results.

More recently, an expanded phylogeny incorporating 102 mitochondrial genomes was constructed, including data from avian species parasites of the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* (Pacheco et al. 2018a). Following logic similar to the previous study (Pacheco et al. 2011), different calibration constraints were compared using three different types of methods: a Bayesian method assuming autocorrelation of evolutionary rates (MCMCTree, Yang 2007), a Bayesian method assuming independence of evolutionary rates (BEAST), and a relative time method (RelTime, Tamura et al. 2012) (Fig. 12.1). In addition to differences in how evolutionary rates are modeled, these molecular dating methods all have other different requirements as well. For example, autocorrelation methods require a time calibration for the root of the tree. In contrast, the independent rates method allows for the estimation of the time of the root using different calibration scenarios. Thus, under the independent rate model, the origin of avian haemosporidian parasites was estimated to have occurred between 57.93 and 81.55 Ma (mean = 68.84 Ma) (Fig. 12.5). This time interval overlaps with a calibration constraint proposed for the origin of palaeognathous birds (56.8–86.8 Ma, Benton et al. 2015). As a result, the time estimated for the root indicated that the diversification of the avian haemosporidian subgenera/genera took place after the Cretaceous-Paleogene (K-Pg) boundary following the radiation of modern birds (Pacheco et al. 2018a).

The time estimate for the root of the haemosporidian time-tree used as calibration further allowed for exploration of the effect of using autocorrelation versus independence assumptions on modeling the rate heterogeneity. Such a comparison showed important differences. For example, autocorrelated models estimated divergence times that were ~40% older for *Haemoproteus* (*Parahaemoproteus*) species and avian/Squamata *Plasmodium*, and slightly younger for primate/rodent malaria parasites and *Leucocytozoon* species, than those obtained by the independent rates models. Thus, the priors used to model evolutionary rate variation across lineages, independent or autocorrelated, have an effect on time estimates (Pacheco et al. 2018a).

Which model is more accurate? In the absence of additional information, such an assessment is not possible. However, assuming independent rates seems more robust

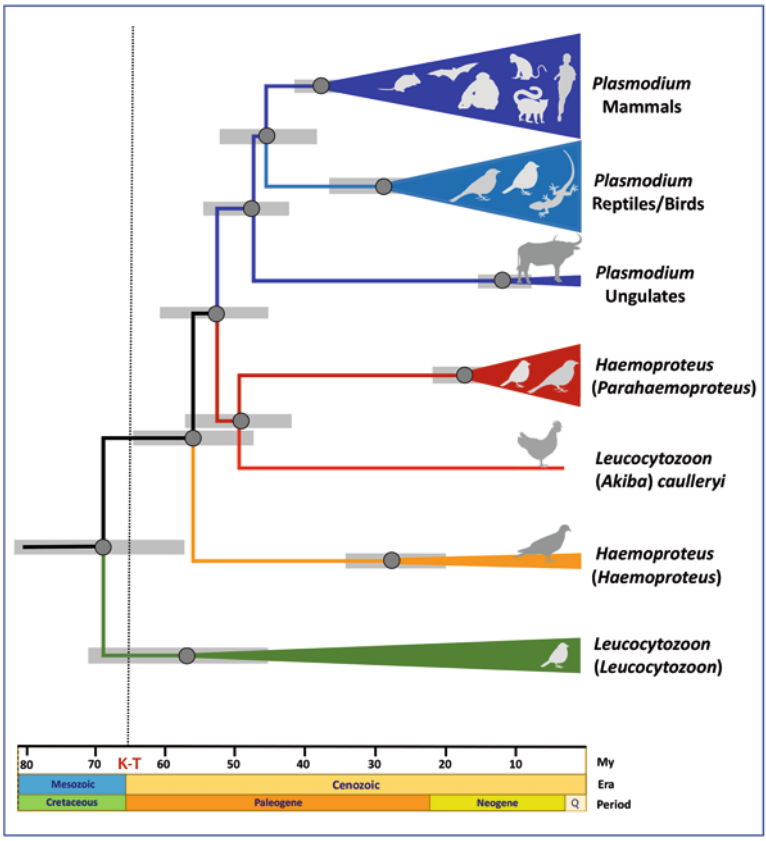

Fig. 12.5 Estimates of the divergence time and the associated 95% credibility intervals of the major clade of haemosporidian parasites. Branch colors indicate parasite genera and the silhouettes represent hosts. Times are shown in My (millions of years). This figure is modified from Fig. 2 published by Pacheco et al. (2018a). Times were estimated using mitochondrial genome sequences and a cross-platform program for Bayesian analysis (BEAST) under a scenario (scenario 1) based on the maximum and minimum divergence of Macaca/Papio using fossils (6–14.2 Ma), with a maximum of 24.44 to a minimum of 34.0 Ma for the human/Macaca split, and a range of 20–65 Ma for the origin of the lemur parasites. These estimates suggest that avian haemosporidians diversified during the radiation of their vertebrate host orders after the K–Pg boundary (66 Ma). Unfortunately, the genera *Polychromophilus* (from bats), *Nycteria* (from bats), and *Haemocystidium* (from reptiles) were not included in this study because no mitochondrial genome sequences were available at that time

simply because of the way autocorrelation is currently modeled (dos Reis et al. 2016). An alternative way to compare these different models is to validate their time estimates using calibration constraints not included in the original analyses (Pacheco et al. 2011). For example, there exists a fossil of *Plasmodium dominicana*, found in a sample of Dominican amber that is considered closely related to the avian parasite species *Plasmodium juxtanucleare* (Poinar 2005). Following some initial controversy, this amber is now commonly dated at 16–18 Ma (Seyfullah et al. 2018),

overlapping with the time estimated for the origin of *P. juxtanucleare*, 14.80 Ma (11.28–18.15) under the independent rate model (Pacheco et al. 2018a). It is worth noting that the time estimates and mitochondrial genome evolutionary rates obtained by Pacheco et al. (2018a) are consistent with other studies that have used different calibration constraints (Sutherland et al. 2010). Furthermore, they are similar to those found in studies carried out in ungulates and rodent parasites (Ramiro et al. 2012; Martinsen et al. 2016). However, discordant time estimates obtained from these different methods (autocorrelation versus independent rates) may indicate uncertainties in evolutionary rates in the mitochondrial genome between clades of haemosporidian parasites (Pacheco et al. 2018a). Mitochondrial genomes likely undergo different selective regimes correlated with the environment within the vector, further evidenced by the fact that the expression of the mitochondrial genome genes is critical in the vector and not so in the vertebrate host (Pacheco et al. 2018a). It is possible that some of the problems of rate heterogeneity could be mitigated by using nuclear genes rather than the mitochondria. Nevertheless, the best way to move the field further is to seek additional calibration constraints, ideally within avian parasite genera such as *Haemoproteus* and *Leucocytozoon*.

This task, however, appears difficult given the extensive migration patterns and broad host range of avian parasites (Bensch et al. 2013). An alternative may be the use of data from malaria parasites of reptiles where geographically constrained clades of parasites and hosts may provide additional calibrations (Bensch et al. 2013). Unfortunately, given the available phylogenetic data (Pacheco et al. 2018a), those clades will likely only improve estimates for *Plasmodium* but not necessarily for the other avian haemosporidian genera, *Leucocytozoon* and *Haemoproteus*.

The effects of model assumptions in the above-described models may be partially worked out when more field data are collected, and the estimated rates validated by exploring scenarios with alternative sources of data (e.g., Fecchio et al. 2018, 2019). Still, the available calibration constraints make some events less likely. As an example, currently available time estimates indicate that haemosporidian speciation is not consistent with the classic hypothesis that parasites radiated with the origin of their insect vectors (Huff 1938; Escalante and Ayala 1995; Bensch et al. 2013), an event that took place 200–240 Ma. Using such an event as a calibration would require a series of *ad hoc* considerations invoking particularly slow rates of the mitochondrial genome at the root of the tree, which is not compatible with what is known in haemosporidians (e.g., Ricklefs and Outlaw 2010; Martinsen et al. 2016; Fecchio et al. 2018, 2019). Furthermore, since many parasite clades would be older than the existing groups of hosts, selective extinctions (or failure to colonize particular groups of hosts) are required to account for the lack of related parasite lineages in a broader group of vertebrate hosts and outside some geographic areas.

Importantly, like in previous work, a remaining limitation is that available calibration constraints are secondary calibrations based on host biology and are specific to the genus *Plasmodium* (Pacheco et al. 2018a). However, an important finding is that each of the calibration constraints used thus far cannot disprove the others. Therefore, given the data, these time priors provide the most biologically plausible framework for the timing of haemosporidian parasites available today.

12.5 Conclusions

Although the preferred method to describe and delimit species integrates morphology and molecular data, the use of single-gene approaches to describe ecological and biodiversity patterns continues to prevail. Thus, it is essential to standardize the criteria used to define lineages and continue to support a unique system for recording detection-based occurrences like the one implemented in MalAvi (Bensch et al. 2009). The studies discussed in this chapter suggest that "community-level processes" dominate the speciation of haemosporidians. These processes cannot be adequately described using only traditional speciation models such as those of allopatric or sympatric speciation. In addition, evidence suggests that codivergence (association of parasite and host clades) is only observed at the level above the species (e.g., families). This observation, together with the fact that parasite lineages are often found in multiple hosts, suggests that avian haemosporidians exhibit, on average, extraordinary phenotypic plasticity. Beyond documenting host switches, it is important to study parasite fitness landscapes on multiple hosts and to reconcile the demographic histories of the hosts and parasites. These studies could focus on well-defined parasite clades rather than biodiversity patterns across many host species, so the factors leading to that parasite clade diversification can be characterized. Although these types of investigations are still unreachable to many, the creation of suitable biobanks with adequate metadata may support such kinds of investigations when the technology and its associated costs become more accessible. In the future, efforts should be made to understand how vertebrate–vector biocenoses emerged. Such studies will provide valuable information about the origin and diversity of haemosporidian parasites. In the context of such an ambitious research agenda, it is also important to improve the framework for molecular dating by procuring additional calibration information.

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Chapter 13
An Introduction to Landscape and Urban Ecology: An Avian Haemosporida Perspective

Ian MacGregor-Fors, Pilar Carbó-Ramírez, and Martha Bonilla-Moheno

Abstract The magnitude of both direct and indirect anthropogenic disturbances has altered all ecosystems across the globe. Human actions have triggered such extensive environmental consequences that an era has been coined to encompass them: the Anthropocene. This myriad of shifts has been abstracted in a unifying "global change" concept. Among the most worrisome components of the ongoing human-induced global change process, the following head the list: climate change, land-use change, biogeochemical cycle shifts, biological invasions, pollution, and urbanization. In this chapter, we (i) review some of the major global change drivers and consequences in Textboxes (i.e., climate change, land-use change, biological invasions, biogeochemical cycle shifts), (ii) introduce the urban ecology and landscape ecology disciplines, as well as their frameworks together with some general avian patterns, and (iii) concentrate on the avian malaria literature from tropical regions, providing an urban and landscape ecology focus to identify main findings and areas of opportunity for future research in the region.

Keywords Avian parasites · *Haemoproteus* · Landscape ecology · Landscape epidemiology · Landscape parasitology · *Plasmodium* · Urban ecology · Urban parasitology

I. MacGregor-Fors (✉) · M. Bonilla-Moheno
Red de Ambiente y Sustentabilidad, Instituto de Ecología, A.C. (INECOL), Xalapa, Veracruz, Mexico

P. Carbó-Ramírez
Red de Biología y Conservación de Vertebrados, Instituto de Ecología, A.C. (INECOL), Xalapa, Veracruz, Mexico

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13.1 Landscape Ecology

There are several definitions of the term landscape that come from different scientific perspectives (e.g., Zonneveld 1989; Meyer and Turner 1994; Farina 1998; Bürgi et al. 2004; Turner 2005). In general, landscape refers to a geographic region where environmental variables and physical forms converge creating recognizable units (e.g., resources, vegetation covers) distributed heterogeneously. Landscape-units are shaped by the continuing interaction of various factors, such as geologic processes, abiotic variability (e.g., gradients in climate, topography, soil type), biotic interactions (e.g., competition, dominance, predation), disturbances (e.g., fires, hurricanes, floods), and very importantly, past and present anthropic activities related to land use (Perry 2002). This complex interaction of variables and processes occurs within spatial (e.g., elevation gradients) and temporal (i.e., short- or long-term periodic fluctuations) scales, favoring or limiting the presence and extension of certain units. The distribution and arrangement of landscape units in an area set the spatial patterns of the landscape and determine three important features that make each landscape unique: structure or configuration, function, and change (Turner et al. 2001).

Landscape configuration is temporally and spatially dynamic and therefore will vary depending on the scale of study (Turner et al. 2001). The scale defines the dimensions where the process of interest occurs and is determined by two main measures: grain and extent. The grain refers to the size of minimal homogenous spatial unit perceived at a certain resolution; satellite images with fine-grain resolution will distinguish smaller elements than those with coarser grain (Turner et al. 1989). The extent refers to the overall spatial dimension considered as the area of interest, which is constrained within the landscape limits. As a result, changes in the spatial scale determine different landscape patterns (Perry 2002): what seems homogeneous at small extents or coarser grain could become heterogeneous at larger extents or finer grain (Fig. 13.1).

Landscape ecology is the sub-discipline of ecology that studies the relationship between the spatial patterns of landscapes and ecological processes (Wu 2013). Studies with a landscape ecology approach provide useful information for the management of natural resources, but also help to understand the causes, both current and historic, responsible for the configuration of the landscape and the consequences of such arrays. An important aspect of landscape ecology that distinguishes it from other sub-disciplines is its focus on the effect of the spatial pattern and temporal heterogeneity on the ecological functions and processes occurring within and among ecosystems, such as energy fluxes, organisms, resources, or species distribution (Turner et al. 2001). In this sense, the appropriate scale of study depends, for example, on the real extension of the studied ecological process or the home range of the organism of interest. If the selected scale is too small or larger than the process of interest, interpretation of the observed processes could be misguided. In this way, the accurate determination of the scale of study will correctly describe scale-dependent processes occurring within the study system. For instance, Tewksbury

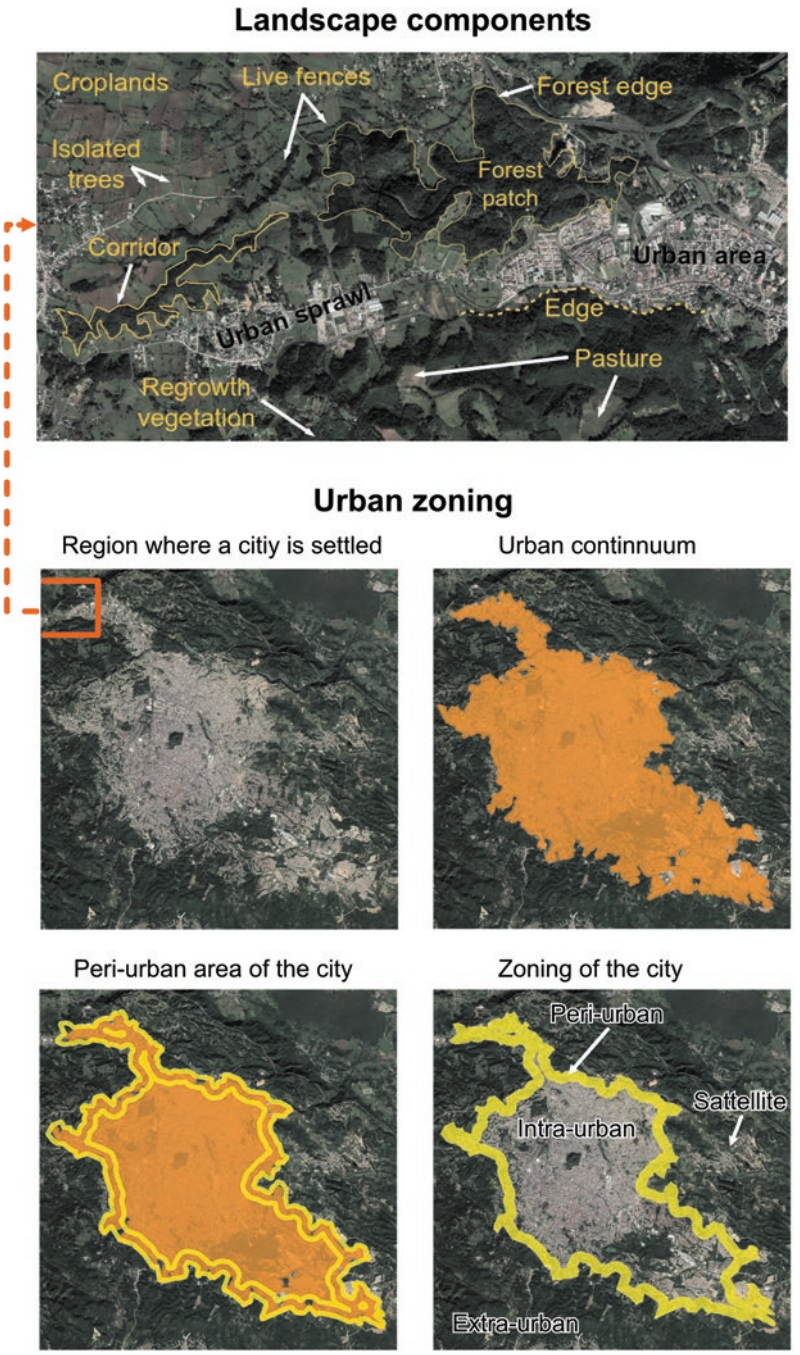

Fig. 13.1 Urban zoning and landscape components. Scaling in the figure allows depicting the variation in the resolution to identify landscape elements

et al. (2006) found strong evidence that depending on the studied scale, the configuration of the landscape mosaic (i.e., forest fragmentation, presence of forest buffers, habitat type) affects nest predation differentially, as it favors the presence of generalist predators. Additionally, local and landscape patterns can differentially influence the diversity and distribution of birds. A study from Brazil showed that the diversity of forest-dependent bird species was negatively related to the simplification in vegetation complexity and fruit availability (local scale factor), while the diversity of non-forest-dependent bird species was positively influenced by the amount of forest edge (landscape scale factor; Morante-Filho et al. 2018).

Currently, the importance of environmental topics, management of resources, and conservation biology, as well as the interest for understanding ecological processes at different scales have contributed to the relevance of the discipline. In addition, technological developments (e.g., satellite images, software development, spatial data) and landscape metrics have allowed to quantify features at several levels (from patches to landscapes), providing accurate information of the factors that can influence the distribution of species or resources, such as the extension of land covers, the level of habitat connectivity, loss or fragmentation, number, size, and distance of patches or the potential for dispersal of exotic species. For example, a study by Brotons et al. (2003) evaluating the effect of landscape matrix on bird numbers, by comparing densities in true islands versus fragmented forests, showed that the quality of the surrounding matrix is an important predictor of species density and therefore this landscape feature should be incorporated into models that assess the effect of habitat fragmentation on species. However, proper interpretation of changes in the configuration and fragmentation on biodiversity will depend on the selection of the correct scale of study. While habitat loss has direct negative effects on biodiversity, fragmentation *per se* (the breaking apart of habitat at landscape level; *sensu* Fahrig 2003) is more inconclusive and in some cases can actually enhance species richness and abundance at the landscape level (Fahrig et al. 2019). Thus, assessing the relationships between habitat fragmentation and biodiversity requires the independent roles of habitat loss and fragmentation to make accurate inferences. Spatial techniques have also allowed mapping the distribution of vector-borne infectious diseases and spatially predict their current and future distribution based on environmental variables or landscape patterns (Sehgal et al. 2011; Loiseau et al. 2012; see Chaps. 7 and 9 for an ecological niche and macroecological perspectives on host–parasite interactions). Some other applications of landscape ecology include determining the optimal arrangements for different land uses in a way that helps to maintain habitat diversity, to identify the potential for dispersal of exotic species, or to guide habitat restoration principles.

Landscape configuration can either directly or indirectly shape the transmission of vector-borne diseases. For example, deforestation can alter the fitness, distribution, and migration patterns of avian populations, which in turn can indirectly affect the feeding ecology of vectors and the infection dynamics of the pathogens they transmit (Sehgal 2010). Landscape configuration can also directly influence the distribution of vectors, such as mosquitoes vectoring avian malaria in native birds, and therefore influence transmission patterns of vector-borne diseases (Ganser et al.

2016). As the configuration of the landscape is highly influenced by anthropogenic activities, one of the most relevant characteristics of current landscapes is the direct or indirect influence of humans; in particular, the different forms in which humans use the land and its elements (Wu 2013). Examples of the latter are led by food production, housing or infrastructure, or recreation. Over the past 100 years, humans have transformed landscapes more rapidly and extensively than in any comparable period of human history. These changes happened largely to meet the increasing demand of resources and have contributed to substantial net gains in human well-being and economic development.

One extreme kind of human-modified landscape is cities, which are considered as landscape entities that can act as habitat filters or barriers for biodiversity (Aronson et al. 2014). Although well-planned cities can provide certain and important ecosystem services (Bolund and Hunhammar 1999; Yu and Hien 2006), the maintenance of the growing urban world population demands resources beyond the local ecosystems (Grimm et al. 2008). In this way, benefits such as the increase in quality of life have been achieved at a high cost in the form of loss of biodiversity and degradation of ecosystems and would likely increase in the coming decades, particularly due to species over exploitation and change in land cover to meet agricultural demands and urban expansion (Maxwell et al. 2016).

13.2 Urban Ecology

13.2.1 The Urban System

Although it may seem intuitive to establish the borders between the urban and non-urban frontiers, this has been a topic of debate with no current consensus. Even more complex yet, academics have debated on the boundaries of urban ecosystems and if only urban centers are part of them or if they include non-urban systems. According to Nilon et al. (2003), urban ecosystems are models based on the interaction between three main spheres: physical, ecological, and social. Thus, several frameworks have been suggested to abstract urban ecosystems, as well as their boundaries, with some tightly related to the ecosystem concept, *sensu stricto*, suggesting that the direct area of influence of a given urban center – generally referred to the watershed or urban metabolism limits – determines its geographic extent (Zhang 2013; Fig. 13.1). Based on the latter, it is clear that many non-urban scenarios occur in an urban ecosystem, even within the urban center, where its environmental heterogeneity includes many types of non-built, but often managed, greenspaces (MacGregor-Fors 2011).

A diverse array of procedures to establish the limits of an urban center have been suggested in the past, most of which are based in remote sensing. Some methods consider built infrastructure in the landscape as a variable to determine the extent of an urban center, while others have considered the use of indirect information to

delimit cities, such as night lighting, as well as the history and geography of the given city (Catalán et al. 2008; Tayyebi et al. 2011; Álvarez-Berríos et al. 2013). Although all methods have their strengths and limitations, it is crucial to use the ones that better fit the aims of any given study. Yet, one important point to consider when delimiting the study area for a project focused on urban patterns and processes is that urban continuums are more ecologically sound as landscape units than governmental or administrative boundaries.

When describing an urban system, it is important to provide basic information for readers to understand the scenario in which a given city is settled. Besides describing the specific sampling or study areas, basic information of the studied city or cities is fundamental, including its geographic location, and the biogeographic realm where it is located, as well as pre-existing natural ecosystems in the area where the city was settled, current surrounding ecosystems, updated population and size, and main land uses and principal economic activities (MacGregor-Fors 2011; Fig. 13.1).

13.2.2 A (relatively) New Discipline

Among the most surprising anthropogenic phenomena has been the massive human migration to urban centers (UN 2015). A complex set of socio-economic and industrial dynamics has generated the rearrangement of the human population, with more than half of humankind living in cities (Grimm et al. 2008). This process is not only related to a rapid urban sprawling, but also to the rise of new urban centers and satellites to current megacities (Seto et al. 2011). Certainly, although the urban extent represents a minimal proportion of the global terrestrial surface (~3%; Liu et al. 2014), the environmental demands and ecological consequences it represents are unprecedented and alarming (Berkowitz et al. 2003). Yet, it is not only the land-use modification itself the reason behind the huge ecological effect of urbanization, but rather the process through which input resources are brought and processed in cities and the resulting outputs that have placed this perturbation in the spotlight during the last decade (Kennedy et al. 2011). This process, which has been framed and abstracted as "urban metabolism" has shown to be so extensive that the food resources, energy, and other materials and goods required to supply regular demands can exceed 200 times the size of any given city (Wigginton et al. 2016).

Considering all of the above, it is not surprising that urbanization has been identified to be tightly related with at least four of the main components of global change (i.e., climate change, land-use change, biological invasions, biogeochemical cycle change; Textboxes 13.1, 13.2, 13.3, and 13.4), as well as with a pattern of species richness loss and endangerment worldwide (Grimm et al. 2008). More than two decades ago Czech and Krausman (1997) identified urbanization as the leading cause of species endangerment in the USA when excluding Hawaiian and Puerto Rican species. Thereafter, Czech et al. (2000) developed on the topic, identifying urbanization, next to agriculture, as a major cause of species endangerment by

Textbox 13.1: Climate Change

Climate change is only one of the many major causes of current global change; yet, it could determine the environmental future of the planet. There is growing and pressing evidence of the direct effect of human activities on Earth's current changing climate (National Academy of Sciences & Royal Society 2014). Albeit the global average surface air temperature has increased in the past century, climate change has been related with other worrisome phenomena, including meteorological instability (Parseman and Yohe 2003). The latter is given by the increase in temperature and moist of the lower atmosphere, providing more potential energy for meteorological events to occur, such as storms and hurricanes (National Academy of Sciences & Royal Society 2014).

Such extreme climatic events have been related with diverse avian patterns and processes at different levels. Among the best-studied avian shifts related to global climate change, the following head the list: changes in distribution and geographical range, phenological shifts (e.g., migration, mating, laying dates), demography, breeding performance, and survival (Crick 2004). Although some species have shown to adapt to the novel climatic scenarios, some will not, and the novel regional climatic conditions will represent an unprecedented environmental scenario in human history.

It has been predicted that climate change will result in large-scale responses of infectious diseases. Evidence suggests that the specific climate change scenarios and the complexity and specificity of host–pathogen systems will determine the outcome (Altizer et al. 2013). Most recently, avian malaria studies have assessed climate change as a driving force of their complex study models. Findings have not been promising, with important evidence suggesting that the risk of avian infection with malaria, for example, will increase with climate change (Garamszegi 2011). Although there is no clear pattern on the future of avian malaria and climate change, the tropics will surely be one hotspot of research in the search of solutions to mitigate potential climate-driven zoonosis, epidemics, and disease emergence.

directly replacing habitat and depleting the resources needed to support modern urban economies. Most recently, Maxwell et al. (2016) assessed the global "big killers", namely the causes of the greatest current impact on biodiversity, focused on species included in the IUCN red list. In their evaluation, Maxwell et al. (2016) identified urban development, mainly housing, tourism, and recreation, and industrial activities as the third most important "big killer", behind the over-exploitation of natural resources (e.g., logging, hunting, fishing) and agricultural activities (e.g., crop and livestock farming, timber plantations, aquaculture). It is notable that both over-exploitation and agricultural activities are closely related to the supply

Textbox 13.2: Land-Use Change

Land use refers to the human employment of the land, while land cover to the physical state of the land. Cultivation, urban settlements, recreation, and forestry, are all different types of uses that humans make from the land, while the vegetation, water, materials, crop types, or impermeable surfaces are the features that define the type of cover. Although same land uses can have different covers, the change in land use is usually followed by the physical change of the land cover (e.g., deforestation, or conversion from croplands to pasture lands). Anthropogenic land modification has occurred for most of human history; however, until recently this was moslty limited to agricultural activities. One main consequence of the diversification of human activities, the proliferation of globalized markets, and the technification of agriculture has been the intensification and extensification of agricultural areas, which has had important impacts for biodiversity and ecosystem cycles. In fact, land use and cover change has been identified as a major driver of ecosystem change, being the conversion of land for agriculture the major driver of this process. Although the rate, direction, and scale of the change can vary across temporal or spatial scales, land-use change has become a particularly important threat in the tropics, where productive forests have been converted into agricultural land, for cropland, or pasturelands, and continuously used for centuries. The spatial arrangements of such systems will most probably mold the occurrence and prevalence of vector borne diseases. In fact, some studies have suggested that the presence of natural elements in human dominated landscapes, such as urban forests, tree patches within agricultural matrices, or forest elements in general, will host higher prevalences of avian malaria (Mendenhall et al. 2013; Hernández-Lara et al. 2017).

Textbox 13.3: Biological Invasions

The spatial dispersion of organisms is a natural process, which in fact happens to be one crucial driver for evolution and the composition of wildlife communities across the globe (Hui and Richardson 2017). During the Anthropocene, the deliberate or accidental dispersion of wildlife beyond their natural geographical limits has occurred as a result of human activities and represents a major issue, with ecological, economical, and even health consequences (Pimentel et al. 2005; Pyšek and Richardson 2010; Bellard et al. 2016). Although biological invasions are increasingly common in a growing number of regions, they are the result of highly complex series of events, where isolated factors rarely play a crucial role. In a nutshell, the process that occurs from the translocation of individuals of a certain species to becoming

invasive is as follows: (i) transport beyond the species natural geographic distribution, becoming introduced individuals or population, (ii) survival to the new environment in the wild and establishment of viable populations, generally named established or naturalized individuals, populations, or even species, and (iii) dispersal and colonization of nearby areas from translocation ones and having viable populations in such new sites or regions, when they can be considered invasive (Blackburn et al. 2011; Lockwood et al. 2013).

It is notable that some invasive species can move through human-altered landscapes, with both biotic and abiotic modifications leading to important invasion processes. For instance, many generalist invasive species used agricultural landscapes to invade new areas, including both native and non-native species (e.g., native: great-tailed grackle – *Quiscalus mexicanus* expanding from southeast Mexico to the USA and Central America, with some known translocations, Haemig 2014; exotic: house sparrow–*Passer domesticus* invading North America in less than two centuries, also with well-known translocations, Baughman 2003; Lowther 2006). Closely related to the central topic of this chapter, there are studies that show the importance of invasive species as vectors of diseases for birds and humans (Pedersen et al. 2006), besides the well-known ecologic and economic effects, including crop and infrastructure damages (Pimentel et al. 2001, 2005; Booy et al. 2017; see Chap. 15 for a synthesis of the role of parasites in invasion biology).

Textbox 13.4: Biogeochemical Cycle Shifts

Exchanges or flows in energy and resources within landscapes are essential processes for the functioning of the ecosystem. However, one major consequence of global change and human activities on ecosystems has been reflected in the change of the recycling of chemical elements (e.g., carbon, nitrogen, water, oxygen), which are crucial for the growth and survival of organisms. In nature, the reuse of these chemical elements has existed since the beginning of our planet and their balance can determine ecosystem functions and clime regulation. The interruption or modification of these cycles by human activities has had some important cascading effects, for example, the change in vegetation cover will modify the amount of atmospheric carbon dioxide (CO_2), as well as carbon storage in the soil, which will affect the biogeochemical cycles of different elements at various scales.

Carbon is the main element of the biotic system; almost half of the Earth's biomass is composed of carbon and represents a major part of the primary productive ecosystems, mainly by photosynthesis and biomass conversion. In addition to above-ground vegetation, soil contains multiple storage sites (e.g.,

roots, mycorrhizae, bacteria) that make its capacity of C storage almost four times larger than that of the vegetation (Ciais et al. 2013). As ecosystems and organisms capture or produce carbon, they represent sources or sinks of this element. In this way, the net balance of carbon within ecosystems is dependent on all sources and sinks from physical, biological, and anthropogenic origins. Although terrestrial and oceanic ecosystems absorb half of atmospheric CO_2, recent human activities, such as deforestation and extraction of fossil fuels, have caused a massive increment in CO_2 and other forms of carbon. In fact, it is calculated that in the last 60 years there has been an increment of >100 ppm of atmospheric carbon (from 280 to 390). Models integrating carbon and climate change indicate that as global temperature increase, the fraction of CO_2 generated by humans will also increase, mainly due to the reduction in terrestrial carbon sinks. This is aggravated in tropical ecosystems where deforestation and land-use change have created new sources and decreased the number of sinks for carbon. In addition, soil respiration, an important source of carbon in boreal regions where high amounts of carbon are stored, is expected to increase as a consequence of global warming.

Another important cycle expected to be disrupted by global change is the nitrogen cycle. Nitrogen is an essential component for the formation of nucleic acids, crucial for plant growth and therefore for the primary productivity of ecosystems. Although nitrogen is the most abundant element of the atmosphere, it is available only as dinitrogen (N_2) and few organisms can absorb it in that form, and only becomes available once soil organisms accumulate it in their biomass. However, expansion of agricultural and industrial activities has produced more nitrogen than what is possible for the ecosystems to absorb. This excess of nitrogen in the form of nitrites leaches to the underground water having a cascaded effect on terrestrial and aquatic organisms, soil acidification, increase in atmospheric nitrogen and effects on the ozone layer.

There is still much uncertainty on the feedbacks that global cycles will experience due to global change, but it is known there will be complex interactions. For example, an experiment by Gregg et al. (2003) showed that plant biomass growth in urban areas was double than that from plant growth in non-urban areas. However, these differences were not due to the higher concentration of pollutants, temperatures, or CO_2 that exists in cities but rather to the high accumulation of ozone (O_3) in non-urban areas (e.g., agricultural or forested areas), which is probably a consequence of urban and industrial emissions. In addition, as energy fluxes shape the functions of ecosystems, they can drive changes in local ecological processes, such as vector–parasite–bird interactions.

component of the "urban metabolism", which does not only consider urban centers but their dynamics within and outside their boundaries, and could be considered as the most impacting anthropogenic process altering global ecological patterns.

As a consequence of the worrisome environmental pressures posed by urbanization in the past decades, an important research wave focused on the ecological patterns and processes that occur in urban systems, consolidating a discipline that started back in the mid-1800s (McDonnell 2011). Ever since, urban ecologists have framed their studies in two main paradigms: ecology in the city and ecology of the city. The former focuses on untangling classical ecological questions in, or considering, urban systems, while the latter concentrates on the systemic understanding of urban systems considering the complexity of the spheres it is made of (i.e., physical, ecological, social), broadening not only the conceptual framework but also the spatial scale (Pickett et al. 1999; Grimm et al. 2000). Most recently, Childers et al. (2015) suggested a third paradigm that seeks to promote the interaction of experts from diverse fields, as well as urbanites and decision-makers, to mold the future of cities: ecology for the city. Given the trans-disciplinarity implied in this novel paradigm, its main philosophy is "from knowledge to action" (McDonnell and MacGregor-Fors 2016; Pickett et al. 2016).

13.2.3 Main Avian Urban Ecology Findings

In 2001, Marzluff, Bowman, and Donnelly gathered most of the relevant and available urban bird ecology information from the globe. In the introductory chapter of their pioneering book, they highlight the 1980s as the decade when urban bird studies started to rise, rapidly increasing during the past decade (Marzluff 2016). Before 2000, Marzluff et al. (2001) identified research to be characterized by temporally short studies (1–2 years) mostly focused on correlational investigation of breeding birds from the USA and West Europe (with studies from the tropics particularly scarce). Five years later, Chace and Walsh (2006) summarized the knowledge regarding the effects of urbanization on native avifauna, identifying several generalizations that applied to the available knowledge before their review was published: (i) a selection of urbanization for omnivorous, granivorous, and cavity nesting species, (ii) bird species richness decreases and total biomass increases with urbanization, (iii) positive relationships between vegetation structure and composition, especially those of native plants, with urban avian diversity, and (iv) some of the urban hazards related to certain ecological patterns are as follows: collision with man-made structures, changes in predator assemblages, food supply, and diseases. Chace and Walsh (2006) also noted the evident absence of information from regions of high avian diversity, mostly in the tropics. Three years later, Evans et al. (2009a) reviewed and assessed the relative importance of regional and local factors, suggesting that local factors (i.e., mostly site-specific features within 1 km of the focal

study site) are better in explaining urban bird species richness, that habitat fragmentation has a strong influence on urban avian assemblages (with patch size being more important than isolation), and that urban bird diversity tends to respond positively to increasing structural vegetation complexity, supplementary feeding and negatively to human activities.

In 2011, Ortega-Álvarez and MacGregor-Fors set the context of the urban bird studies from outside the USA, Canada, and West Europe, with an important focus on tropical and sub-tropical urban centers. Although their findings show that there were $\geq$ 10 publications for only four countries (i.e., Australia, Brazil, Argentina, China), they pinpoint differences found in relation to some patterns that were previously thought as general. For instance, they identified studies reporting nectarivorous, frugivorous, and even insectivorous species to conform the predominant guilds within urban areas of Australia, Singapore, and Mexico, respectively. They also reviewed studies that found differing patterns of biomass, such as that of Posa and Sodhi (2006) where reduced total abundance was recorded in an urban area when compared to a non-urban control in the Philippines.

Fortunately, urban bird studies have populated the literature in the past decade; yet, they are still mostly focused on patterns rather than processes. Within the tropics, an important amount of knowledge has resulted from research performed in Latin American cities. In fact, a recently published book synthesized most of the current urban bird knowledge from urban Latin America (MacGregor-Fors and Escobar-Ibáñez 2017). One important point that the editors of the book stress in their introductory chapter, that applies to most of the tropical Global South, is that Latin America is a region "where economic inequality and urbanization meet with biodiversity." In a nutshell, the book makes it evident that although urban bird ecology in Latin America is still underrepresented, it is gaining strength and recognition. Some countries are well represented (i.e., Brazil, Mexico, Argentina) and others are starting to contribute with valuable information (e.g., Barbados, Guatemela, Bolivia). Studies are increasingly focusing on a more diverse array of topics (e.g., bioacoustics, parasitism, and demographics) and starting to consider long-term and multi-taxonomic approaches. Thus, although still scarce and biased toward large cities of a handful of countries, it is clear that urban bird ecology patterns can hold or change in tropical cities when contrasted with those from temperate regions.

Closely related to the central topic of this book, Santiago-Alarcon and Delgado-V (2017) considered most of the parasitological urban bird studies from Latin America, mainly from Brazil and Mexico. They recognize the need to follow urban–non-urban contrast systematic studies, as well as urbanization intensity gradients, to understand bird parasitism in urban systems. Additionally, they pose three research questions that could lead to future directions of the topic in the region, targeted on: (1) the host species that are being infected by parasites that are potential zoonoses for humans, (2) parasite shifts following urbanization, and (3) the role of hosts as super-spreaders or diluents.

13.3 Avian Malaria in the Tropics: A Landscape and Urban Glance

With the aim of gathering a representative idea of the field, we performed an intensive literature review focused on avian haemosporidian blood parasites globally. For this, we used an advanced keyword combination in the Web of Science database to retrieve as many studies as possible, as follows: TS = ((malaria OR haemosporidia* OR haematozoa* OR hematozoa* OR "blood parasite*" OR parasites* OR haemoproteid* OR Plasmodium OR Haemoproteus OR Leucocytozoon) AND (bird* OR avian) AND (*urban* OR "urban parasitology")). Complementarily, we searched the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi/).

After removing those studies that did not fit our topic but were included in our searches, we gathered a total of 431 publications, of which 207 were developed uniquely in tropical and subtropical regions. This set of publications goes back to 1939 with a study focused on the blood parasites of caged birds in Mexico City, with a steady increase in the number of publications until the beginning of the twenty-first century, when the total number of publications increased exponentially (Fig. 13.2; see Chap. 1 for a review of the avian haemosporidian research during the twentieth century). Regarding the geographic representation of the reviewed literature from the tropics and subtropics, we identified more than 50 countries, with Spain, Mexico, Brazil, and Colombia heading the list, as well as some tropical and subtropical cities from the USA and Japan where avian malaria studies have been performed since the late 1930s.

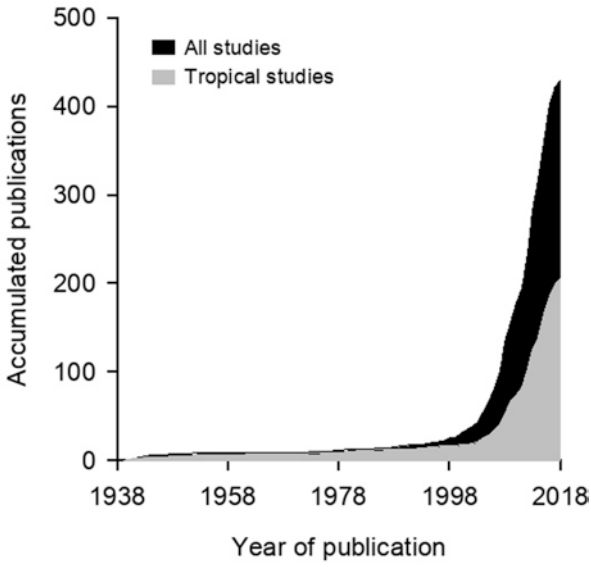

Fig. 13.2 Temporal increase in the number of avian malaria studies worldwide contrasting all the retrieved studies with those performed solely in tropical and subtropical regions

In the following sections, we develop on the set of publications focused on urban environments and including a landscape ecology approach. Given that zoos are closely related with urban settings, but have different implications (mostly veterinary), we provide some information of studies from tropical and subtropical zoos in Textbox 13.5.

13.3.1 Urban Environments

Although avian malaria studies have largely focused on their patterns and processes outside urban settings, an increasing number of publications performed within cities has increased across the globe (some including urban–non-urban gradients, reviewed in Sect. 13.3.2 Landscape approach). In tropical areas, bird haemosporidian parasite studies from urban areas have mostly assessed parasite screening (Toro et al. 1999; Yeltatzie and Fedynich 2001; Mohammad and Al-Moussawi 2012; Mantilla et al. 2013; Carbó-Ramírez and Zuria 2015), as well as vector screening involved in the transmission of haemosporidian parasites (Njabo et al. 2009). Regarding parasite infection studies, comparing urban and non-urban areas in single bird species have

Textbox 13.5: Zoos

Given the nature of zoos, with constant human presence exposed to a high density of captive wildlife animals from different regions, among other causes, they have been targets for avian parasitism sites as they can favor infections in both wild and captive individuals (see Chagas et al. 2013). Studies focused on avian haemosporidians from tropical areas have reported individuals positive for malarial infection in Magellanic penguins (*Spheniscus magellanicus* by *P. relictum*; Bueno et al. 2010) and a dead Egyptian goose (*Alopochen aegyptiacus* by *Plasmodium* sp. and *P. nucleophilum*), as well as infections in free-living resident and migratory birds that visit the zoo (Chagas et al. 2013).

Other studies have reported haemosporidian parasite infections in endangered bird species under special *ex situ* conservation programmes. Some examples of the later are the white-eared-pheasant (*Crossoptilon crossoptilon*, Murata et al. 2008) in a Japanese zoo, the masked bobwhite quail (*Colinus virginianus ridgwayi*, Pacheco et al. 2011) in a Southwestern US zoo, as well as crane species in a Beijing zoo (Jia et al. 2018), which were probably infected via a parasite transfer from wild birds in the zoo or via exotic bird exhibits. These reports highlight the risk that captive specimens pose and the importance of periodic health examination of avian collections and the establishment of proper protocols of quarantine in zoos (Bueno et al. 2010; Pacheco et al. 2011; Chagas et al. 2016, 2017).

found contrasting results, such as: (i) higher infection status in non-urban areas when compared to urban areas (Fokidis et al. 2008; Evans et al. 2009b; Santiago-Alarcon et al. 2018) and (ii) similar parasite composition between urban and non-urban areas (González et al. 2015). Additionally, Bonier et al. (2007) reported that urban white-crowned sparrow (*Zonotrichia leucophrys*) females with parasite infection had few mates and fewer offspring than females without parasites, while male reproductive success did not differ. Other studies have shown that the exotic and invasive urban-adapted house sparrow (*Passer domesticus*) has lost its native parasites when colonizing new areas, facilitating its invasion and therefore in agreement with the "enemy release hypothesis" (Lima et al. 2010; Marzal et al. 2011). Finally, contrasting avian haemosporidian communities among urban greenspaces from different geographical areas (i.e., Nearctic, Neotropical, Palearctic) showed similar infection prevalence, abundance structure, and assemblages dominated by widespread generalist parasites (Carbó-Ramírez et al. 2017).

13.3.2 Landscape Approach

Research efforts have also been devoted to compare parasite infections using a landscape scale. One pattern that has been reported in several studies is the higher prevalence of *Haemoproteus* in sites with simple vegetation, whereas *Plasmodium* prevalence is higher in sites with more complex vegetation structure within a landscape (Tunisia: Ayadi et al. 2017; Cameroon: Bonneaud et al. 2009; Ghana: Loiseau et al. 2010). Yet, another study from Cameroon showed higher prevalence of *Haemoproteus* and *Leucocytozoon* infections in patches with more complex vegetation structure within a landscape (Chasar et al. 2009). Regarding land uses, several tropical studies have reported higher infection rates in urban areas when compared to non-urban ones (Brazil: Belo et al. 2011; Ferreira et al. 2017; Mexico: Hernández-Lara et al. 2017; Tinajero et al. 2019). Yet, a study from South Africa reports higher infection prevalence in non-urban areas (i.e., croplands) when contrasted with urban settings (Okanga et al. 2013).

Other research efforts have been directed toward untangling the role of abiotic and biotic factors on haemosporidian infections. Gonzalez-Quevedo et al. (2014) found that temperature and the distance to artificial water bodies were related in differing ways, both positively and negatively, to malaria regarding the analysed scenario in the Berthelot's pipit (*Anthus berthelotii*) in Tenerife Island. Ferraguti et al. (2018) reported a positive relationship between the distance to man-made water reservoirs with the prevalence and diversity of *Plasmodium* parasites in Southwest Spain in the house sparrow. Sehgal et al. (2011) found in Central and West Africa, across different habitat types (i.e., primary forest, secondary forest, ecotone), that temperature was the most important abiotic factor related to rises in avian malaria prevalence in the olive sunbird (*Cyanomitra olivacea*), even when contrasted to other biotic factors (e.g., vegetation characteristics). In agreement with the later study, studies from the Peruvian Andes have identified abiotic factors

(i.e., temperature, precipitation) as primary drivers of parasite infection, particularly for the rufous-collared sparrow (*Zonotrichia capensis*, Jones et al. 2013) and house wren (*Troglodytes aedon*, Galen and Witt 2014).

Regarding urban–non-urban gradients, a study focused on the black sparrowhawk (*Accipiter melanoleucus*) in South Africa reports no variation of the prevalence and parasitemia by *Haemoproteus nisi* or parasitemia by *Leucocytozoon toddi* across the gradient; however, the risk of infection by *L. toddi* did decline with increasing urban cover (Suri et al. 2016). In agreement, Jímenez-Peñuela et al. (2019) found that infection prevalence of the house sparrow was similar along an urban gradient of Southern Spain (see Chap. 14).

A set of studies has focused on elevation gradient shifts of haemosporidian infection on birds. A study from Australia reported that low temperatures typical of higher elevations could reduce parasite prevalence (Zamora-Vilchis et al. 2012). A study performed in Colombia showed that the phenomenon is not that simple, reporting that *Leucocytozoon* prevalence was positively associated with altitude, whereas those of *Haemoproteus* and *Plasmodium* showed no relationship (González et al. 2014). In Central and South American there was a decrease in *Plasmodium* prevalence with elevation, while that of *Haemoproteus* parasites increased at higher altitudes (Doussang et al. 2018). In Chile there was a positive relationship between prevalence and latitude for *Leucocytozoon* lineages and a negative relationship for *Haemoproteus* and *Plasmodium* lineages (Merino et al. 2008; see Chap. 10 for a thorough revision of tropical avian haemoparasite studies in relation to ecological gradients).

Finally, regarding the vectors of haemosporidian parasites across land uses of a landscape (i.e., well-preserved montane cloud forest, peri-urban forest, urban forest), a recently published study from Mexico found the presence of avian malaria DNA in *Culex restuans*, *Aedes quadrivittatus*, and *Wyeomyia adelpha*, with mosquito composition similar across land use types but with stark changes in abundance and dominance by generalist and urban adapted mosquito species (Abella-Medrano et al. 2018) (see Chap. 6 for an in-depth revision of Diptera studies in relation to avian haemosporidians).

13.4 Concluding Remarks

In this chapter, we have introduced the landscape and urban ecology disciplines in general and through an avian–parasite perspective (see Chaps. 7 and 9 for a niche ecology and macroecological perspectives), afterwards focusing on the avian haemosporida literature for tropical regions (see also Chap. 14 for avian haemosporidian and vector studies in relation to anthropogenic impacts). Future directions in the tropics that could strengthen our understanding of avian haemosporidians include multiple spatial scales and drivers that could affect their prevalence, such as habitat change, species distribution, physiological stress, and diseases, as well as change in the strategies of parasite transmission (Brearley et al. 2013). Furthermore, it has

been suggested to adopt interdisciplinary approaches to studying urban human-wildlife-livestock interfaces along gradients of landscape transformation, together with the descriptions of the biological organization and community ecology at these interfaces to develop appropriate interventions that can be used to reduce risk of disease transmission (Hassell et al. 2017). Another interesting topic that requires attention is the invasion and emergence of diseases, where landscape modifications (e.g., urbanization) can play a key role (Dunn and Hatcher 2015). Actually, we are still in the early stages of understanding the complex interaction between host–parasites and anthropogenic environmental changes, the reason why this does not only apply to tropical regions but also for other areas from across the globe (Sehgal 2015; Santiago-Alarcon and MacGregor-Fors 2020).

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Chapter 14
Anthropogenic Effects on Avian Haemosporidians and Their Vectors

Martina Ferraguti, Carolina Hernández-Lara, Ravinder N. M. Sehgal, and Diego Santiago-Alarcon

Abstract With a population of nearly 8 billion humans, the planet is going through rapid unprecedented change. Human activities cause deforestation, desertification, urbanization, and climate change, all of which are affecting the tropical regions of the world. For example, it is clear that anthropogenic disturbance in tropical forests can rapidly increase biodiversity loss, and global environmental change may severely further degrade forests in the future. With regard to avian haemosporidians, it is not entirely clear how these changes will affect the prevalence, diversity, and pathogenicity of the parasites, but several studies have provided insights into how human impacts in the tropics will affect birds, vectors, and blood parasites. This chapter summarizes recent work that investigates the human effects on haemosporidian disease ecology.

Keywords Anthropocene · Diptera · Haemosporida · Land use change · Landscape epizootiology and epidemiology · Parasite ecology · Urban parasitology

14.1 Multiple Threats to Tropical Biodiversity

Human activities have impacted natural environments for at least 10,000 years, mostly through changing landscapes in the form of agriculture, forest management, building of canals and terraces, and more recently through the construction of cities (Piperno 2007; DeClerck et al. 2010; Forman 2014; MacGregor-Fors and

M. Ferraguti (✉)
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain

C. Hernández-Lara · D. Santiago-Alarcon
Red de Biología y Conservación de Vertebrados, Instituto de Ecología, Xalapa, Mexico

R. N. M. Sehgal
Department of Biology, San Francisco State University, San Francisco, CA, USA

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Escobar-Ibáñez 2017). The main threats to tropical biodiversity are deforestation and habitat fragmentation (Pfeifer et al. 2017), followed by other factors such as land use changes (e.g., agriculture, urbanization) (Aratrakorn et al. 2006; Shochat et al. 2010), species introductions (Marzal et al. 2015), and climate change (Ramírez-Villegas et al. 2014).

14.1.1 Deforestation and Habitat Fragmentation

Rainforest fragmentation is rapidly affecting all tropical areas of the world, and the number of forest fragments will increase substantially over the next decades (Taubert et al. 2018). The increased numbers of forest edges will impact the overall biodiversity and restructure ecological communities on a global scale (Pfeifer et al. 2017). In general, it is thought that deforestation has contributed significantly to the emergence of diseases, both in humans and in wildlife (Patz et al. 2000; Stephens et al. 2016). For example, we now know that HIV-AIDS originated in chimpanzees (Gao et al. 1999) and that human contact with wildlife in the tropics may lead to further outbreaks of unknown diseases.

Birds are affected by many of the same types of pathogens as humans (viruses, bacteria, and parasites), and thus they can serve as sentinels for the study of emerging diseases. Avian communities are significantly affected by tropical rainforest fragmentation (Ferraz et al. 2007); therefore, we expect that their parasite communities will be similarly impacted. The avian haemosporidian blood parasites have complex life cycles that include two hosts: an insect and a bird (see Chap. 2 for an introduction on avian haemosporidian life cycles and study methods). Based on their ecology, insects and birds will be affected by changing environments differently. Insects will be more sensitive to changes in temperature and microclimates (see Chap. 6 for a synthesis on vectors of avian haemosporidians in the tropics), whereas birds will be affected by changes in forest patch size, shape, isolation, and other landscape features (Carrara et al. 2015). To date, it has been difficult to model exactly how changes in habitat, and in particular deforestation and forest fragmentation, will affect parasite communities.

The major questions that researchers are studying regarding avian haemosporidians can be distributed into three broad areas:

1. How do forest fragmentation and habitat destruction affect the prevalence and diversity of parasite communities?
2. What will be the impact on the distribution of parasite species and will certain generalist species dominate?
3. How will changes in haemosporidian communities affect overall avian health?

Several research studies addressing these questions have been published, but although there are trends in the data, it is clear that more investigations are needed.

Previous research has been done in several tropical areas of the world to answer these questions (Sehgal 2015). In Sub-Saharan Africa, studies have shown that

overall, the prevalence and diversity of avian haemosporidians is quite variable, but deforestation appears to affect parasite communities. For example, in Ghana, in one bird species (*Cyanomitra olivacea*), a decrease in the prevalence and parasitemia of avian malaria was associated with increased forest disturbance (Loiseau et al. 2010). When comparing pristine forests to deforested areas in Cameroon, results showed that the prevalence of the genus *Plasmodium* in the community of birds was higher in the intact forested areas (Bonneaud et al. 2009). Another study in Cameroon analyzed the diversity and prevalence of *Plasmodium* and *Haemoproteus* in two widespread species of African rainforest birds from paired disturbed and undisturbed habitats. Results revealed that in deforested areas, the prevalence of some parasite species increased, while the prevalence of others decreased, highlighting the importance to treat each parasite species/lineage separately (Chasar et al. 2009). More recently, in the Caribbean islands of Guadeloupe and Martinique, areas of high habitat fragmentation were associated with a higher prevalence of *Plasmodium* and *Haemoproteus*. Interestingly, habitat fragmentation was more important than habitat loss in predicting increased parasite prevalence (Pérez-Rodríguez et al. 2018). Thus, the loss of forests and the increase in forest edges with fragmentation will have a profound impact on the overall composition of haemosporidian communities. With these changes in prevalence and parasite diversity, it is becoming evident that wild birds will be affected. The ramifications of these global changes for overall avian health will be an important area of future studies.

14.1.2 Agriculture

Archeological evidence (e.g., Mesoamerica) demonstrates that humans have altered tropical habitats in a way that landscapes are dominated by a few tree species favored by humans (DeClerck et al. 2010). Hence, much of the original vegetation in tropical areas has been lost, and most of the human uses include vast extensions of monocultures (e.g., pineapple, banana, oil palm, and cattle ranches creating severe habitat loss and fragmentation; Harvey et al. 2005a). In the future, tropical forests of South America and Africa will be threatened by oil palm development: both regions are characterized as harboring high species biodiversity (Vijay et al. 2016). In Thailand, the conversion of forests to oil palm and rubber plantations reduced bird species richness by 60%. Species of insectivores and frugivores appear to be most affected (Aratrakorn et al. 2006). The same pattern has also been reported for other vertebrate (Danielsen and Heegaard 1995), as well as invertebrate taxa (i.e., beetles) (Chung et al. 2000). However, there are crops that are more biodiversity friendly, such as shade coffee and shade cacao plantations, that when dominating the landscape matrix provide refuge to wildlife unable to thrive in habitats that differ from the original ones (e.g., sun coffee; Rice and Greenberg 2000; Harvey et al. 2005a; Bisseleua et al. 2008; DeClerck et al. 2010; Clough et al. 2011; MacGregor-Fors et al. 2018). At smaller regional and local scales (range between <10 m and 200 km), it is important to consider heterogeneity in land uses. At such

scales, landowners do not equally manage bio-friendly plantations where land use change, soil type, and biotic interactions become more relevant, and their influence will have different impacts depending on the taxonomic group or interaction under study (Greenberg et al. 1997a; Cruz-Angón and Greenberg 2005; Harvey and González Villalobos 2007; Philpott et al. 2008; also see Peterson et al. 2011 for a review). For instance, at a local scale, birds were more abundant in forests, bats in riparian forests, and beetles in secondary forests (Harvey et al. 2006). Moreover, without a tree stratum in the matrix, biodiversity will decline, and ecosystem services will be lost (DeClerck et al. 2010), which is particularly relevant for avian species, such as frugivorous birds, that are sensitive toward deforestation (e.g., Luck and Daily 2003). Landscape features, such as forest patches and living fences (i.e., fences delimiting private properties constructed using live trees) connecting such patches, are relevant for biodiversity conservation (Harvey et al. 2005b). In open habitat types (e.g., cattle ranches), the presence of living fences or some isolated trees are required for maintaining higher species richness and connecting local forest patches (Greenberg et al. 1997b; Lang et al. 2003; Harvey et al. 2005b). Therefore, it is a priority to understand how the different land use types change the landscape matrix and affect biodiversity (see Chap. 13).

Focusing on birds, it is known that responses to landscape matrix composition will depend on local conditions and functional groups (e.g., Deconchat et al. 2009). For instance, in the Atlantic forest of Brazil, small isolated native vegetation patches surrounded by a low permeability matrix (e.g., sugar cane) were particularly detrimental for understory insectivores due to their low dispersal capacity, making apparent the need to create a connected landscape (Uezu and Metzger 2011). In some regions, it is more beneficial for birds to have a large connected landscape of small native vegetation patches compared to a single large patch, in particular when species assemblages are not deterministically structured and nested (i.e., species found in several small patches are not a subset of species found in a large forest patch; several small patches can contain more species than a single large one, e.g., Mohd-Azland and Lawes 2011). Generalization of biodiversity patterns at large geographical scales can be misleading; therefore, for effective biodiversity conservation, the study and understanding of the local landscape context is necessary.

14.1.3 Urbanization

The urbanization process has rapidly increased during the last decades, and cities around the world are becoming a common feature of landscapes (Alberti 2008; Forman 2014). More than half of the human population will be living in cities by the year 2050, imposing environmental demands at different spatial and temporal scales (McDonnell and Pickett 1990; Grimm et al. 2008; Montgomery 2008). Due to major landscape modifications related to the settlement of novel urban structures and to cover human basic needs (e.g., food, energy, water; McDonnell and Pickett 1990), urbanization has been identified as a major threat to biodiversity (Czech et al.

2000; McKinney 2002; Berkowitz et al. 2003; Alberti 2008; Shochat et al. 2010), directly affecting >3000 species listed as threatened or near-threatened according to the IUCN Red List (Maxwell et al. 2016). The most important threats to birds arising from the urbanization process are predation by cats, collisions with windows and with vehicles; together these factors amount to billions of death birds in the USA and Canada (Loss et al. 2015). Although less well documented, the same pattern arises in Latin American cities (Santiago-Alarcon and Delgado-V 2017).

At large spatial scales, there are high similarities in bird species richness between urbanized and semi-natural systems (Pautasso et al. 2011). When zooming into the urban landscape, complex patterns can be identified. For example, in the southwestern part of Mexico City, bird communities are dominated by a few generalist species in areas with commercial components, whereas there is higher evenness in green areas, suggesting that species richness and abundance are sensitive to site-specific characteristics (Ortega-Álvarez and MacGregor-Fors 2009). Within the urban landscape, granivores and insectivores dominate the avian community in the green areas, whereas omnivores are the dominant guild in the residential and commercial areas (Ortega-Álvarez and MacGregor-Fors 2009). In India, urban bird communities are dominated by granivores and omnivores with clear absences of insectivores, which are present in forested rural areas (Sengupta et al. 2014), suggesting that urbanization tends to homogenize avian communities. Hence, urban features of the landscape must be considered in biodiversity studies, as some characteristics of the urban matrix, such as urban forests and greenspaces, are highly valuable for bird conservation (Croci et al. 2008; Aronson et al. 2014; MacGregor-Fors et al. 2018).

14.1.4 Introduction of Species

Another factor relevant for the conservation of native birds is the introduction of exotic species or biological invasions due to human activities (Mack et al. 2000). When organisms reach new areas they have to face novel environments and challenges, and the few that become naturalized may turn into a latent threat to native species (e.g., *Passer domesticus*, MacGregor-Fors et al. 2010) because they have escaped from their natural enemies (e.g., Torchin et al. 2003; Marzal et al. 2011) and found areas where they can thrive and displace native birds (MacGregor-Fors et al. 2010; Jiménez-Peñuela et al. 2019; Santiago-Alarcon et al. 2019). In general, invasive organisms can cause extinctions of native species via predation, competition for food, habitat modification, alteration of species interactions, and introduction of novel pathogens (Mack et al. 2000; Roemer et al. 2002; Perkins et al. 2008; Marzal et al. 2015). In the case of birds, the successful establishment of introduced species hinges on their population of origin in terms of growth rate, dispersal capacity, and life history (e.g., minimum population size to avoid Allee effects, geographical range size), characteristics of the place of introduction (e.g., climate), and specific features of the introduction event, such as the number of birds introduced to the new location (Duncan et al. 2003). Because most bird introductions begin with

small numbers, they usually fail to establish (Pimm 1991). The success of an introduction also decreases when there are large differences in terms of physical conditions between the original and introduced environments (Duncan et al. 2003). Given that human activities homogenize the environment (Aronson et al. 2014), bird species able to adapt to such conditions (Fischer et al. 2015) will be more likely to become invasive outside their native ranges. The invasive birds could potentially be more resistant to both native and exotic pathogens as compared to native birds (e.g., avian malaria; van Riper et al. 1986; see Chap. 15 for a review of invasion biology and parasites).

14.1.5 Climate Change

Climate change in the last century caused by anthropogenic activities has generated shifts in the range, abundance, survival, phenology, disease emergence, reproductive success, and extinctions of many species (Condit et al. 1996; Pounds 2001; Hickling et al. 2006; Lavergne et al. 2006; Menéndez et al. 2006; Parmesan 2006; Wilson et al. 2007; Lurgi et al. 2012). Though climate change is generally not considered to be the primary threat to biodiversity at present, it will become the main conservation concern in future decades (Jetz et al. 2007). In general, species will move to higher latitudes and higher elevations in order to find "similar" climatic conditions (Wilson et al. 2007; Chen et al. 2011; Ramírez-Villegas et al. 2014). Studies in the tropics indicate that species from lowlands would have to travel longer distances in order to find suitable climatic conditions, which would increase their vulnerability to climate change depending on their dispersal capacity (Bertrand et al. 2011). Perhaps the most endangered habitats are the alpine forests, grasslands, and shrublands because of the drastic and accelerated reduction of suitable climatic conditions: it is likely that high altitude species will face higher extinction risks (Pounds 2001; Williams et al. 2007).

14.1.6 Conclusion

Summarizing, all of the above-discussed human factors that impact biodiversity via global environmental changes have led to the new geological epoch known as the Anthropocene, mainly due to increases in global temperature, climate anomalies, pollution, and species declines (Waters et al. 2016). For example, historical museum bird collections (spanning 100–150 years) have demonstrated increases of toxic chemicals that bioaccumulate (e.g., mercury), increases in prevalence of infectious diseases, as well as changes in diet and migratory routes (Schmitt et al. 2018). One of the most threatened avifauna is the endemic Hawaiian honeycreepers that currently face habitat loss, invasive species including predators and pathogens, and their precarious situation will be exacerbated by climate change (Paxton et al. 2018).

Conservation management suggestions include mosquito control, protection of key habitats for forest birds, disentangling the genetic underpinnings of disease immunity, predator control, reintroductions and translocations, and increasing captive breeding efforts among others (Paxton et al. 2018). A prime example of conservation success is the avifauna of the Galapagos Islands, where no recorded extinctions exist and successful eradication programs of non-native species (e.g., goats, pigs, pigeons) have been successfuly completed (Parker 2018). However, more severe El Niño events due to climate change could endanger endemic birds that have small population sizes such as the Galapagos penguin (*Spheniscus mendiculus*) and the Galapagos cormorant (*Phalacrocorax harrisi*), an effect that can act in synergy with native and non-native parasites (Santiago-Alarcon and Merkel 2018). Moreover, a host generalist ectoparasitic fly found in nests, *Philornis downsi*, causes high mortality in chicks of various bird species and is currently the ectoparasite which poses the most serious conservation challenges for native birds in the Galapagos archipelago (Fessl et al.

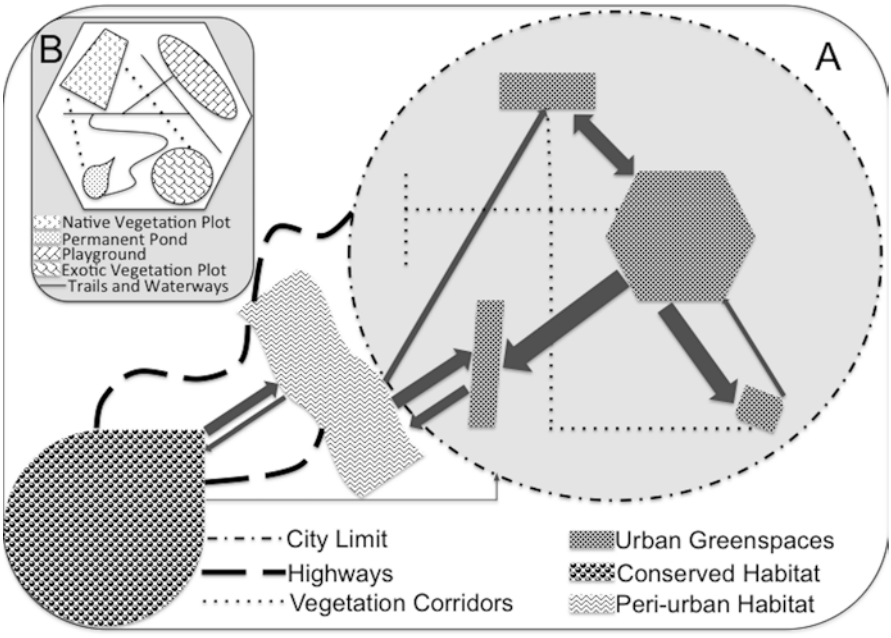

Fig. 14.1 (**a**) Regional landscape showing native pristine and modified habitats (e.g., conserved forests, peri-urban forests, city), connected by animal dispersion (arrows; the thickness of arrows indicates influx rate among habitats). (**b**) Detail of an urban greenspace, where a large degree of heterogeneity is observed in habitat structure at this local scale. This scheme conveys a visualization of available regional species pools and potential ecological interactions at different spatial scales, where metapopulation and metacommunity dynamics can easily develop, even within cities (see **b** for the heterogeneity of an urban greenspace; e.g., Carbó-Ramírez et al. 2017), for both free-living organisms and their symbionts

2018). Hence, a current major research focus in bird conservation is the ecology of pathogens, particularly in relation to anthropogenic impacts (Fig. 14.1).

14.2 Consequences of Human Activities on Host–Parasite Dynamics

Environmental changes and biodiversity loss favor generalist vectors and parasites, increasing the risk of host-switches and emerging diseases in wildlife (Hernández-Lara et al. 2017; Abella-Medrano et al. 2018). Homogenization of host communities could lead to an amplification effect, meaning there would be more competent hosts (or vectors). The increase in the abundance of competent hosts means that the chance of a vector feeding on an infected individual and passing the parasite to another susceptible host is higher, having as a result an increase in infection rate (see Ostfeld et al. 2008). Genetic diversity is also affected by alteration and destruction of natural habitats; a decrease in hosts' genetic diversity could have a negative effect on their ability to respond to infections (Vourc'h et al. 2012). Particularly, endemic species and isolated populations could be at risk of extinction and should become a conservation priority. Transformation of natural habitats into agricultural lands, cattle ranches, and cities can pose a threat to less adaptable species in terms of behavior, for example, by increasing competition for resources (e.g., food, water, breeding sites). This competition and resource scarcity may generate significant stress on birds, causing potential negative effects on immune responses and host health.

14.2.1 Abiotic Factors Are Determinant for Parasite Distribution

Some of the factors explaining the prevalence of avian Haemosporida through disturbance gradients are temperature (Sehgal et al. 2011; Gonzalez-Quevedo et al. 2014), precipitation (Jones et al. 2013), proximity to water bodies (Mendenhall et al. 2013), and pollution (Bichet et al. 2013) (Table 14.1). Temperature, precipitation and water bodies are mainly related to the parasite's development within the vector. Temperature limits the completion of sexual life stages of haemosporidians within the vector (Paaijmans et al. 2013). Particularly, *Plasmodium* spp. require warmer temperatures (LaPointe et al. 2010), while species of *Leucocytozoon* are favored by lower temperatures (Carlson et al. 2018). But even species from *Leucocytozoon* and *Haemoproteus* have a temperature threshold under which they cannot complete their life cycle within the vector. Precipitation is another abiotic factor closely related to the transmission of avian

Table 14.1 Studies of avian Haemosporida focusing on the effects of habitat anthropogenic changes to host-parasite interactions in tropical regions

Site	Vegetation type/land use	Host species	Parasitological parameter	Response direction	Explaining variables	Reference
Cameroon	Mature rainforests and agroforests	Tufted olive sunbird (Nectariniidae), yellow-whiskered Greenbul, and little Greenbul (Pycnonotidae)	Pr (P)	+	Undisturbed sites	Bonneaud et al. (2009)
Cameroon	Undisturbed rainforests and agricultural sites	Olive sunbird (Nectariniidae)	Pr (H, L)	+	Undisturbed sites	Chasar et al. (2009)
			Pr (P)	=	Disturbed vs. undisturbed	
		Yellow-whiskered Greenbul (Pycnonotidae)	Pr (L)	+	Undisturbed sites	
			Pr (P)	+	Disturbed sites	
Cameroon	Undisturbed forests, fragmented forests, and young palm oil plantations	85 species (27 families)	Pr (P)	−	Fragmented forest and young oil palm plantation	Tchoumbou et al. (2020)
			Pr (H)	=	Disturbance	
Ghana	High deforestation and drier, low deforestation, intermediate	Olive sunbird (Nectariniidae)	Pr (P)	+	Tree cover and precipitation	Loiseau et al. (2010)
			Pa	+	Least disturbed site	
		Yellow-whiskered Greenbul (Pycnonotidae)	Pr	=	Disturbance	
			Pa	+	Most disturbed site	
Brazil	Intact and disturbed Cerrado, transition area Amazonian rainforest-Cerrado	122 species (29 families)	Pr	+	Disturbed (urban) vs. intact Cerrado	Belo et al. (2011)
			Pr	=	Transition area and intact Cerrado	
			Pr	=	Transition area and disturbed (urban) Cerrado	

(continued)

Table 14.1 (continued)

Site	Vegetation type/land use	Host species	Parasitological parameter	Response direction	Explaining variables	Reference
Costa Rica	Landscape features, spatial scales, and landscape habitat configurations	Orange-billed nightingale-thrush (Turdidae)	Pr (P)	+	Proximity to the nearest river	Mendenhall et al. (2013)
				+	Increased forest element edge and decreased forest element area	
				+	Smallest forest elements and most deforested agricultural plots	
Tenerife	Anthropogenic, natural, biotic, and abiotic environmental factors	Berthelot's pipit (Motacillidae)	Pr (P)	+	Minimum temperature of the coldest month	González-Quevedo et al. (2014)
				+	Interaction distance to artificial bodies of water and minimum temperature of the coldest month	
				=	Host density	
				=	Vegetation type	
				−	Distance to artificial bodies of water	
				−	Distance from the nearest poultry farm	

Mexico (contrasted with Germany)	Urban greenspaces from three biogeographical regions	27 species (17 families)	Pr	=	Nearctic and Neotropical sites	Carbó-Ramírez et al. (2017)
			Pr	–	Palearctic vs. Neotropical	
			Pr (P)	+	Neotropical vs. Nearctic	
			Pr (P)	+	Neotropical vs. Palearctic	
			Pr (P)	=	Nearctic vs. Palearctic	
					Host community composition higher at Neotropical site	
					Parasite lineage richness higher at Palearctic site	
					Three parasite communities dominated by generalist haemosporidians	
Mexico	Cloud forest, shade coffee plantation, periurban forest, urban greenspace, and cattle ranch	Chestnut-capped Brushfinch (Emberizidae)	Pr	+	Urbanization	Hernández-Lara et al. (2017)
			Pr (P)	–	Bush cover[a]	
			Pr (P)	–	Canopy cover[b]	
			Pa	+	Urban forest	
			Pa	–	Shade coffee plantation	
			Pa	–	Bush cover	
			Pa	+	Leaf litter ground cover	
			Ag	–	Cattle field[b]	
			Ag	+	Preserved forest[a]	

(continued)

Table 14.1 (continued)

Site	Vegetation type/land use	Host species	Parasitological parameter	Response direction	Explaining variables	Reference
Ecuador	Preserved rainforest and forest fragments	18 species (8 families)	Pr (P, H)	=	Disturbance gradient	Rivero de Aguilar et al. (2018)
Mexico	Non-urban (agricultural) and urban	House sparrow (Passeridae)	Pr	−	Urban vs. non-urban	Santiago-Alarcon et al. (2018)
Mexico	Cloud forest, shade coffee plantation, periurban forest, urban greenspace, and cattle ranch	Common chlorospingus (Passerellidae)	Pr (H)	−	Urbanization	Hernández-Lara et al. (2020)
			Pr (P)	+	Urbanization	
			Parasite richness	−	Urbanization	
Islands of Guadeloupe and Martinique	Habitat loss, habitat fragmentation, and landscape heterogeneity	Antillean bullfinch (Thraupidae)	Pr (P, H)	+	Forest fragmentation	Pérez-Rodríguez et al. (2018)

(*Pr*) prevalence, (*Pa*) parasitemia, (*Ag*) aggregation, (P) *Plasmodium*, (H) *Haemoproteus*, and (L) *Leucocytozoon*

[a]Dry season

[b]Rainy season

Haemosporida, as well as the presence of permanent water bodies. During the rainy season, precipitation creates temporal ponds and small water reservoirs in plants (i.e., phytothelmata) that are necessary for the development of the larval stages of dipteran vectors. This, in turn, allows an increase in blood-sucking dipteran populations and subsequently the transmission of blood parasites. Therefore, at sites with marked seasonal precipitation, there is generally a peak of infection during the rainy season (Cosgrove et al. 2008). Another factor is environmental pollution, which can reduce the ability of a host to fight off infections, exposing birds to pollutants that make them prone to acquiring infections (Bichet et al. 2013), and dying due to high parasitemia.

14.2.2 Alteration of Forest Structure Modifies the Host–Parasite Interaction

Vegetation structure is related both to the life history of insect vectors and vertebrate hosts and is therefore a complex variable to study. For example, a forest with dense tree cover would produce a thick layer of leaf litter, favoring the development of food resources (e.g., arthropods) for some bird species. In addition, a forest with dense tree cover would have more breeding and perching sites for birds and, in theory, would yield flowers, nectar, and fruits (important food sources for birds). Thus, we could expect that birds living in a habitat with plenty of resources would be in better condition and immunologically better prepared to fight off infections. On the other hand, a dense tree cover would create favorable microclimatic conditions for the development of dipterans, which could increase the risk of infection if abundant species are competent vectors. Proximity to the edge of the forest has been identified as another important variable explaining the parasitemia of avian Haemosporida, where parasitemia increases as birds are closer to the edge (Knowles et al. 2010). Near the edge of the forest, food resources become scarce and predation increases, both considered to be stressing factors for birds (Knowles et al. 2010). As stress negatively impacts the host immune response (Sapolsky 1992; Loiseau et al. 2008), it leads to a higher probability of a bird acquiring an infection, presenting high parasitemia, and even dying from the infection. Pollutants have been related to a suppressed immune response, reducing host fitness, and increasing host mortality (Sehgal 2015).

There are only a few studies in tropical areas focusing on the effects of land use change (Hernández-Lara et al. 2017) and urbanization (Carbó-Ramírez et al. 2017; Santiago-Alarcon et al. 2018) on host–parasite dynamics of avian haemosporidians (Table 14.1). In some cases, prevalence increases with habitat disturbance (Belo et al. 2011), but in others, the result is the opposite (Bonneaud et al. 2009), or even prevalence may remain similar across disturbance levels (Chasar et al. 2009). So, it seems that responses to habitat disturbance are dependent on the specific host–parasite system and location under study. The ability of a host to control an

infection is related to its ability to cope with habitat disturbance in terms of its stress response, adaptability, tolerance to infection, as well as its competence to host generalist parasites (as they become dominant in disturbed habitats; Hernández-Lara et al. 2020).

14.2.3 Urbanization, an Extreme Habitat for Parasites

Cities represent the most drastic habitat alteration because all the environmental factors (temperature, water availability, vegetation structure) and host and vector diversity are considerably altered when compared to their undisturbed counterparts. The effects of urbanization on the dynamics of bird-Haemosporida systems have mainly been studied in temperate regions, where the diversity of hosts, parasites, and vectors is much lower than in tropical areas (e.g., Fokidis et al. 2008; Evans et al. 2009). Another important difference is that urbanization develops differently across countries. In some places, it develops in an organized manner, but in others the development is erratic without much urban planning (MacGregor-Fors and Escobar-Ibáñez 2017). Therefore, results from different countries, in particular between temperate and tropical regions, should not be generalized.

Within cities, only a handful of native species remain and are subject to new threats and stressing factors (Santiago-Alarcon and Delgado-V 2017), having to compete for resources with non-native species that are well adapted to cities (e.g., pigeons). They have to face new predators (domestic cats and dogs), pollution (e.g., chemicals, noise, light), and other urban elements such as buildings and cars (Seress and Liker 2015). One would think that this is not a problem for birds because they can fly long distances, but in fact, some forest birds may not be able to move from one patch to the other (e.g., chestnut-capped brush finch). Hence, isolation of small populations could potentially lead to local extinctions. However, in some cases, birds are confined to parks and greenspaces, which can serve as refuge for birds not adapted to highly built areas (e.g., urban avoiders, urban utilizers). Actually, urban greenspaces have been shown to harbor high richness of avian haemosporidian lineages across different biogeographical regions, suggesting that urban greenspaces are important reservoirs for biodiversity and are able to keep similar ecological dynamics to nonurban counterparts (Carbó-Ramírez et al. 2017; Fig. 14.1). Furthermore, environmental conditions within urban areas differ from those in the surrounding environments, which can generate urban heat islands (i.e., increased temperature at local and micro spatial scales within cities), improving conditions for parasite year-round transmission (e.g., Buczek et al. 2014). For example, higher temperatures within cities and stable water sources can allow the permanent presence of insect vectors throughout the year in temperate areas, opening opportunities for continued parasite transmission, which might negatively affect hosts during winter months when resources are scarce, damaging their immune response as a result of lower body condition compared to conspecifics inhabiting more suitable nonurban habitats (e.g., Jiménez-Peñuela et al. 2019; Fig. 14.1). At the individual level,

there are immunological responses to habitat alteration, in particular to factors (e.g., chemicals, pollution) that directly affect organisms' health (e.g., Martin and Boruta 2014). In the case of host–vector–parasite interactions, depending on the system under study, it would be possible to observe amplification or dilution effects in response to human habitat alteration (Fig. 14.1).

As mentioned above, anthropogenic changes have facilitated the invasion and colonization of introduced species (Mack et al. 2000). When a bird species expands its distribution or is introduced to new sites, sometimes it carries with it its parasites. Host-switching of these parasites to naïve hosts can have catastrophic consequences if they are competent (i.e., novel weapon hypothesis). A very well-studied case is the introduction of avian malaria (*Plasmodium relictum*) and its vector, *Culex quinquefasciatus*, into the Hawaiian Islands. The infection of naïve competent hosts caused an alarming decrease and extinction of several populations of Hawaiian endemic forest birds (Atkinson and Samuel 2010). Fortunately, some of the endemic honeycreepers have adapted to the parasite and started to recolonize lowland areas where avian malaria has become endemic (e.g., Woodworth et al. 2005; see Chap. 15).

14.2.4 Spread of Parasites with Climate Change

Understanding the key variables explaining host–parasite dynamics are crucial to predict future outcomes to specific anthropogenic changes and also to climate change. Some studies have identified temperature and precipitation (Jones et al. 2013; Gonzalez-Quevedo et al. 2014) as key variables explaining the prevalence of avian Haemosporida. With climate change, temperatures are expected to increase at rates never presented before. Higher temperatures would promote the development of Haemosporida within the vector in sites that are now too cold to allow completion of sexual stages. Likewise, areas that are currently too dry might become ripe for avian haemosporidian invasion if precipitation increases. On the other hand, regions that are presently ideal for vectors may in the future become inhospitable for parasite transmission (Lafferty and Mordecai 2016). Overall, however, we could expect a geographical expansion and/or relocation of the distribution of avian Haemosporida from the tropics into temperate regions, and the colonization of higher elevations and areas that are currently arid (see Chap. 9 and 10 for an introduction to macroecology and a synthesis on environmental gradients in relation to avian haemosporidians).

Several studies have begun to interpret how climate change will affect avian haemosporidians. For example, one work modelled prevalence data from 70 years and reported that avian *Plasmodium* prevalence mirrors the recent increases in temperature associated with global climate change (Garamszegi 2011). Pérez-Rodríguez et al. (2014) investigated how climate change may affect the three major genera of avian haemosporidians. The authors modelled that, with climate change, the overall diversity of parasite lineages will decrease, with an overall homogenization of

parasite diversity. A more recent study predicts that host specialization will also be affected by climate change, given that at present, host specialists are generally found in areas that exhibit more pronounced rainfall seasonality and wetter dry seasons (Fecchio et al. 2019). Thus, with increased temperatures, the ratios of generalists to specialists are likely to increase, which could impact bird populations that have not previously been exposed to certain lineages of parasites.

Two examples of areas of concern for bird populations are Hawaii, where endemic birds suffer severely from avian malaria, and also the high latitudes, where birds may never have been exposed to avian haemosporidians. In the islands of Hawaii, the mosquito *Culex quinquefasciatus* populations are expected to reach higher elevations, thus threatening the remaining honeycreepers (Fortini et al. 2015). Current research has focused on predicting and mitigating the impact of climate change on the remaining birds, possibly by translocating threatened populations, releasing sterile male mosquitoes, and allowing for the evolution of tolerance (Fortini et al. 2017; Liao et al. 2017). In addition to populating higher elevations, avian malaria will likely spread to higher latitudes. Models have shown that in Alaska, and also in France, avian malaria will expand northwards with climate change (Loiseau et al. 2012, 2013). Regarding climate change, it will be important to recognize that each bird species, insect vector, and parasite may respond differently and have differing thermal tolerances. Thus, although predictive models are useful, it is likely that the rapid changes will have unpredictable consequences in terms of changes in overall haemosporidian diversity and prevalence.

14.2.5 Conclusion

Habitat destruction and transformation into agricultural lands and urban areas pose a threat to biodiversity and will affect host–parasite interactions, leading to a homogenization in the diversity of hosts, vectors, and parasites. Also, more frequent contact between native and introduced fauna could lead to parasite host switches and disease emergence. It should be noted that not all bird species respond equally to habitat disturbance. Therefore, a specific disturbance could have a negative effect on the prevalence, parasitemia, and diversity of haemosporidians for one species, but others could benefit from the same disturbance or even have no response at all. Within habitat transformation classifications, urbanization is the most drastic of them, given that only a few species can thrive in the typical city landscape (i.e., urban exploiters). However, preserving well-connected forest patches and other green spaces (e.g., parks) within cities can preserve an important amount of native bird and parasite diversity. Increases in temperature and precipitation due to global warming would favor the development of vectors and haemosporidians (*Plasmodium* spp.) within them, increasing the probability of transmission. Increases in temperature and precipitation could also expand the distribution of parasites and vectors, affecting bird populations that are not currently exposed to specific parasite lineages. Therefore, in order to take successful conservation actions for birds and other

fauna, it is imperative to understand the processes driving parasite transmission and host susceptibility that could help predict and mitigate possible disease outbreaks.

14.3 An Integrative Approach for the Study of Vector-Borne Diseases

In recent years, the number of emerging and re-emerging diseases, many of them caused by vector-borne pathogens, has dramatically increased. The emergence of most of these diseases has been associated with factors of global change such as habitat alteration, growing human densities, biodiversity loss, invasion of alien species, and climate warming operating on a global scale (Jones et al. 2008). Approximately 75% of emerging infectious diseases affecting humans are of zoonotic origin (i.e., wildlife diseases that spillover from animal populations into humans) and circulate in the wild in populations of non-human vertebrates (Vorou et al. 2007). Many of these zoonotic pathogens require vectors, like mosquitoes or ticks, to be transferred from one host to another (Gubler 2009). Hence, vector-borne diseases have had a severe impact on human populations throughout history and represent an important public health issue with significant wide-ranging economic

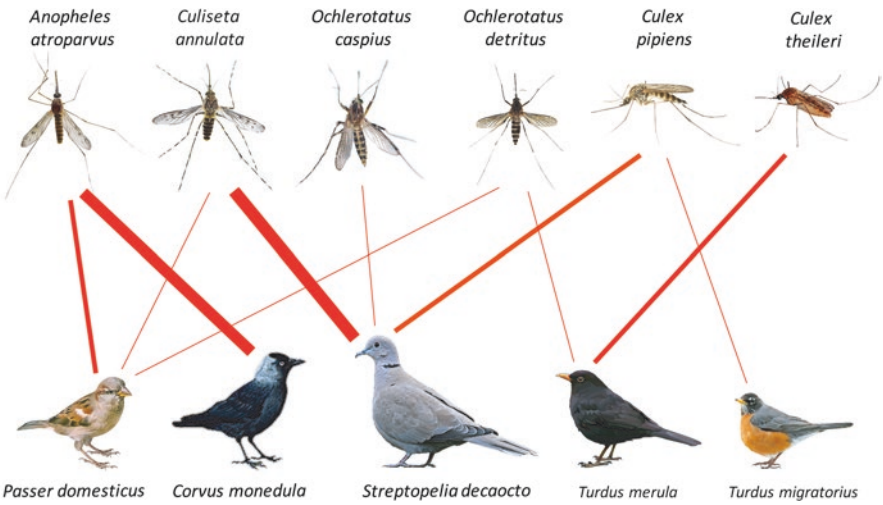

Fig. 14.2 One of the most recent emerging vector-borne pathogens is the flavivirus West Nile virus (WNV), maintained in nature in a bird–mosquito–bird transmission cycle. Birds are the main reservoirs of WNV, while other hosts like humans and horses are considered "dead-end" hosts (i.e., they become infected but do not spread the infection onwards). In the theoretical example of WNV transmission dynamics, mosquitoes (top) are micro-predators feeding on birds (bottom) as their resource. Persistence of WNV depends on the existence of sufficiently competent vectors and hosts able to maintain at least one full transmission cycle (e.g., species connected by thick red lines)

implications. Under a current globalized scenario, understanding which factors regulate pathogen transmission is of great importance for ecological, evolutionary, health, and economic reasons. Emerging vector-borne diseases are usually accompanied by a "reservoir" of many host species and many vector species (i.e., an ecological reservoir), without which they could not persist (Fig. 14.2).

Overall, a One Health integrated approach is relevant for dealing with vector-borne diseases, particularly when mosquito-borne pathogens are involved due to their great sensitivity to direct and/or indirect changes in their environmental conditions (particularly the microclimate), including habitat characteristics and land use (Dobson 2009). In these cases, details about the interface among the vertebrate hosts (both wildlife and livestock), the insect vectors, and the environment they share could be critical for understanding transmission patterns of pathogens. Traditionally, the study of pathogen transmission has been focused on just one or two of these actors, and the remaining ones have been largely ignored. However, from an ecological point of view, studying the interactions among each one of these three players (i.e., vertebrate host, insect vector, parasite), as well as understanding the environmental context in which ecological processes take place, is essential for controlling emerging diseases (see Ferraguti 2017 for a review). Thus, the study of vector-borne diseases requires an integrative approach that combines information on the pathogens circulating between the communities of both vectors (mosquitoes) and vertebrates (hosts) and the environmental characteristics potentially affecting this complex interaction (Fig. 14.3).

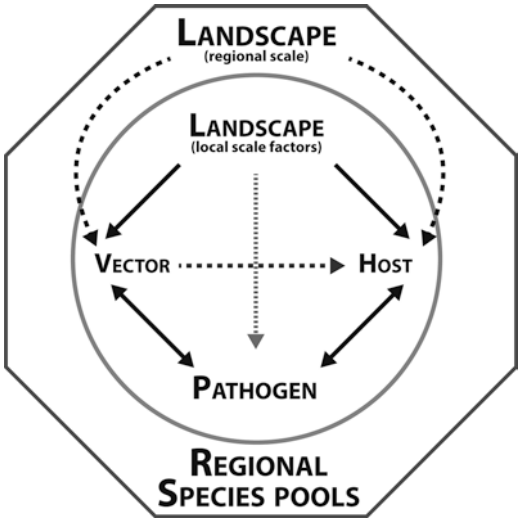

Fig. 14.3 Schematic representation of the main relationships affecting the transmission dynamics of vector-borne diseases. Shown are the direct interactions among organisms and between local environmental conditions and organisms (solid lines), the indirect effect of the local environment on pathogen transmission and on interactions between vector-host, and the indirect effect of the regional landscape on pathogen transmission (dotted lines)

14.3.1 Urbanization Effects on Insect Vectors

Recent studies have shown that environmental anthropization leads to a change in distribution and abundance of mosquito species in a local community (e.g., Abella-Medrano et al. 2015; Sehgal 2015; Ferraguti et al. 2016), and hence as a by-product affects the potential spread of their dependent pathogens. With over 3500 known species described worldwide, mosquitoes are involved in a vast majority of parasite transmission, including metazoan (e.g., filarial worms), protozoan (e.g., malaria parasites), and numerous viruses (e.g., Dengue, Rift Valley and West Nile viruses; see Chaps. 5 and 6 for a thorough review of Diptera families involved in pathogen transmission and recent synthesis of research on vectors of avian haemosporidians). Mosquitoes have a wide variety of habitat requirements and feeding behaviors, which can vary greatly among closely related species (Becker et al. 2010). Consequently, environmental characteristics strongly affect the vector community composition and abundance, shaping the prevalence of vector-borne pathogens (Patz et al. 2000; Sehgal 2015; Ferraguti et al. 2016).

14.3.1.1 Negative Relationship Between Urbanization and Mosquito Abundance

Generally, mosquito abundance and species richness are higher in natural and rural areas than in urban ones, as supported by studies conducted in Europe (Ibañez-Justicia et al. 2015; Ferraguti et al. 2016) and Australia (Johnston et al. 2014). In addition, anthropogenic habitats showed the lowest values of abundance and richness in comparison with natural areas, characterized by more diverse and favorable breeding environments for insects with freshwater and brackish water wetlands (but see Abella-Medrano et al. 2015, 2018, as an example of how seasonality can affect such general trends). For example, while mosquitoes of the *Mansonia* genus predominated in rural habitats, *Culex* mosquitoes are usually more common in urban sites (Johnson et al. 2008).

Also, higher richness has been found near urban environments (i.e., periurban forests) or within urban greenspaces, though abundance was higher at the better-preserved natural areas (Abella-Medrano et al. 2015, 2018). Ferraguti et al. (2016) found negative associations between mosquito abundance and richness and urbanization in southern Spain (Fig. 14.4), a temperate area of Mediterranean climate in which several pathogens affecting humans, wildlife, and livestock circulate (Figuerola et al. 2007). Also, negative relationships were found between both mosquito abundance and species richness and human population density, showing that the relationship between mosquito and human variables had a threshold at areas with approximately more than 50 inhabitants/250m^2 (Ferraguti et al. 2016). Previous studies have found strong support for the negative effect of human population abundance and density on the transmission of vector-borne pathogens (Padmanabha et al. 2012), which may be partially due to the detrimental impact of more densely

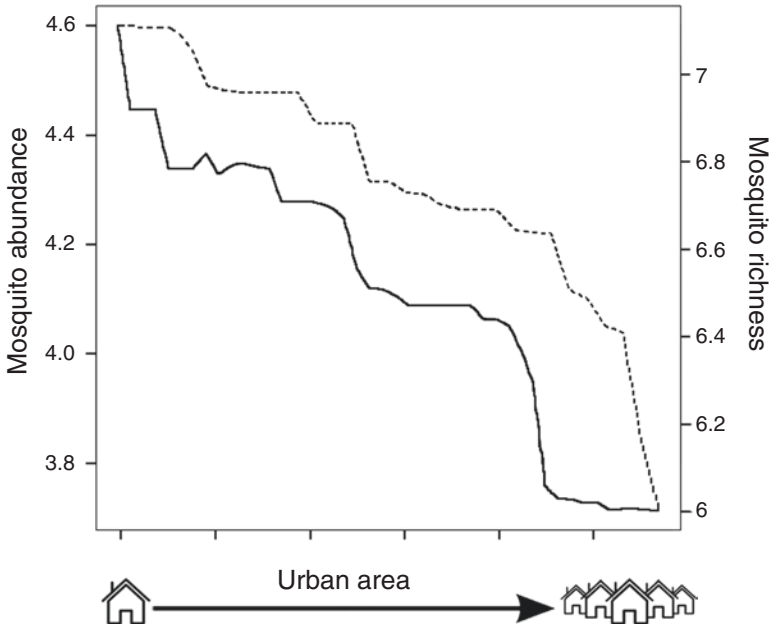

Fig. 14.4 The mosquito abundance and species richness decrease as the degree of urbanization of the environment increases, although not all species are affected equally. Abundance (solid line) refers to the logarithm of the average number of mosquitoes captured at each locality, richness (dotted line) refers to the number of different mosquito species at each locality. (Adapted from Ferraguti et al. 2016)

populated areas on mosquito abundance given a reduction on the availability/suitability of breeding areas, as well as to the implementation of mosquito control programs in cities and peri-urban areas. However, such impacts will change on different cities because urban ecosystems are not homogeneous across the world, particularly in developing regions (e.g., Latin America; MacGregor-Fors and Escobar-Ibáñez 2017), having sometimes opposite trends depending on the species (e.g., Abella-Medrano et al. 2015, 2018).

Similarly, in South Australia, Johnston et al. (2014) found high abundances of different mosquito species in rural areas (further from the city center and closer to saltmarshes), and mosquito species richness was negatively correlated to factors such as the area occupied by urban land, human density, and the distance between urban areas and marshlands. Hence, the presence of salt marshes may provide a suitable environment for halophilic (salt-loving) species of mosquitoes (Leisnham and Sandoval-Mohapatra 2011; Johnston et al. 2014), strongly affecting the abundance of *Culex modestus* and *Ochlerotatus caspius*. Interestingly, in South Spain, the relationship between mosquito abundance and distance to marshlands was not linear, thereby showing a marked threshold at distances of about 2.5 km for *Cx. modestus* and 10 km for *Oc. caspius* (Ferraguti et al. 2016). These differences fit well with the estimated flying distances of mosquitoes, which range between

0.16 and 1.98 km for *Culex* (Ciota et al. 2012) and up to 12 km for *Oc. caspius* (Bogojević et al. 2011). To reduce the nuisance of mosquitoes in localities close to marshlands, larvicide treatments have been used, as in the case of treatments with *Bacillus thuringiensis isra* in urban areas from southern Spain (S. Ruiz *pers. com.*).

14.3.1.2 Adaptation to a New Environment

Regarding insects, urbanization has formed many new habitats and modified existing ones: simplification of habitat structures (Shochat et al. 2006) and the alteration of trophic interactions (Faeth et al. 2005) may lead to an increase in the abundance of a few key species that may affect species interactions (Frankie and Ehler 1978; McKinney 2008). Indeed, this anthropogenic-mediated loss of biodiversity often influences the dynamics of wildlife diseases by regulating the distribution of many mosquito species (Gilioli and Mariani 2011). Urban ecosystems can effectively act as a regulating factor of mosquito population growth, for example, residents who irrigate gardens during summer (Becker et al. 2014). Also, urban areas can have temperature and precipitation regimes that are considerably distinct from the surrounding regions, with important consequences for all organisms inhabiting them (Frankie and Ehler 1978). Here, human behaviors can alter habitat availability and ecosystem quality through changes in water sources, resource accessibility, and vegetation coverage, resulting in a severe impact on mosquito populations (Gilioli and Mariani 2011; Becker et al. 2014; Li et al. 2014), thus, affecting directly and/or indirectly their community ecology and pathogen transmission risk (for a review, see LaDeau et al. 2015). Urban characteristics, including the availability of artificial habitats such as deposits of water, gardens, and subterranean water systems, can provide alternative breeding sites for mosquitoes, therefore leading to an alteration on populations of vectors. Overall, numerous insects are associated with human waste production, and higher mosquito abundances are associated with poverty and degraded urban neighborhoods (LaDeau et al. 2013). For instance, rubbish from domestic animals, garbage dumps, sewage outfalls, and drainage ditches have provided a suitable habitat for a great variety of arthropod species for many decades (Tischler 1973; see Fig. 14.1 for a theoretical approach to tackle these multifactorial interactions in urban sites and in environments surrounding cities).

In cities, some mosquito species are favored by urbanization, especially during the dry season when surface water is otherwise scarce. From Europe to Oceania, going through Asia, some mosquito species of the genera *Culex* (Byrne and Nichols 1999), *Aedes* (Kay et al. 2000), and *Anopheles* (Overgaard et al. 2003) increased their abundance in urban areas, thus facilitating transmission success of pathogens between reservoir vertebrate hosts, or also acting as bridge vectors between infected competent-vertebrates and humans. This is particularly relevant in the case of pathogens circulating among birds that occasionally infect humans, as in the case of West Nile virus (WNV) (Hayes et al. 2005).

14.3.2 Changes in Blood-Feeding Preferences

In addition to species composition and abundance, anthropization may also affect the mosquito blood-feeding preferences, identified as a key factor modulating the amplification and transmission success of vector-borne pathogens (Kilpatrick et al. 2006). Mosquito diet represents an essential step in epidemiological studies allowing the identification of risks of transmission of vector-borne pathogens to human and other animals of both economic and conservation importance. It is known that mosquito species can feed on a large array of hosts, but they show clear feeding preferences, with some species biting mainly birds, i.e., ornithophilic species, and others preferring mammals to obtain their blood meals, i.e., mammophilic species (Santiago-Alarcon et al. 2012a; Takken and Verhulst 2013). Consequently, different feeding behaviors will determine contact rates between mosquitoes and infected/susceptible hosts, thus affecting pathogen dynamics (Kilpatrick et al. 2006; Muñoz et al. 2012). Therefore, some mosquito species may facilitate the transmission of pathogens between reservoir hosts, particularly relevant in the case of pathogens circulating among birds, which occasionally infect humans (e.g., WNV). For example, for the case of southern Spain, there was a characterization of the feeding patterns of the most common mosquito species potentially affecting the transmission dynamics of vector-borne pathogens (e.g., *Anopheles atroparvus*, *Ochlerotatus caspius*, *Culex modestus*, *Culex pipiens*, *Culex perexiguus*, and *Culex theileri*; Martínez-de la Puente et al. 2018a). In this study, there were clear interspecific differences in the feeding patterns of mosquitoes, describing the mammophilic behavior of *An. atroparvus*, *Cx. theileri*, and *Oc. caspius*; and the ornithophilic ones of *Cx. perexiguus, Cx. pipiens*, and *Cx. modestus*. This study revealed the importance of those species feeding mainly on birds that could disproportionally contribute to the amplification of disease such as WNV. Moreover, the mosquito species with an opportunistic behavior frequently fed on mammals and birds, such opportunistic species can play a key role in the transmission of such diseases to humans and horses. However, the differential feeding behavior of mosquito species is also dependent on the availability of potential hosts in the area, as supported by Muñoz et al. (2012) who found that differences in mosquito feeding patterns were significantly explained by both the mosquito species and the sampling locality. Furthermore, mosquitoes also showed clear feeding preferences on particular host species. Studies that integrate knowledge about the origin of mosquito blood meal with information on the host densities present in an area allows identifying both preferred and avoided vertebrate species. For instance, in a study from North Italy, by combining information between avian community with mosquito blood meal identification, authors showed how eurasian blackbirds (*Turdus merula*) and magpies (*Pica pica*) were preferred by insect vectors, while common starlings (*Sturnus vulgaris*) and rock doves (*Columba livia*) were bitten less frequently than expected according to their relative abundance (Rizzoli et al. 2015). Overall, several host variables can influence mosquito selection among species, including factors such as host phylogenetic relationships and phenotypic or behavioral traits (VanderWaal and Ezenwa 2016;

Yan et al. 2017). Obviously, temporal dynamics of host populations may affect the feeding patterns of mosquitoes and, consequently, the transmission dynamics of the pathogens that they are able to transmit. For example, the feeding patterns of *Cx. pipiens* changed seasonally in USA, with mosquitoes feeding mainly on American robins (*Turdus migratorius*) from May to June, and on humans and other mammals from July through September (Kilpatrick et al. 2006). Curiously, American robins represent less than 4% of the available hosts in the area and the disappearance from the environment of this preferred bird species due to migration coincides with the change of mosquito diet. Such changes in seasonal mosquito diet overlap with peaks of human WNV cases, determining the occurrence of epidemic outbreaks of mosquito-borne virus. Thus, knowledge on host preferences of insect vectors is essential to identifying key species and quantifying the risk of pathogen amplification and transmission by mosquitoes to humans or other species of interest. This is also the case of other insect vectors such as *Culicoides* (i.e., biting midges, Diptera: Ceratopogonidae; see Chap. 5 for an in-depth treatment of Diptera families of medical and veterinary importance) where infected vectors sucking blood from humans also feed on other vertebrate animals, thus representing a potential bridge vector of pathogens between wildlife and people (Santiago-Alarcon et al. 2012b). However, despite the importance of the availability of food resources, studies on mosquito feeding preferences, with few exceptions, suffer from the substantial limitation of not considering the availability (density and/or relative abundance) of hosts present in the local community (see Santiago-Alarcon et al. 2012a and Chap. 6 for a thorough review on current Diptera vector feeding preferences with emphasis on tropical regions).

14.3.3 Habitat and Host Specificity of Culex pipiens *Subspecies (*pipiens and molestus)

Differential ecological requirements have been reported within mosquito species. This is the case of *Cx. pipiens*, a worldwide spread mosquito species, which includes two different forms: the molestus and the pipiens that frequently hybridize (Vinogradova 2000; Martínez-de la Puente et al. 2016). Following their differential habitat requirements, the molestus biotype is able to mate in confined spaces (stenogamy) and can lay their eggs without a previous blood meal (autogeny), while mosquitoes of the pipiens form prefer open environments for mating and require a blood meal for oviposition (Fonseca et al. 2004). Overall, in North European countries, the molestus form prefers living in urban and underground environments, such as those in the London underground railway tunnels (Byrne and Nichols 1999) while mosquitoes of the pipiens form are mainly present in above ground and in natural large breeding habitats (Vinogradova 2003; Fonseca et al. 2004). Otherwise, in countries of the Mediterranean basin, mosquitoes of the two forms are sympatric, and hybrids are frequently found (Amraoui et al. 2012; Gomes et al. 2013; Krida et al. 2015; Di Luca et al. 2016). Hybridization has been also reported under natural

conditions in countries such as the Netherlands (Reusken et al. 2010), UK (Danabalan et al. 2012), Germany (Rudolf et al. 2013), Portugal (Osório et al. 2014), Austria (Zittra et al. 2016), Italy (Di Luca et al. 2016), Spain (Martínez-de la Puente et al. 2016), and Tunisia (Beji et al. 2017). Finally, in the United States, hybrids between these forms are ubiquitous (for a review, see Fonseca et al. 2004). Interestingly, under an epidemiological perspective, in addition to the living habitat differences between *Cx. pipiens* forms, it has been proposed that molestus form feeds mainly on mammals while pipiens prefers birds (Osório et al. 2014; Fritz et al. 2015), while hybrids may have an intermediate feeding behavior, potentially playing a role in the transmission of pathogens between humans and birds (Fritz et al. 2015). Nevertheless, a study conducted in Spain did not find significant differences in the feeding patterns of *Cx. pipiens* forms, with birds dominating the diet of the forms molestus, pipiens, and their hybrids. Consequently, a similar contact rate between mosquito forms and bird vector-borne pathogens was reported (Martínez-de la Puente et al. 2018b).

14.3.4 An Emblematic Case of an Urban Species: The Asian Tiger Mosquito

Environmental anthropization of the landscape often impact mosquito community composition and potentially alters the transmission rate of vector-borne pathogens (Ferraguti et al. 2016). For example, the spread of invasive mosquitoes and the pathogens they transmit has become a global health concern (Kraemer et al. 2015). In this context, understanding the global invasion of the Asian tiger mosquito, *Aedes albopictus* represents an important public health challenge (Bonizzoni et al. 2013). The *Ae. albopictus* was originally considered a rural mosquito in its native distribution in Southeast Asia due to its preference to breed in natural habitats and its occurrence at forest edges (Higa 2011). However, during the last decades the species has dramatically spread its range to different continents including Africa, America, Europe, and Australia, establishing nowadays stable populations in most of the Mediterranean basin, being the most widespread species in suburban and urban environments (Bonizzoni et al. 2013). As a result, the Asian tiger mosquito has been catalogued as one of the world's 100 most invasive alien species (Lowe et al. 2000), representing one of the major threats to public health (Medlock et al. 2012).

The success of this invasive mosquitoes lies in their ability to use small temporal water reservoirs for their larvae development, which are common in urbanized areas (e.g., pots, sewers, tires). Therefore, they are easily transported by the trade of used ornamental plants and tires or by the passive transportation in cars (Roche et al. 2015; Eritja et al. 2017). In invaded areas, *Ae. albopictus* females prefer to feed on humans (Faraji et al. 2014; Martínez-de la Puente et al. 2015). Even so, domestic animals (e.g., dogs and cats) and wildlife are also common hosts of *Ae. albopictus*,

but bird blood meals are usually avoided (Martínez-de la Puente et al. 2015). However, in natural areas where a high density of birds can be found, the percentage of avian-derived blood meals could reach up to 70.0% (Hess et al. 1968). Based on preferences of *Ae. albopictus* to feed on humans, and given the low representation of bird blood in their meals, the importance of this mosquito species for the transmission of pathogens circulating between birds and mammals can be considered low (Martínez-de la Puente et al. 2015). Nevertheless, under a global change scenario, this mosquito species may represent a key potential vector for the transmission of human pathogens such as Chikungunya virus (Paupy et al. 2010), Dengue virus (La Ruche et al. 2010; Paupy et al. 2010), Zika virus (Grard et al. 2014; Gutiérrez-López et al. 2019), and *Dirofilaria* worms (Cancrini et al. 2003; Gratz 2004), then requiring further studies.

14.3.5 Conclusion

To sum up, the study of vector feeding preferences through blood meal analyses is of great help to comprehend how the presence of invasive mosquitoes may affect the local transmission of pathogens with medical and veterinary relevance. Indeed, the presence of a highly competent vector such as *Ae. albopictus* in dense urban areas combined with its highly anthropophilic behavior can amplify its potential negative effect on public health. Moreover, it is important to remember that the establishment of an exotic vector species can create new epidemiological and epizootiological scenarios in the invaded range, with important ecological and health consequences for humans, wildlife, and domestic animals.

14.4 Future Research Directions

The effects of habitat disturbance on avian infections are not totally clear, since many biotic and abiotic factors are involved in parasite transmission, which create contrasting responses. Current studies in the tropics have mainly considered two contrasting habitat conditions (e.g., undisturbed vs. disturbed), leaving aside a variety of intermediate habitats (see Table 14.1 for a synthesis of studies on avian malaria and habitat disturbance in the tropics). Future research should focus on the identification of those biotic and abiotic factors explaining parasitological parameters in a gradient of disturbance, from undisturbed (or well preserved) to urban areas, considering the urbanization grade as the percentage of gray or green along the gradient.

Within cities, it is critical to conduct studies that take into account both the heterogeneity of the urban environment (e.g., greenspaces, permanent water bodies,

buildings, roads with tree lines, number of vehicles, heat islands) and the assemblage of urban species (i.e., urban dwellers, utilizers, avoiders; *sensu* Fischer et al. 2015). This will allow the understanding of how anthropogenic activities modify the ecological dynamics of host–vector–parasite interactions. Within the urban ecosystem, parks and natural reserves (greenspaces) may act as biodiversity reservoirs, mainly for native species not adapted to built areas, and (i) serve for conservation of vulnerable species at different functional levels (e.g., host, parasites, vectors, reservoirs, dead-end hosts), (ii) provide suitable habitats for urban utilizers and urban avoiders, and (iii) contribute to a better host condition and health. In addition, it is important to determine if parasite exchanges occur among the different kinds of urban organisms (i.e., urban dwellers, utilizers, avoiders) to evaluate the risk of emergent diseases from exotic species (both vertebrate hosts and insect vectors) into native ones.

Given that responses of parasite population parameters (i.e., prevalence, parasitemia, aggregation) to environmental changes largely depend on the identity of species involved in the interactions, it is essential to identify host species (i) sensitive to infections (e.g., high mortality and morbidity) in order to develop prevention and conservation actions or (ii) tolerant to infections, because they can act as reservoirs and superspreaders of virulent pathogens. Most studies that include habitats of different disturbance levels focus only on the prevalence of avian haemosporidians, leaving aside their parasitemia. Even though high parasitemia is often associated with an early infection, taking into account parasitemia would considerably improve our understanding of the host's ability to fight infections as a function of habitat types. It is important to identify those sites where higher parasitemias are expected, given that such host populations may suffer more severe consequences in terms of health, which under unfavorable habitat and climatic conditions can undergo population size reductions, and even face local extinctions.

Finally, in terms of studies of avian haemosporidians and land use changes, there is currently little research in the tropical areas of Asia and northern Australia (i.e., Queensland). Southeast Asia is undergoing high rates of habitat destruction and modification and many novel emerging diseases have derived from this region (Aguirre et al. 2012); therefore, it is of outmost importance to implement studies in the region addressing how habitat modification alters host–vector–parasite dynamics.

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Chapter 15
The Role of Malaria Parasites in Invasion Biology

Alfonso Marzal and Luz Garcia-Longoria

Abstract Invasive organisms are non-indigenous species that are introduced outside its natural range, where they expand and establish. These non-indigenous species colonize new habitats and undergo rapid proliferation, imposing severe ecological and health impacts on invaded ecosystems. However, not all the translocated species beyond their native range become successful invaders and, hence, particular features may allow some alien species to establish and spread in the new geographical range. Some evolutionary hypotheses have proposed that pathogens may play an important role in this context, explaining the invasion success of their hosts. Since bird species harbor a wide variety of malaria and related haemosporidian parasites, the bird-malaria interaction represents an ideal model to test these evolutionary hypotheses. The *Enemy Release Hypothesis* proposes that invasive species are more competitive in newly colonized habitats because they left behind their natural parasites in their original geographic range. Alternatively, the *Novel Weapon Hypothesis* builds on the idea that parasites are co-transported by the invasive species into newly colonized habitats, and these parasites can act as biological weapons if they infect and harm native competitors. Conversely, the *Biotic Resistance Hypothesis* claims that parasites from native community may reduce the fitness of potential invasive species and hence prevent their colonization in the new environments. In this chapter we will review the main scientific contributions showing the role of avian malaria parasites in the global spread of their bird hosts, assessing the features of both bird hosts and malaria parasites to become a fruitful invader. We will also highlight the extinctions and endangerment of numerous native species provoked by avian malaria invasions globally.

A. Marzal (✉)
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain

L. Garcia-Longoria
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain

Molecular Ecology and Evolution Lab, Department of Biology, Lund University, Lund, Sweden

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15.1 Introduction

Exotic, alternatively called non-indigenous, alien, or non-native species, are defined as those species that colonize an area beyond their natural geographic range, where they reproduce and establish a population (see review in Blackburn and Ewen 2014; Table 15.1). These alien species constitute one of the most important threats to biodiversity, imposing severe ecological and health impacts (Ricciardi et al. 2013; Jeschke et al. 2014; Tsiamis et al. 2016). The invasive species negatively affect biodiversity through competition and displacement of indigenous species, and eventually causing extinctions of these native species. Also, the introduction of pathogens coming along with domestic and wildlife exotic species may provoke emerging diseases with tremendous costs in terms of global health. For example, some studies have shown that zoonosis is linked to birds spreading diseases to humans. In this sense, it is thought that the West Nile virus, a bird pathogen causing encephalitis and mortality to humans, was introduced in New York by migratory or invasive bird species in 1999 (Calisher 2000). Also, the avian influenza viruses (H5N1), which have been transported by invasive bird species, provoked 861 cases and 455 human fatalities worldwide between 2003 and 2019 (WHO 2019). In addition to these biodiversity and health impacts, invasive species may also entail tremendous costs in

Table 15.1 Key terms in biological invasions

Term	Definition
Alien (exotic) species	A species living outside its native distributional range by human assistance (either deliberate or accidental)
Native species	A species living within its natural range including the area which it can reach and occupy as a result of only natural processes
Introduced species	Alien species that has been transported outside its distribution range
Established species	Alien species that has been successfully translocated into new regions and set up a viable population
Invasive species	Exotic species that has been introduced outside its native distribution, establish a viable population, and afterward spread with harmful effects on native species
Co-introduced parasites	An exotic parasite that has been transported by an alien host species beyond its original range
Co-invasive parasites	A co-introduced parasite that has been able to switch to native hosts and spread in the new range
Emerging infectious diseases	Infections that have newly appeared in a population, or those who are rapidly increasing in incidence or geographic range or could upsurge in the near future

terms of economic expenses. For example, the annual expenses estimated by European Union on damages provoked by invasive species exceed €12 billion (Kettunen et al. 2009).

Because of the health, economic, and ecological importance of invasive species, biological invasions have captured the attention of the scientific community during the last decades. Despite the efforts from scientists to understand and control biological invasions, there is an increasing number of intentionally and unintentionally introduced alien species (Essl et al. 2015; Roques et al. 2016), and the mechanisms that allow one species to become invasive are still poorly understood. The ecological theory of ten rules states that there is a filtering process for alien species during invasion process (escaping, establishing, and becoming a pest) (Fig. 15.1; Table 15.1). This theory predicts that of 100 imported species or individuals introduced or escaped into an environment, about 10 of these introductions become established, and of these only 1 will become an invader (Williamson and Fitter 1996; Jarić and Cvijanović 2012). The question arising is: which features cause an individual/species to become a successful invader?

The identification of factors explaining establishment and spread of alien species has become a crucial issue to identify invasion risk and design interventions. For example, it has been proposed that some ecological attributes, such as colonial nestlings, behavioral flexibility and feeding preferences (e.g., granivorous) may promote survival and reproductive success of some exotic bird species in new areas and become successful invaders (Sodhi 2010). Also, introduced individuals may face new parasites and pathogens in the new areas during the colonization process. It has been predicted that native pathogens in the new environments may decrease survival or induce costly immune responses in introduced naïve hosts, thus reducing the fitness of potential colonizers and preventing their spread and establishment (*Biotic Resistance Hypothesis*, Elton 1958; Table 15.1). With the aim to successfully counteract the challenge from new pathogens, an increased investment in immune defences may allow colonizers to combat these new pathogens. Therefore, individuals with better immune defences may enjoy better invasion success (*Invasive Immunity Hypothesis*, Lee and Klasing 2004). This hypothesis has been surveyed in a study examining spleen size in house sparrows (*Passer domesticus*) in Kenya along their invasive range (Martin et al. 2014). They showed that Kenyan house sparrows had larger spleens near the range edge than sparrows from Mombasa, where they were originally introduced in approximately 1950. These results suggest that sparrows at the expansion front, where they are more exposed to novel parasites, may be more immunocompetent. More recently, Marzal et al. (2018) explored the size of the uropygial gland and the antimicrobial activity of its secretions in house sparrows from Spain and Peru to explore whether these defence mechanisms may have facilitated the invasion of individual sparrows into South America. Their results showed that Peruvian sparrows had larger uropygial glands and higher antibacterial activity in its secretion than sparrows from Spain, thus suggesting that defensive traits may favor sparrows to colonize and spread in the new environments.

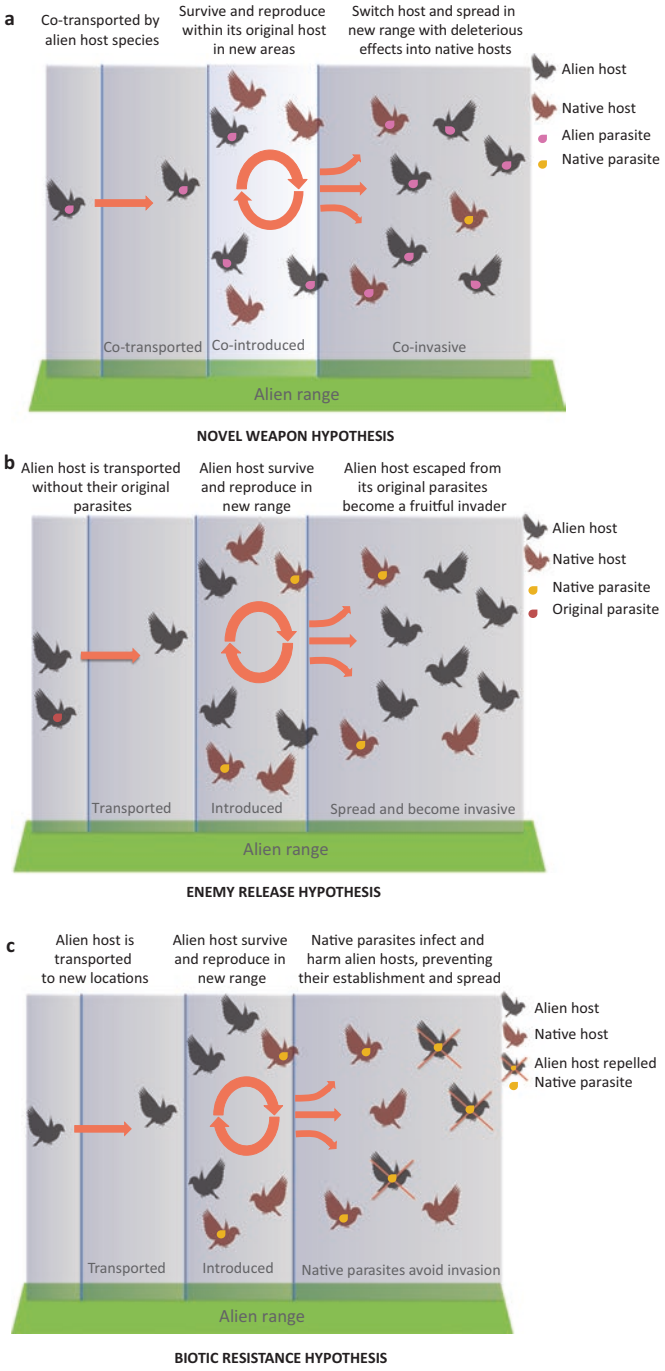

Fig. 15.1 Illustrations showing the role of parasites in invasion and co-invasions of alien species. The rectangular blueish area represents a new area outside the natural range of exotic species. Alien bird species are shown in light black, while brown figures represent native bird species.

Parasites have also been proposed to play a key role on biological invasions, facilitating or limiting colonization and spread of their hosts in new continents and on islands (Perkins et al. 2008; Tompkins et al. 2011). In this sense, it has been suggested that parasites may mediate the biological invasions at any of the three phases of introduction, establishment, or spread (Lymbery et al. 2014). Three major hypotheses have been suggested to explain the role of parasites in facilitating the successful colonization of their hosts. The *Novel Weapon Hypotheses* (hereafter, *NWH*) rely on the idea that invasive species can have a competitive advantage against native species in the exotic environment because they possess unique parasites to which naïve natives have not adapted (Fig. 15.1; Table 15.1). Thus, exotic species can act as a sort of "Trojan horse" bringing alien parasites and pathogens inside them. Once in the new environments, these co-transported parasites may switch to native host species and spread in the new communities, hence becoming themselves invasive parasites and provoking population declines in native species (Callaway and Ridenour 2004; Prenter et al. 2004). Conversely, parasites may also prevent the establishment and dispersal of introduced hosts in new environments. Following this idea, the above-mentioned *Biotic Resistance Hypothesis* (hereafter, *BRH*) (also known as *Diversity-Invasibility Hypothesis*) describes that native communities are able to repel invasive species through a combination of processes of their native fauna (e.g., predation, competition and parasitism; Fig. 15.1; Table 15.1) (Elton 1958; see Chap. 11 for an explanation of avian haemosporidian parasite dispersal and host specialization).

NWH and *BRH* represent two ways in which parasites influence competition between native and invasive species by causing differential mortality of one competitor. But the role of parasites on biological invasions may extend beyond the direct effects of parasites on hosts into new areas, and hence indirect effects on species may also be expected (Hatcher et al. 2012). Following this idea, the Enemy Release Hypothesis (hereafter *ERH*) states that exotic species become successfully established because they have lost their natural parasites during the process of colonization (Keane and Crawley 2002; Colautti et al. 2004; Fig. 15.1; Table 15.1). Because the loss of parasites may positively affect some host traits such as reproduction and survival, the competitive ability of exotic species may increase and thus displace native species in the new areas (*Evolution of Increased Competitive Ability Hypothesis*; Blossey and Nötzold 1995).

Fig. 15.1 (continued) Arrows indicate movement between each step in the process: introduction, establishment and (if proceed) invasion of the native habitat. (**a**) Schematic diagram of the *Novel Weapon Hypothesis*. Alien parasite (in pink) is co-transported and co-introduced by its original alien host, switch into new native hosts provoking harmful effects on them. (**b**) Schematic diagram of the *Enemy Release Hypothesis*. Alien host is transported beyond its natural range without its original parasite (in red). Native parasites (in yellow) do not harm alien host. The escape from its own original parasite facilitates the invasion in the new range. (**c**) Schematic diagram of the *Biotic Resistance Hypothesis*. Alien host is transported and introduced into new range. Native parasite (in yellow) infects and harm alien host, thus preventing the biological invasion in the new range. (These figures are based on figure 1 from Lymbery et al. 2014)

Malaria and related haemosporidian parasites are among the most pathogenic species of poultry and wildlife birds. They are widespread and harmful organisms (Valkiūnas 2005), provoking negative effects on the life history of their avian hosts by reducing survival (Martínez de la Puente et al. 2010; Marzal et al. 2016), reproductive success (Merino et al. 2000; Marzal et al. 2005, 2008) and body condition (Valkiūnas et al. 2006; Palinauskas et al. 2008; see Chap. 17). Avian malaria parasites are responsible for economic losses, mass mortality, population declines and even extinctions of many bird species worldwide after their emergence outside their native range. For these reasons, the International Union for Conservation of Nature (IUCN) considers some avian malaria species within the 100 world's worst invasive species (Lowe et al. 2000). In this chapter we will recapitulate the main scientific contributions explaining the importance of malaria parasites on the global invasions of their avian hosts. We will also review some examples of extinctions and endangerment of native species as a consequence of the pathogenicity of invasive avian malaria.

15.2 The Novel Weapon Hypotheses: Avian Malaria as an Emerging Infectious Disease

Co-transported parasites by exotic hosts are expected to be relatively benign to these introduced hosts, because otherwise these alien hosts probably would die in transit (Strauss et al. 2012). Therefore, if co-transported parasites can switch into native hosts species and infect them in the new environment, then these co-transported parasites become co-invasive parasites and act as novel weapons, representing the origin of emerging infectious diseases (EIDs) (Daszak et al. 2000).

In this scenario (invasive parasites and EIDs) occurs a large number of novel and evolutionary untested host-parasite-vector interactions. In such first-time interactions, theory predicts that the virulence of parasites will be low in co-evolved hosts, but high in naïve hosts in the new areas because of lack of evolved protective immunity (Schmid-Hempel 2011). Thus, a high rate of fatalities provoked by invasive pathogens is expected among naïve native hosts (e.g., aggressive symbiosis hypothesis; Ryan 2009). In support of this prediction, Lymbery et al. (2014) compared the pathogenic effect of 16 co-introduced parasites that switched to native hosts, estimating that 85% of these co-introduced parasites showed higher relative virulence in native hosts than in the co-introduced alien host. An example of this phenomenon is the reduction of indigenous populations by diseases brought by European colonizers into the Americas, such as measles, influenza, typhus, bubonic plague and smallpox (Diamond 1997). Dramatically, it is estimated that more than 90% of native populations in the Americas died because of these endemic European diseases in just a few hundred years (Mann 2005).

Avian malaria parasites (genus *Plasmodium*) provide an excellent model system to study invasive pathogens from the concepts of EIDs. These pathogens are globally distributed, including several hundred species infecting many taxa of birds, and

being responsible of biodiversity losses worldwide. *Plasmodium relictum* is among the most pathogenic species of bird malaria (Martínez-de la Puente et al. 2020). It has been documented that this malaria parasite might easily switch to new hosts as they spread into new areas (Beadell et al. 2006; Hellgren et al. 2009), provoking mass mortality, population declines and even extinctions of many bird species in association with other anthropogenic impacts such as habitat destruction (Van Riper et al. 1986; Valkiūnas 2005). Next we will describe some features of parasites increasing their likelihood to successfully establish and spread in new environments, as well as some studies illustrating the dramatic outcomes of *P. relictum* infection after its introduction outside its native range.

15.2.1 Attributes of Exotic Invasive Parasites

Parasites are the most abundant and widespread organisms in the world, constituting around 50% of all species (Dobson et al. 2008). However, not all co-transported parasites successfully spread and establish in new environments. Actually, theory predicts a range of about 5–20% of translocated parasites to establish and spread in the invaded areas (Jeschke 2014). Therefore, some parasite characteristics would increase the probabilities to become a fruitful invader. The assessment of these drivers in haemosporidian parasites facilitating biological invasions would be essential to identify potential invaders and design interventions.

Ewen et al. (2012) explored the traits that enabled the successful establishment of exotic avian malaria parasites (*Plasmodium* spp.) in New Zealand. According to their prediction, they found that *Plasmodium* parasites that fruitfully invaded New Zealand were more globally generalist (were recorded from most host species and were also more widely distributed outside New Zealand) than avian malaria parasites not co-introduced to New Zealand. They also found that haemosporidian parasites introduced to New Zealand showed higher prevalence in their native range in Europe than malaria parasites found infecting birds in Europe but not in New Zealand. These features could increase the chances of parasite transmission, since it would be easier to find susceptible hosts to infect in the new location. In agreement with these ideas, Garcia-Longoria et al. (2019) have recently analyzed infection status and host range of haemosporidian parasites in a bird community of Southern Europe, showing that generalist parasites (with a broader host range) usually display higher prevalence of infection. Moreover, it is predicted that a parasite showing lower virulence in their original hosts than in naïve hosts in the new areas would promote the establishment of its original hosts, and hence would have greater opportunities to become a successful invader in the new environments (Schmid-Hempel 2011; Blackburn and Ewen 2017). Therefore, it is expected that alien invasive parasites should have relatively high prevalence and low virulence in their native hosts, because they must be present in the small number of the invasive founder population. The third prediction assumes that exotic parasites should have suitable competent vectors in the new area to allow parasite transmission. In the case of invasive

Plasmodium in New Zealand, Ewen et al. (2012) pointed out evidence of populations of both native and exotic mosquito species (*Culex quinquefasciatus*) suitable for *Plasmodium* lineages transmission in New Zealand (Tompkins and Gleeson 2006; Massey et al. 2007). Finally, exotic haemosporidian parasites require the presence of suitable bird hosts in the new range to complete their asexual life cycle and produce viable gametocytes circulating in the peripheral blood in order to spread (see Chaps. 2, 5, and 6 for a thorough treatment to the life cycle of avian haemosporidian parasites). In this sense, experimental investigations have demonstrated that not all bird species are susceptible hosts for *P. relictum*, probably because the development of this parasite might be abortive in such species (Dimitrov et al. 2015; Valkiūnas et al. 2018).

Summarizing, more widespread and abundant haemosporidian parasites (generalist parasites with high prevalence) are more likely to be successfully co-transported with their original hosts. In addition, a lower parasite virulence of these haemosporidian parasites in the co-introduced host species would be desirable to avoid failing during transit. Moreover, with the aim to establish a viable population and spread in the new environments following translocation, suitable vectors and susceptible naïve hosts are required. All these features would increase the risk of alien haemosporidians to become successful invaders.

15.2.2 Invasive Malaria Parasites Provoking Emerging Infectious Diseases

15.2.2.1 Avian Malaria Outbreaks in Hawaiian Islands

For the outbreak of any vector-borne diseases, the right assemblage of different elements is needed: a source of infected individuals, a group of suitable vectors to transmit the parasite and a naïve host community to establish and spread the parasite. Hawaiian Islands were discovered by Captain Cook in 1774 (Obeyesekere 1994). This archipelago includes hundreds of islands with a considerable high number of endemic species, thus representing an important source of biodiversity (Carlquist 1980). Most probably avian malaria parasites have been present in Hawaii for hundreds of years arriving via migratory birds that annually visit the islands. However, native bird communities were free from avian malaria, probably because of the absence of suitable vectors transmitting the disease.

The nineteenth century came along with the growth of the international commercial trading. This increased the movements of ships among islands and continents, which in turn boosted the opportunities for worldwide spread of parasites and pathogens. In 1826, the shipping vessel HMS Wellington arrived at the Hawaiian Islands coming from Mexico (LaPointe et al. 2005; Beadell et al. 2006). It is thought that in the ballast water of ships, there were larvae and eggs of the mosquito *Culex quinquefasciatus*, the primary vector of avian malaria. With the aim of refilling the ship's water kegs with freshwater, the crew of the ship dumped the mosquito-laden remnants of their water casks into a Hawaiian stream (Leighton 2002). Since then,

populations of avian malaria vectors established and spread in Hawaii, representing the first and one of the most serious insect pests arriving at Hawaii. Many endemic Hawaiian bird species are highly susceptible to avian malaria (Atkinson et al. 2005), but we still need to know the origin of the third element for the right assemblage leading to the avian malaria outbreak: the source of invasive avian malaria parasites. It has been suggested that reservoirs of avian malaria parasites started with the co-transport of exotic host species during colonial times. Additionally, it has been proposed that thousands of ducks and shorebirds which annually migrate to Hawaii from the Arctic may act as natural reservoirs (Beadell et al. 2006). Regardless of their origin, in 1941 six species of *Plasmodium* spp. were recorded for the first time from endemic and exotic birds in Hawaii (see review in Laird and van Riper 1981). Since theory predicts that alien parasites should have minimal impact on co-evolved non-native birds (Schmid-Hempel 2011), these exotic species may act as effective reservoirs for avian malaria (Atkinson et al. 2005; Atkinson and Samuel 2010). In this line, van Riper et al. (1986) conducted laboratory and field experiments in 16 different areas of the Hawaiian Islands to examine the pathogenicity of avian malaria on forest birds. As expected, they showed that native forest birds were more susceptible to malaria parasites than introduced species, concluding that avian malaria was partly responsible for the population decline and extinctions of some native bird species during previous years. After the successful establishment of exotic avian malaria and its vector in the archipelago (around 1920s), more than a half of over 100 endemic bird taxa in Hawaii have been driven to extinction by a combination of habitat loss, introduced species and diseases (LaPointe 2000).

Also, *Plasmodium* parasites may act as a barrier to the movement of the native bird species (Dobson and Hudson 1986), thus leading to a reduction in habitat use by endemic bird species. The optimal breeding grounds of many native bird species are at lowlands; the problem is that *Culex* mosquitoes are also abundant at such places and the fitness of many native birds breeding at their optimal habitats may decrease because of the harmful effects of avian malaria. Hence, these native bird species may be displaced to sub-optimal habitats at higher elevations, where food and cover may be scarce. This situation has generated population declines and restriction of the distribution of many susceptible Hawaiian bird species (Samuel et al. 2011). Currently, it has been documented that the prevalence of *Plasmodium* spp. in Hawaiian forest birds at 1900 m asl has more than doubled over a decade, revealing the fast spread of avian malaria (Freed et al. 2005). On the positive side is the fact that some avian endemics are developing resistance and/or tolerance to avian malaria, which is allowing them to recolonize the lowlands (e.g., Woodworth et al. 2005).

15.2.2.2 Avian Malaria Outbreaks in New Zealand

New Zealand was one of the last lands to be colonized by humans (Higham et al. 1999). Because of this historic and geographical isolation, New Zealand had a large number of endemic fauna (Daugherty et al. 1993). However, since its human colonization in about 1280–1300 AD (Wilmshurst et al. 2008), many different exotic

species have settled in New Zealand facilitating the spread of novel parasites such as some species of avian malaria, resulting in disease outbreaks that threaten native avifauna (Tompkins and Gleeson 2006). In the earliest years of the twentieth century, Doré (1920) documented the first records of avian malaria in New Zealand via imported bird species. Thirty years later, Laird (1950) described blood stages of haemosporidian infections in introduced birds, though he was unable to find any evidence of malaria infection in endemic birds from New Zealand. However, he was the first to suggest the likelihood of alien avian malaria as a causative agent of local native bird extinctions, and he recommended further studies on bird blood parasites given the small sample size of his study. In 1970s, Fallis et al. (1976) analyzed blood samples from more than 40 native and exotic bird species, but he did not find any evidence of avian malaria infection in the blood smears, perhaps also due the low sample size of 1–10 samples per bird species and possibly also to low parasitemias undetectable by microscopy.

In contrast, more recent studies have found avian malaria parasites in 35 different endemic, native, and exotic bird species in New Zealand, advising that avian malaria could be an emerging disease affecting New Zealand avifauna (Barraclough et al. 2012; Schoener et al. 2014). For example, it has been reported that avian malaria killed more than 90% of the population of the endemic yellowheads (*Mohoua ochrocepahala*) in Christchurch Wildlife Park (Derraik 2006). Similarly, five yellowheads at Orana Wildlife Park died because of *Plasmodium* spp. infection (Alleya et al. 2008). Furthermore, Howe et al. (2012) reported the death of native and alien bird species due to acute *Plasmodium* spp. infections. Likewise, Baillie et al. (2012) reported three exotic parasite lineages belonging to the morphospecies *Plasmodium (Huffia) elongatum*, *Plasmodium (Novyella) vaughani*, and *Plasmodium (Haemamoeba) relictum* infecting the endemic New Zealand passerine bellbird *Anthornis melanura*.

Most of these avian malaria parasites are not known to be endemic to New Zealand (Ewen et al. 2012; Schoener et al. 2014). Therefore, it is believed that these parasites were co-transported with their alien host species during colonial times (Thomson 1922) and arrived in New Zealand via multiple independent events (Ewen et al. 2012). Also, 12 indigenous and 4 exotic species of mosquitoes are established in New Zealand, mainly due to transport by aircrafts and ships coming from Asia and the South Pacific (Derraik 2004a). Among them, *Culex quinquefasciatus* is an exotic mosquito species that has rapidly spread across New Zealand (Tompkins and Gleeson 2006), and it is likely to be the vector responsible for some avian malaria outbreaks (Derraik 2004b).

15.2.2.3 Invasive Avian Malaria in Native Birds of Peru

Previous examples have shown that *Plasmodium* parasites are cosmopolitan pathogens responsible for mass mortality, population declines, and even extinctions of many bird species after its establishment beyond their native range (van Riper et al. 1986; Valkiūnas 2005). Among the diversity of avian malaria species, *Plasmodium*

relictum is a highly invasive and globally distributed species with extraordinary virulence (LaPointe et al. 2005; Valkiūnas 2005; Beadell et al. 2006; Palinauskas et al. 2008; Marzal et al. 2011; Martínez-de la Puente et al. 2020).

Studies analyzing the genetic variation of *P. relictum* have revealed a huge genetic diversity of this pathogen, with distinctive mitochondrial haplotypes (Hellgren et al. 2015). These different parasites lineages also showed significant differences in their global distribution and areas of transmission. For example, *P. relictum* GRW4, the parasite lineage responsible for massive bird mortalities reported in Hawaii, has a wide geographical range including Africa, Asia, New Zealand, and the Americas (Beadell et al. 2006; Marzal et al. 2011). It is closely related to lineage *P. relictum* SGS1, which is an abundant and actively transmitted parasite lineage in Europe, Africa, Asia, and Europe (Palinauskas et al. 2007), though it has also been recorded infecting native and indigenous birds in Oceania (Howe et al. 2012). Both parasites lineages show a vast invasive potential, since they can easily switch to new hosts as they spread into new areas (Beadell et al. 2006; Hellgren et al. 2009). *P. relictum* lineage SGS1 has been reported in over 70 species of birds, but until very recently this invasive lineage had not been reported in the mainland Americas (Beadell et al. 2006; Durrant et al. 2006; Merino et al. 2008; Marzal et al. 2011; Lacorte et al. 2013).

Marzal et al. (2015) documented the first report of *P. relictum* SGS1 in the mainland Americas. They examined 102 blood samples from 18 native bird species from two different areas of Peru (Lima and Huanuco) searching for haemosporidian infections. They identified five *Haemoproteus* lineages and five *Plasmodium* lineages infecting 12 different Neotropical bird species. Invasive *P. relictum* SGS1 was the most generalist and the most prevalent parasite lineage in the study, infecting 13 individuals from eight host species (almost 40 % of the total infections). Moreover, SGS1 was also a geographically widespread parasite, being the only *Plasmodium* lineage infecting birds in both study areas. The presence of the invasive avian malaria parasite *P. relictum* SGS1 in the Americas has been more recently confirmed by Turcotte et al. (2018) analyzing the prevalence and lineage diversity of haemosporidian parasites in a population of tree swallows (*Tachycineta bicolor*) living in an agricultural landscape in southern Québec, Canada.

15.2.2.4 Studies of Avian Malaria in the Galapagos Islands

The Galapagos Archipelago is a remote group of volcanic islands with unique sets of environmental conditions and ecological isolation that have facilitated the development of high levels of endemism (Gibbs et al. 1999). Since the international recognition after the publication in 1859 of Darwin's book *On the Origin of Species*, the native ecosystems on the Galapagos Islands are suffering massive destruction as a result of the introduction of alien species (feral goats, pigs, dogs, cats, and rats, among others) that have altered ecosystem characteristics and driven endemic species, such as several species of the Galapagos tortoise, to extinction (Schofield

1989; Mauchamp 1997). Fortunately, there have been no extinctions of avian endemics in the Galapagos despite anthropogenic factors (e.g., urbanization, domestic species), but some species are endangered and in need of immediate protection (Parker 2018).

Endemic populations of islands usually suffer more from introduced pathogens than mainland populations (Fromont et al. 2001). Because of their isolation and high endemism, island species usually exhibit weaker immune systems (Matson and Beadell 2010; Lobato et al. 2017), which could contribute to the susceptibility of the population to infectious disease. For example, Galapagos penguins exhibit low levels of genetic diversity (Nims et al. 2008) and very low variation in major histocompatibility complex (MHC) genes (Bollmer et al. 2007). Therefore, the introduction of pathogens, which may be highly virulent for the immunologically naïve endemic birds, could be a cause of major population declines and extinctions in Galapagos avifauna (Parker 2018; see Chap. 8).

Initial microscopic and molecular studies failed to detect malaria infection in many Galapagos birds, including the Galapagos penguin (Miller et al. 2001; Travis et al. 2006). But some years later, Levin et al. (2009) presented the first evidence of a *Plasmodium* blood parasite found in the Galapagos Archipelago, detecting 5% of the 362 analyzed penguins infected with a sister lineage of *Plasmodium elongatum*, which is one of the most dangerous lineages of *Plasmodium* known to provoke severe mortality and morbidity in captive penguin populations (Fleischman et al. 1968). In addition, these *Plasmodium* infections were widely distributed across nine sites of five islands in the Archipelago. However, none of the studied penguins showed disease symptoms, and no obvious impact on their survival and reproduction was recorded. In addition, infected individuals diagnosed by polymerase chain reaction (PCR) did not show any gametocytes or other blood stages in the microscopic evaluation, and hence it is not clear whether the parasite could complete its life cycle in Galapagos penguins to ensure its transmission. Furthermore, no competent vector for *Plasmodium* was identified on the islands where penguins are located.

Later, Santiago-Alarcon et al. (2010) studied the phylogenetic relationship of *Haemoproteus* parasites in the endemic Galapagos dove (*Zenaida galapagoensis*), showing that haemosporidian parasites infecting endemic Galapagos doves were not unique to the archipelago and were widely distributed and prevalent across the islands and the Americas. These outcomes suggested multiple colonization events of haemosporidian lineages existing on the continent. More recently, Levin et al. (2013) screened more than 3700 samples of endemic birds of 22 Galapagos species for *Plasmodium* spp. They found four different genetic lineages of *Plasmodium* parasites infecting Galapagos birds (lineages A, B, C, and D). Lineage A was first described only from Galapagos penguins (Levin et al. 2009), infected more than one species at multiple locations and across multiple years, thus implying that lineage A is established and is transmitted regularly in the archipelago. On the contrary, infections by lineages B, C, and D were transient infections of parasites not established on the archipelago, and birds may be dead-end hosts for these *Plasmodium* lineages. They also revealed similarities between *Plasmodium* spp. found in Bobolinks

(*Dolichonyx oryzivorus*) with Galapagos lineages B and C, which may suggest that North American breeding Bobolinks, which regularly migrate through Galapagos, could be responsible for infecting local vectors that transmit the parasite to local birds. In this sense, the mosquito *Culex quinquefasciatus* was first reported in Galapagos in 1989, and it was well established by 2003 (Whiteman et al. 2005). However, they failed to find gametocytes in blood smears of infected individuals, and hence could not confirm that the parasite is completing its life cycle (see Parker 2018 for an in-depth summary of the avian haemosporidian work conducted in the Galapagos Islands, and see Chap. 8 for an island biogeographic analysis of avian haemosporidians).

15.3 Parasites Preventing Biological Invasions: The Biotic Resistance Hypothesis

The mechanisms involving *NWH* favoring the invasion of alien species on new areas could be also applied conversely to explain why native communities may have the ability to keep out invasive species. Thus, the *BRH* indicates that strong biotic interactions with native species may slow the progress of invaders or fully hinder their establishment and spread (Elton 1958). Bearing in mind that parasite virulence will be lower for co-evolved than for novel naïve hosts (Schmid-Hempel 2011), *BRH* states that biological invasion of alien species is prevented because native parasites have higher impact on exotics than on native hosts. Alternatively, a lack of co-evolution between co-transported parasites and the new native hosts may hinder the establishment of a parasite in the new areas because parasites may have lower fitness in the novel hosts (Dunn 2009). This is the case of abortive infections of some avian malaria parasites that are unable to complete their life cycle on some not co-evolved hosts because of an inability to infect red blood cells on these new hosts (Valkiūnas and Iezhova 2017; Valkiūnas et al. 2018). Therefore, more than explaining the success of biological invasions, *BRH* can explain why some exotic species fail to invade communities.

The effects of biotic resistance may be critically miscalculated because failed introductions are not usually seen, whereas successful invasions are mainly reported. In addition, most studies of biotic resistance in animal communities have focussed on elements of the native biota other than parasites, such as predators (Carlsson et al. 2010; MacNeil et al. 2013) or competitors (Levine et al. 2004). For example, it has been documented that a native predator, the blue crab *Callinectes sapidus*, has provided biotic resistance to invasion and prevented the spread and establishment of the introduced European green crab *Carcinus maenas* in eastern North America (Rivera et al. 2005).

As far as we know, there is no study with results clearly supporting the *BRH* in a bird-malaria system. Of course, this lack of evidence could be due to the fact that failed invasions are often not reported in scientific studies, and so this bias is not

well documented. Comparative analyses of successful introductions of sister host populations in areas with different parasite assemblages would gain some insights into this question. The best approach showing that bird haemosporidian parasites could provide biotic resistance avoiding the establishment of an alien bird species has been described by Ricklefs (2010) while studying host-pathogen co-evolution, secondary sympatry and species diversification in birds from the West Indies. Speciation process requires achieving complete reproductive isolation followed by secondary sympatry. It has been suggested that secondary sympatry might be prevented by between-population competition through co-evolved pathogens. These pathogens might have locally co-evolved with one population of hosts, but they could be harmful to sister populations (Ricklefs and Bermingham 2007; Ricklefs 2010). For example, it has been shown that some haemosporidian parasite species of *Plasmodium* and *Haemoproteus* show significant avian host species by island interactions in prevalence in the Lesser Antilles (Apanius et al. 2000; Fallon et al. 2003). These findings imply that these parasites have evolved locally to host populations, and hence local parasites could prevent the invasion of an island of sister host populations coming from other islands in the archipelago, as predicted by the *BRH* (see also Chap. 12).

15.4 Avian Malaria Parasites and the Enemy Release Hypotheses

The above studies illustrate some of the most recognized examples of each proposed hypothesis, but the role of avian malaria parasites in the biological invasions of their hosts may extend beyond these effects. Flourishing colonizers frequently harbor a reduced parasite fauna compared with their native counterparts leaving behind their natural parasites (*ERH*), either because they were absent in the arriving individuals or because they were lost during transit or after arrival (Torchin et al. 2003; MacLeod et al. 2010). Because the presence of malaria parasites decreases host fitness and requires a host investment concerning limited resources (e.g., nutrients, energy), the loss of natural enemies may result in re-allocation of resources away from these costly defences into other important biological functions (e.g., reproduction and growth). In consequence, invasive species would gain a competitive advantage allowing them to become established in the new areas.

15.4.1 Which Processes Are Driving Parasite Loss?

According to *ERH*, introduced populations may lose their native parasites during the invasion process. However, our knowledge about which processes may cause parasite loss during host introduction is still limited. In this sense, three main

processes have been proposed to explain parasite loss during colonization (Torchin and Lafferty 2009; Ewen et al. 2012). First, introduced populations normally derive from a subset of the original source population, and thus founding populations of exotic hosts may not carry the complete range of parasites found in the source location simply as a result of biased sampling (*missing the boat*) (Torchin et al. 2003; Paterson and Gray 1997; MacLeod et al. 2010). Second, if infected hosts fail to survive and persist following introduction, then their parasites will also fail (*sinking with the boat*) (MacLeod et al. 2010). Third, even if infected founding individuals can survive to establish a population, parasites may be unable to complete their life cycle in the new environment (i.e., insufficient or a complete lack of transmission among hosts owing to the absence of an intermediate host or vector) and hence could fail to persist (*drowned on arrival* or *lost overboard*) (MacLeod et al. 2010). In other words, not all co-introduced parasites become co-invasive parasites. Hence, understanding the processes that drive parasite loss in introduced populations is crucial for protecting biodiversity and designing interventions to reduce the impact of an invasive species, as well as to help conserve newly established populations of an endangered species (i.e., conservation programs of translocations of individuals to create new populations of a species).

The main problem of the majority of co-invasive parasites is the complete absence of information about founding host individuals because the establishment phase of introductions is rarely observed (Torchin and Mitchell 2004), thus making impossible to identify the mechanisms involved in biological invasions. However, conservation-driven translocations could provide a unique opportunity to analyze mechanisms involved in parasite loss after host introduction since it would allow the identification of founding individuals and the parasites co-transported with them. Likewise, longitudinal studies may allow tracking the process of parasite loss immediately after, and for several years, following translocations to investigate patterns of parasite prevalence in newly established populations.

With this aim, Fairfield et al. (2016) analyzed more than 3800 blood samples collected over 22 years to investigate the prevalence of *Haemoproteus nucleocondensus* in translocated populations of Seychelles warblers (*Acrocephalus sechellensis*), a small passerine endemic to the Seychelles in the western Indian Ocean. This species was almost extinct in the middle of twentieth century (the population size was reported to be less than 30 individuals confined to the island of Cousin; Crook 1960). From 1988 to 2011, four conservation programs aiming to preserve Seychelles warbler and population expansion successfully translocated 88 individuals from the last remaining population on Cousin Island to other islands (Aride, Cousine, Denis, and Frégate) (Komdeur 1994; Richardson et al. 2006; Wright et al. 2014). In this longitudinal study, they sampled translocated warblers to Denis and Frégate before and after translocation, showing that the prevalence of *H. nucleocondensus* decreased dramatically over time. About 50% of sampled individuals were infected with *H. nucleocondensus* before they were translocated, therefore denying the *missing the boat* hypothesis. In addition, they confirmed that infected hosts could survive following translocation, thus rejecting the *sinking with the boat* hypothesis. Finally, they stated that the lack of suitable insect vectors for

transmitting *H. nucleocondensus* on Denis and Frégate islands was probably responsible for the decrease in prevalence on the translocated areas (*drowned on arrival* or *lost overboard* hypothesis).

15.4.2 Testing the Predictions of Enemy Release Hypothesis

To evaluate the role of *ERH* in the successful establishment of invasive species, we need to test two different predictions, which are not comparable or mutually exclusive (Torchin and Lafferty 2009). One original prediction of the *ERH* is that introduced populations lack natural enemies compared to populations within their native range (Williams 1954; Elton 1958). To test the validity of this first prediction, we require a comparison of the genetic diversity of parasites within the same species across native and introduced populations. The other prediction of the *ERH* is that introduced species should gain fitness advantage in the enemy-mediated competition because they are less likely to be affected by natural enemies than their native competitors (Elton 1958; Keane and Crawley 2002). This second prediction requires a comparison of the parasite fauna, the prevalence and/or parasitemia (i.e., infection intensity or load) in populations of an introduced species with populations of one or more native species within the same community as the invader. Avian malaria and related haemosporidian parasites are known to exert detrimental effects on their host populations by decreasing host fitness (Asghar et al. 2015; Marzal et al. 2016), therefore providing an excellent host-parasite model to study the *ERH* (see Chap. 17).

To test these predictions, different studies have compared the prevalence and genetic characterization of avian haemosporidian parasites in many continents and islands. For example, Ventim et al. (2012) compared the prevalence and genetic diversity of haemosporidians (genera *Haemoproteus* and *Plasmodium*) in four exotic passerines (*Estrilda astrild*, *Amandava amandava*, *Ploceus melanocephalus,* and *Euplectes afer*) and with those of native marsh warblers and sparrows (*Acrocephalus arundinaceus*, *Acrocephalus scirpaceus*, *Cettia cetti*, *Locustella luscinioides*, *Passer domesticus*, and *Passer montanus*) in four coastal wetlands of Portugal colonized by the exotics. Exotic passerine species exhibited lower haemosporidian prevalence than native bird species. In addition, they found no evidence of exotic haemosporidian parasites established in the Portuguese study sites. Moreover, invasive bird species were infected by two local *Plasmodium* lineages (SGS1 and PADOM01). In agreement with *ERH*, their outcomes suggest that alien bird species have lost their original blood parasites when colonizing Europe, and were parasitized by blood parasites with local transmission. The importance of being released from parasites in invasive common waxbills (*Estrilda astrild*) when colonizing Southwest Europe was later confirmed by Lopes et al. (2018). They screened 617 waxbills in 23 sites in Portugal, showing that the prevalence of haemosporidians in waxbills from colonized areas was significantly lower than in native grounds.

Recently, Antonini et al. (2019) tested the *ERH* in relation to haemosporidian parasite infection (prevalence and parasitemia of *Plasmodium* spp. and *Haemoproteus* spp.) of two phylogenetically closely related species of thrushes (*Turdus leucomelas* and *T. merula*) and invasive house sparrows in Portugal and Brazil. They found that both house sparrows and thrushes in their native range (Portugal) showed a higher prevalence of haemosporidian infection than in their non-native range (Brazil). In addition, the house sparrows from their native range had higher parasitemia than sparrows from invaded areas, which is also consistent with the *ERH*.

The *ERH* also predicts that non-native hosts are less impacted by generalist parasites (e.g., resulting in lower prevalence) than native hosts (Keane and Crawley 2002). This prediction was explored by Lima et al. (2010) by examining the prevalence of *Plasmodium* spp. and *Haemoproteus* spp. in introduced house sparrows and native urban birds of central Brazil, as well as a comparison with European house sparrows. Their outcomes showed that native birds from Brazil presented significantly higher parasite prevalence. They also found that European house sparrows exhibited significantly higher parasite prevalence than introduced house sparrows from Brazil. All these results support enemy release as the most likely hypothesis, which may have been important for the success of house sparrows as an invasive exotic species.

Likewise, Marzal et al. (2011) studied the potential contributions of the *NWH* and the *ERH* to the global spread of house sparrows. They analyzed whether this invasive species left their natural parasites behind when colonizing new areas, and/or acquired new local parasites. By examining prevalence and genetic diversity of malaria and related haemosporidian parasites in 58 localities on six continents, they showed that the malaria prevalence was higher in sparrows from native populations than in sparrows from invasive populations. Moreover, house sparrows did not retain their native parasites in newly colonized regions, thus providing evidence supporting the hypothesis that the release from these natural enemies favored the global spread of sparrows during the last two centuries.

More recently, Marzal et al. (2018) explored if the variation in prevalence and genetic diversity of malaria parasites may explain the invasion of house sparrows in Peru, which was introduced in parks of Lima around 1950 (Leck 1973). Invasive house sparrows from Peru showed lower prevalence and genetic diversity of haemosporidian parasites than sparrows from their natural range (Spain). Moreover, none of the haemosporidian lineages infecting sparrows from Spain was found in sparrows from Peru. The only haemosporidian parasite found in sparrows from Peru was *Plasmodium relictum* GRW4, a wide generalist parasite that is mainly transmitted out of Europe (Hellgren et al. 2015). These patterns are in accordance with *ERH*, showing that the spread of invasive populations is accompanied by a reduction of parasite prevalence compared to conspecific populations from the native range, and by a loss of their native parasites and assimilation of generalist parasites of the newly colonized region.

Urban development has enormously increased during the last decades, entailing striking ecological changes in resource availability and habitat quality (Zhou et al. 2004; Grimm et al. 2008). Despite the fact that species of some taxa have been

dramatically reduced in highly urbanized areas (mainly urban avoiders), a small number of species (e.g., house sparrows, rock doves) have successfully colonized these urban ecosystems and adapt to them, thus occurring at higher densities in these novel man-made environments than in their original more natural habitats (MacGregor-Fors et al. 2017). But the mechanisms driving these colonization events, such as the potential role of avian haemosporidian parasites, are still poorly understood. This novel approach has been recently tested by Santiago-Alarcon et al. (2018) analyzing the role of avian malaria parasites in the successful urban invasions of house sparrows in Mexico City. By comparisons of haemosporidian prevalence between sparrows from one urban and one non-urban (agricultural) site, they showed that sparrows inhabiting highly urbanized areas of Mexico City have lower infection prevalence compared with their non-urban counterparts. This finding supports the idea that leaving behind their natural parasites might be a key factor to explain house sparrow invasiveness in urban areas.

One important issue regarding the *ERH* is that the lower prevalence found in invasive species when compared to their native ranges could be due to the lower number of these exotic species in local assemblages in the new areas (Murray and Lepschi 2004; McGill et al. 2007). This is because transmission rates depend on host population density in natural parasite communities, and hence parasite prevalence is expected to be positively related to host population density (Arneberg et al. 1998). However, very limited studies have examined the relationship between parasite prevalence and the abundance of host species at a particular site (e.g., Santiago-Alarcon et al. 2016). With this aim, Ellis et al. (2017) analyzed the relationship between avian host abundance and the prevalence of haemosporidian parasites (genera *Plasmodium* and *Haemoproteus*), comparing the prevalence between non-natives and native bird species in the same assemblage (Chicago, USA). They showed that the three non-native host species (*Passer domesticus*, *Sturnus vulgaris,* and *Haemorhous mexicanus*), despite being relatively abundant, had lower prevalence than did native species, as predicted by the *ERH*.

15.5 Mixed, Inconclusive Results and Possible Synergies Between ERH and NWH Processes

The above examples described the role of avian malaria parasites in the invasions of their hosts in new environments. As we have seen, there are several examples in which haemosporidian parasites (or the lack of them) may facilitate the spread and establishment of their avian hosts beyond their original range, thus showing that both mechanisms are valid to explain invasion success. However, perhaps these mechanisms (*ERH* and *NWH*) may not work in isolation from one another, and hence synergies between them could explain the success of bird invasive species (Lau and Schultheis 2015; Zheng et al. 2015).

Ishtiaq et al. (2006) were the first to test the *ERH* in bird-malaria systems. They conducted a comparative study to assess the prevalence and distribution of *Plasmodium* and *Haemoproteus* parasites in native and six introduced populations of the common myna *Acridotheres tristis*, a common passerine native to southern Asia that has been considered by the IUCN as one of the world's most invasive avian species (Lowe et al. 2000). Their findings reported little evidence that the release from malaria parasites could explain the invasion success of common myna, as not all comparisons of introduced populations to the native populations were consistent with the predicted *ERH*. For example, native populations showed greater overall parasite prevalence than introduced populations. However, when prevalence data from introduced populations on oceanic islands (Fiji and Hawaii) were excluded from analyses, prevalence did not differ significantly among native and continental introduced populations. Additionally, they found mixed results regarding *ERH* and *NWH*, since they showed some evidence that introduced populations may have become infected with novel parasite lineages, but also that common mynas carried parasite lineages from native to introduced locations.

These mixed results were later confirmed by Clark et al. (2015) in Australia, where common myna were primarily introduced from India and was secondarily expanded to the eastern Australian coast. They screened introduced mynas and native Australian birds in the myna's secondary introduction range in Australia for avian haemosporidian infection. They found that introduced mynas carried significantly lower parasite diversity than native mynas. Also, *Haemoproteus* spp. were only recorded in introduced mynas in the primary introduction range, but were apparently lost during secondary expansion, thus suggesting that invasive mynas apparently escaped from specialist *Haemoproteus* lineages in eastern Australia during secondary expansion. However, *Plasmodium* patterns support *NWH*, introduced mynas carried two invasive potentially harmful *Plasmodium* strains (*P. relictum* GRW04 and *P. elongatum* GRW06). They also found evidences that *Plasmodium* prevalence was significantly higher in both introduced and native myna than in native Australian birds, suggesting that introduced mynas could act as important reservoirs for *Plasmodium* infections.

In another investigation, Baillie et al. (2012) tested the *ERH* after a recent colonization event of an endemic New Zealand passerine, the bellbird *Anthornis melanura*. They studied patterns in *Plasmodium* spp. infecting bellbirds at multiple-host subpopulations simultaneously. According to Phillips et al. (2010), they predicted that colonies either should have lower parasite species diversity and prevalence than the source population. They documented a decrease in parasite species diversity from source to founder sites. However, they did not find any significant decrease in the prevalence of *Plasmodium* parasites from colonies to founder populations. Hence, their findings did not provide strong support for the *ERH*.

House sparrows were introduced to Kenya via the eastern port city of Mombasa in the 1950s, from where they have spread westward across the country. Coon and Martin (2014) explored the changes in haemosporidian prevalence in house sparrows across their range expansion in Kenya, using small spatiotemporal scales to

evaluate whether *NWH* or *ERH* enabled the colonization success of sparrows in Eastern Africa. They found poor evidences of enemy release or use of novel weapons by range-expanding house sparrows in Kenya. Haemosporidian prevalence in Kenyan house sparrows appears to be more strongly associated during the invasion process with host characteristics (e.g., physiological response to parasites) and external environmental factors (e.g., rainfall) rather than with time since introduction.

15.6 Conclusions and Future Directions

In the era of globalization, rapid and profound environmental changes affect host-parasite interactions resulting in disease outbreaks threatening global health and biodiversity. The joint action of increasing globalization (facilitating the arrival of exotic species to new areas) and environmental disturbances (promoting the colonization and spread of alien species) are increasing the processes of biological invasions worldwide, thus constituting one of the main risks to biodiversity, economies, and human health (Early et al. 2016).

Many bird species have been introduced and become invaders worldwide, both naturally and with human assistance (Blackburn et al. 2009). For example, since the eighteenth century, more than 1400 human attempts to introduce 400 bird species have been recorded in many parts of the world (Sodhi 2010). In many of these introductions, haemosporidian parasites (or the lack of them) might have played a major role facilitating the establishment and spread of their bird hosts beyond their natural range. In addition, with the support of native and introduced mosquito vectors, some co-transported avian malaria parasites have host-switched and infected native bird species in new areas, also becoming invasive parasites and provoking emerging infectious diseases. The outcomes of these biological invasions are devastating, with decimation and even extinctions of populations of many native birds.

In light of current and future global change scenarios, future research should be directed to characterize the malaria invasive species, identify their potential vectors, and develop an appropriate management strategy to avoid its colonization and spread. The establishment of exhaustive monitoring of exotic and native species and border surveillance is therefore the next important step to prevent new avian malaria outbreaks. In addition, mapping the expected impacts on biodiversity, economy, and health of invasive species of birds and their haemosporidian parasites would be essential to assess potential risks. All these efforts in biological invasions and avian disease management must be coordinated over broad landscapes, and will require the cooperation of researchers, communities, and governmental agencies.

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Chapter 16
Bird Migration and Vector-Borne Parasite Transmission

Farah Ishtiaq and Swen C. Renner

Abstract Migration plays a significant role in the ecology and evolution of hosts and – consequently – their parasites. Migratory birds have evolved to cope with physiological and ecological demands of long-distance migration. While migrant hosts are expected to harbor a large diversity of parasites and thus facilitate cross-species transmission, migration allows hosts to escape parasites by moving away from high parasite pressure habitats during crucial stages of the migration cycle. In the context of vector-borne parasites, three factors play an important role in successful parasite transmission – the level of parasite host-specificity, the ecological and phylogenetic similarities with current and potential hosts, and parasite biology. Parasites with extremely high host-specificity are naturally limited in their ability to switch to new hosts compared to more generalist parasites. However, having closer phylogenetic relationships among hosts increases the likelihood that parasites are shared. Hence, if the migrant host belongs to a phylogenetic group represented in the wintering range, there may be more opportunity for both generalist and specialist parasites to expand their host range, whether from the wintering grounds to the breeding grounds or vice versa. Much of the literature is focused on bird movement and the exchange of parasites between regions, but we need a more in-depth study on the phenology (seasonal timing) of vector emergence and quantifying host and vector abundances, which in turn determines the interactions across taxa. Such data will help to develop a model to identify ecological factors associated with migration that influence host–parasite dynamics, which can further predict the spread of disease with climate change.

Keywords Avian haemosporidians · Climate change · *Haemoproteus* · *Leucocytozoon* · Migration · *Plasmodium* · Vector-borne parasites

F. Ishtiaq (✉)
Tata Institute for Genetics and Society, Bangalore, India

S. C. Renner
Institute of Zoology, University of Natural Resources and Life Sciences, Vienna, Austria

Ornithology, Natural History Museum, Vienna, Austria
e-mail: swen.renner@nhm-wien.ac.at

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16.1 Introduction

Millions of birds endure long, demanding, and spectacular annual journeys between breeding and wintering grounds – a phenomenon that has evolved to cope with changing seasonal demands, to exploit food resources (Dingle 1996; Alerstam et al. 2003) and to escape parasites (Loehle 1995). Indeed, migration is a costly life-history trait that not only requires long-distance movements with substantial allocation of energy (Alerstam and Lindström 1990), but it also increases the risk of predation (e.g., Lindström 1989) and competition both at wintering (Price 1981; Rabol 1987; Jones et al. 1996) and breeding grounds (e.g., Bensch and Hasselquist 1991; Hasselquist 1998; Hermes et al. 2015). It is generally expected that migratory populations would be exposed to a larger array of parasites as they traverse through diverse habitats and environmental conditions (e.g., temperature, humidity, and altitude). Therefore, one can predict that migrants must have evolved mechanisms to cope with a huge diversity of parasites compared to resident species. For instance, host aggregation during migration (stopover sites) or at wintering sites exposes individuals to a higher infection risk (Krauss et al. 2010) compared to resident bird species (Loehle 1995; Møller and Erritzøe 1998).

Migration has been perceived as a major phenological trait, which plays an important role in parasite movement across large geographical scales and facilitates cross-species infection (Altizer et al. 2011). Alternatively, parasite avoidance or escape from parasites has been considered a potential driving factor in migration strategies (Box 16.1) for hosts to escape from habitats where parasite pressure is higher during a certain time of the year (migratory escape; Loehle 1995), and in the process helps reduce disease levels as infected individuals do not migrate successfully (migratory culling; Klassen et al. 2012), permitting evolution of less-virulent and benign interactions with parasites (i.e., aggressive symbiosis hypothesis; Ryan 2009) (Fig. 16.1).

Migration has evolved as multiple independent evolutionary events (convergence) across avian hosts and is a labile life-history trait that can respond quickly to

Box 16.1
Birds primarily follow two movement strategies, sedentary (residents) and migration (either short- or long-distance movement). Long-distance movement can increase exposure to diverse parasites, but can also reduce infection risk by weeding out infected individuals or escape from peak parasite transmission period. Many birds follow a partial migration with varying degrees of strategies that can switch over time (e.g., a migratory bird species can become resident) or space (e.g., parts of the population switch in part of the distributional range from migrant to resident). In addition, the strategy can be different between sexes or can switch from the juvenile to the adult stages

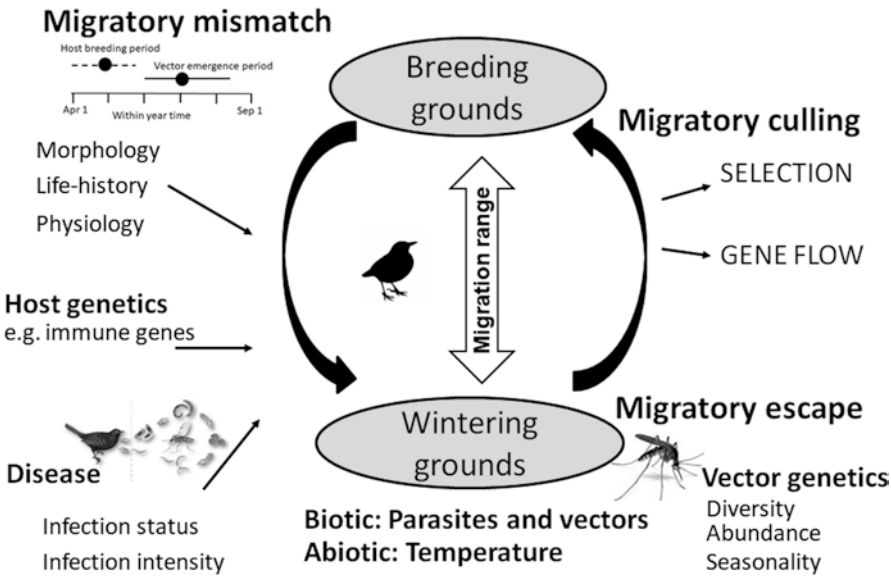

Fig. 16.1 Annual migratory cycle: a host species endures a seasonal migration journey to a wintering site and is potentially exposed to a large suite of vectors and parasites. However, to avoid high parasite pressure (i.e., migratory escape), the host species migrates back to breeding sites, and, in the process, weeds out highly infected individuals (i.e., migratory culling)

change in environmental conditions (Charmantier and Gienapp 2014). In the context of climate change, many birds have advanced their breeding time by shifting the egg-laying date with changes in spring temperatures and food availability. Charmantier et al. (2008) showed using a 47-year-long population study on British great tits (*Parus major*) that individual adjustment to the egg-laying date allowed the tit population to keep track of rapidly changing environmental conditions. However, a similar phenological shift by migratory pied flycatchers (*Ficedula hypoleuca*) breeding in the Netherlands was found to be inadequate in keeping pace with the changing environment (Both and Visser 2001). Furthermore, there is no information on how the phenology of avian haemosporidians and their vectors is influenced with a change in the breeding time of their hosts across resident and migrant avian hosts.

Based on a large body of research on avian haemosporidians in temperate regions, it is suggested that migratory birds acquire infections during the breeding season in the temperate zone. Both migration and breeding are physiologically stressful, which leads to a suppressed immune system and triggers relapses of chronic infections (Beaudoin et al. 1971; Sheldon and Verhulst 1996; Valkiūnas 2005; Cosgrove et al. 2008). In addition, migration and breeding must coincide with vector emergence in order to maintain infection dynamics (i.e., circulation of parasites in the local host assemblages). As haemosporidians can only be transmitted when they are present in the blood as an infective stage (i.e., mature gametocytes),

parasite biology/phenology plays a significant role in a migrant bird. A parasite must evolve strategies that facilitate year-round transmission in a migrant species while in the breeding, wintering, and transit localities. From a climate change perspective, the diurnal variation in temperature has been shown to influence the rate of parasite development (e.g., Paaijmans et al. 2009) and vector emergence, which may allow for a longer breeding season of arthropod vectors and exposure of susceptible avian hosts (see Chaps. 5 and 6). While the birds and vectors are shifting their range in response to temperature changes (see Chap. 7 for a perspective on niche ecology and climate change in relation to vector-borne parasites), the immunity toward novel pathogens is unlikely to be evolving at the same pace as pathogens adapt to novel conditions, which could be potentially detrimental for naïve birds (e.g., Atkinson et al. 2000; Woodworth et al. 2005; Freeman et al. 2018).

Given that migration has evolved as an adaptive response to maximize fitness in space and time, the selection on immune genes in species must vary between high pathogen areas and low pathogen areas (e.g., O'Connor et al. 2018), and thereby the response to novel virulent parasites or protection against lethal infections (Westerdahl et al. 2005). During the last decade, few studies have examined the role of latitudinal migration and the regular movement of birds on disease dynamics and evolution of pathogens, particularly within host populations across a spectrum of host-specificity as well as the response of migratory species to infection risks. In this chapter, we review the role of latitudinal migration in the movement of vector-borne avian haemosporidians. We address some of the outstanding questions in disease ecology:

(i) How do migration and parasites influence hosts?
(ii) How does migration drive parasite ecology?
(iii) How do parasites influence migration behavior across species?

16.2 How Do Migration and Parasites Influence Hosts?

Migrant birds connect continents and their different ecosystems each year (Hahn et al. 2009). From ecological and evolutionary perspectives, migration has a significant influence on the ecology of parasites and birds – empirical data has shown that migrants harbor higher parasite diversity, prevalence, and parasite load than sedentary birds (Emmenegger et al. 2018; Jenkins et al. 2012; Slowinski et al. 2018). Through the transportation of parasites (Waldenström et al. 2002; Hubalek 2004) and the presence of competent vectors (Comstedt et al. 2006) migrants' seasonality modifies the disease dynamics of local bird communities during winter, stopover, and breeding times of the year (e.g., Møller et al. 2004a). Such seasonal effects of migration would eventually lead to host–parasite co-evolutionary dynamics that vary in space and time (Jenkins et al. 2012).

Migration is physically strenuous and infection with pathogens having a negative influence on host physiology and ecology (fatal and morbidity effects; decrease in

reproductive success and poor body condition), but could also influence the time and direction of migration, or the decision to leave or stay at wintering sites (e.g., Santiago-Alarcon et al. 2013; Møller et al. 2004a). There are many examples of how high-intensity infections can delay migration and in consequence, the infected birds suffer further from suboptimal habitats at breeding grounds or face suboptimal refueling sites during migration because noninfected birds already occupy optimal winter habitats (Fig. 16.2). Møller et al. (2004b) showed that the spring arrival date to the breeding grounds in barn swallows (*Hirundo rustica*) is predicted by the parasite load (i.e., parasitemia). Early arriving males usually gain access to a superior habitat, mates thereby having a much higher reproductive success than average individuals (Møller et al. 2004a).

While there seems to be a well-established conceptual framework for limiting factors influencing migrant individuals during the varying stages of migration (stop-over), wintering, and breeding grounds (Faaborg et al. 2010; Sherry and Holmes 1995), studies of how parasites affect migrants are relatively scarce. Most studies

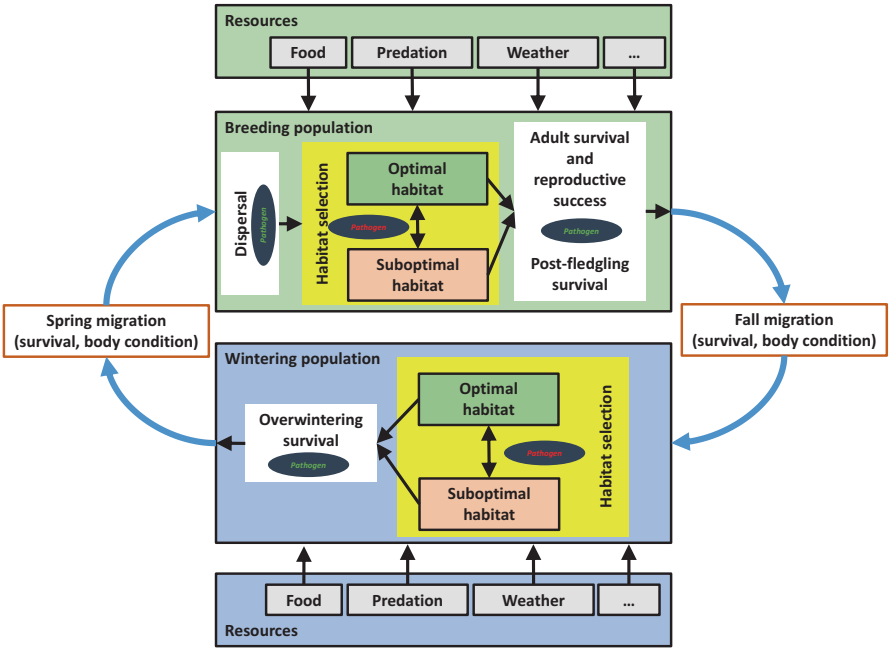

Fig. 16.2 Migration framework showing important factors for bird infection risk. Modified from Faaborg et al. (2010), but considerably amended by the major potential localities where pathogens can infect hosts and consequently affecting host health. "Pathogen" (in red if relatively high infection risk and in green if relatively low infection risk) refers to localities where a risk of infection is expected (i.e., at specific locations and periods of the migration cycle). For example, during the breeding season, more vector–host interactions are possible compared to the migration period, characterized by relatively short stays at stop-over habitats, many of which are not visited during the peak of vector emergence (e.g., late winter and early spring; that is, a migratory mismatch, see Fig. 16.1)

consider the breeding success as a parameter to evaluate the impact of pathogens at the population level (e.g., Davidar and Morton 1993; Marzal et al. 2008), but the shortage of energy due to parasitism has been hypothesized but not demonstrated empirically to negatively influence bird migration (but see Hahn et al. 2018). Depending on parasite biology, infection with certain parasite genera can be detrimental for a host species. For example, species of the genus *Plasmodium* cause more severe anemia than the other parasite genera due to its asexual reproduction in peripheral blood. Asexual reproduction of *Plasmodium* species can be found both in internal organs and in the blood of vertebrates, whereas *Haemoproteus* and *Leucocytozoon* are only found in the gametocyte stage in the blood (asexual reproduction takes place in the tissues of internal organs, known as exoerythrocytic stages; Valkiūnas 2005; see also Chap. 2 for an introduction to haemosporidians' life cycle). However, infection in blood cells causes cell destruction and potentially different degrees of anemia in the host (Atkinson and vanRiper 1991). To date, most changes inflicted by parasites on migratory birds concern selection of migration routes, delayed migration, or prolonged stopover times (D'Amico et al. 2008; DeGroote and Rodewald 2010; cf. discussion in Fuller et al. 2012). Immune-challenged birds prolonged their stopover duration on average by 1.2 days in long-distance and 2.9 days in short-distance migrants (Hegemann et al. 2018a, b). During prolonged stopovers, immune-challenged birds reduced their local movements, independent of migration strategy. In addition, birds infected with avian malaria-like parasites showed that a higher parasite intensity delayed flight initiation by 2.5 h on average than uninfected birds (Hegemann et al. 2018a). The differences affect overall migration speed and can in the long run decrease migration success and delayed return to the breeding grounds (Hegemann et al. 2018a), which in turn affects the selection of locally optimal habitats (Fig. 16.2; Faaborg et al. 2010), which again can affect infection risk by habitat types (Lüdtke et al. 2013; Renner et al. 2016). Hence, morbidity due to pathogens can affect migratory behavior (Hegemann et al. 2018b), which in combination with more stable resources throughout the year in some tropical areas can lead to the development of resident populations (e.g., Salgado-Ortíz et al. 2008) that can even hybridize with closely related resident species (e.g., MacKinnon-Haskins and Dzib-Chay 2017). However, the detrimental effects of pathogens on migrating individuals remain as correlative evidence needing experimental exploration. In addition to the effects of pathogens on migrants, pathogens can be transmitted during several life-stages of the bird host (Fig. 16.2). Hence, most migrants have a higher spatiotemporal risk of acquiring an infection than residents because in many tropical areas transmission occurs year round (except at high elevation environments and plateaus, and in seasonally dry environments where environmental conditions limit the presence of arthropod vectors; see Chap. 6 for a synthesis on Diptera vector research in relation to avian haemosporidians and Chap. 10 for a summary of avian haemosporidians across environmental gradients). For example, in shorebird species (freshwater and marine), prevalence is higher in lower latitudes compared to higher latitudes (cf. Fig. 4 in Clark et al. 2016); but at the same latitude, freshwater waders seem to have a higher prevalence than marine ones (Mendes et al. 2005; Clark et al. 2016).

16.3 How Does Migration Drive Parasite Ecology?

Migration drives the ecology and evolution of parasites by providing opportunities to exploit multiple habitats, which offers a more diverse set of host species and potential interactions. Migrants can serve as a reservoir for diverse pathogens and transport mild infections over large distances (e.g., Waldenström et al. 2002; Hasle 2013). Migrating birds can transmit pathogens, particularly those that do not significantly affect their own health and therefore do not interfere with migration (Olsen et al. 2006). Hence, migrant birds can be considered as vectors (termed also "bird-borne diseases"; Jourdain et al. 2007) because they could be involved in large-scale dispersion of pathogens. One example in the Palearctic involves the transport of low pathogenic avian influenza viruses from Southeast Asia to Europe; birds ringed in Camargue, France, have been infected with Avian Influenza A from northern East Asia at around 110° East (Jourdain et al. 2007). Furthermore, the local avian assemblage in Camargue usually acquires infection from birds migrating from Southeast Asia (Alexander 2007; Gilbert et al. 2008; cf. findings by Winker et al. 2007 on origin of avian influenza in Alaska). Thus, birds contribute to the global spread of emerging infectious diseases in a manner analogous to humans traveling on aircrafts (Reed et al. 2003; Waldenström et al. 2002; Elfving et al. 2010). A better understanding of avian migration patterns and their infectious diseases would be particularly useful in helping to predict future outbreaks of emergent zoonotic pathogens (Reed et al. 2003).

Each year, billions of migratory birds move between tropical/subtropical wintering areas to temperate/boreal summer breeding grounds (McClure, 1974). There are six major flyways, which connect the breeding and wintering grounds of birds, potentially allowing parasites to extend their geographic and host range (Webster et al. 2002; Ricklefs et al. 2017). Among these, Americas Flyways and Africa-Eurasia Flyways (Palaearctic-African) are relatively well studied on infection dynamics of avian haemosporidians (Fallon et al. 2005; Waldenström et al. 2002; Hellgren et al. 2007; Ricklefs et al. 2017). The genetic data are key to understanding a parasite's movement and can be used as markers to understand the origin of parasite lineages in migrant birds (Hellgren et al. 2013; Pulgarin-R et al. 2019; but see Fallon et al. 2006) and are important from a co-evolutionary perspective. The role of birds in disease ecology largely depends on migration strategies and host traits that influence host–parasite interactions and infection dynamics (Møller and Szep 2010; Clark et al. 2016). In the Palaeartic-African bird migration system, resident birds in Africa can act as reservoirs for tropical avian blood parasites (Valkiūnas 1993), increasing the risk for Palaearctic migrants to become infected from tropical bird species at overwintering African quarters (Waldenström et al. 2002). This is in addition to parasites already harbored by European birds in northern latitudes where they do not experience year-round transmission. Hellgren et al. (2007) examined the host and geographical shift in haemosporidian lineages in birds from Palaearctic and sub-Saharan Africa to determine the degree to which migrants are involved in the movement of parasites across continents. Using a phylogenetic approach, this

study confirmed only two lineages to have transmission in resident bird species in both geographical areas. The extent of parasite sharing across three parasite genera varied – *Plasmodium* appeared to have reduced transmission barriers between the continents, whereas *Haemoproteus* and *Leucocytozoon* rarely changed transmission area and appeared to have been restricted to one resident bird fauna over a long evolutionary time span. Findings differ between the Americas and the Euro-African migration system. In the Americas, the parasites exhibit more geographical diversity, representing the combinations of resident and migrant host species in both temperate and tropical latitudes. Furthermore, the taxonomic relatedness of avifauna and the relatively short migration distances of many species that winter in the Caribbean and Central America probably drive the dispersal of parasite lineages due to the availability of similar vector species. Waldenström et al. (2002) found that closely related bird species of migratory and resident status shared similar parasite lineages, which implies the presence of competent vectors for the transmission of parasites at breeding and wintering grounds. Similar patterns have been observed in the Central Asian Flyway. For example, India is a staging and wintering ground for many European passerines. Blyth's reed warbler (*Acrocephalus dumetorum*) breeds in Europe and Russia and winters in India. A recent study identified six *Haemoproteus* lineages and one *Plasmodium* lineage infecting the Blyth's reed warbler. Of these only two *Haemoproteus* lineages identified as *Haemoproteus belopolskyi* (hACDUM1 and hACDUM5) were recovered from resident Himalayan birds at a low frequency and without infective stages (i.e., no gametocytes; Ishtiaq 2017), suggesting that these could be spillover infections in a dead end host. The rest of the *Haemoproteus* lineages were shared with warblers of the genus *Acrocephalus* in the breeding grounds. Hence, the presence of phylogenetically related species makes the transmission of lineages more feasible between migrant and resident bird species.

16.4 How Do Parasites Influence Migration Behavior Across Species?

Most studies focus on the effects of food availability, mates, or changing climate as main factors influencing the evolution of migration. However, the negative interactions with parasites or predators are often not considered (Shaw et al. 2019). Pathogens drive the evolution of migration strategies – from high disease risk wintering areas to low pathogen pressure areas (pathogen escape/release) or infection with novel pathogens in new areas (pathogen exposure) influencing selection regimes on diverse immune genes (O'Connor et al. 2018). On the one hand, migratory behavior exposes bird species to a number of ecologically relevant infectious diseases (e.g., Avian malaria, West Nile Virus, Avian influenza) and increases the probability of pathogen transmission following aggregation or contacts with conspecifics or heterospecifics at breeding or stop-over sites (Figuerola and Green

2000; Waldenström et al. 2002; Clark et al. 2016). On the other hand, migration can reduce the infection risk either by removing infected individuals (migratory culling) or by interrupting pathogen transmission for part of the year (migratory scape). For a given pathogen, transmission may occur at a particular stage in the migration cycle because of constraints from pathogen biology due to either geographical factors or host-driven constraints. For example, *Borrelia burgdorferi* infection in black-legged kittiwakes (*Rissa tridactyla*) occurs at breeding sites via the tick vector *Ixodus uriae* that lives in the substrate of sea bird nesting sites (McCoy et al. 2005). Since the tick takes only one blood meal per year during each life-history stage and spends its life in the breeding colonies, the probability of *Borrelia* transmission occurs only during the breeding period. Similarly, low pathogenic avian influenza infections occur in high aggregation sites of bar-tailed godwit *Limosa lapponica* in breeding and wintering grounds (Hansbro et al. 2010). *Haemoproteus payevskyi* infections occur only in adult great reed warblers (*Acrocephalus arundinaceus*) and prevalence decreases with no new infections recorded during the course of the breeding season in Europe. This implies that *H. parayevskyi* infections occur on wintering grounds in sub-Saharan Africa or stop-over sites (Hasselquist et al. 2007).

Based on theoretical work by Hall et al. (2016), parasites influence the migratory propensity of their hosts by reducing the transmission risk – infection prevalence was lower for populations that left the breeding grounds sooner. Alternatively, environmental changes leading to a lower migratory propensity (or eliminating migratory behavior altogether) would cause a higher prevalence for parasites transmitted during the breeding season. In addition, sick or parasitized individuals may not be able to survive the migration journey, leading to a higher mortality (migratory culling), which reduces the prevalence of parasites and reduces the infection risk to noninfected hosts, probably eliminating highly pathogenic parasite variants. The concept of migration culling has been well documented in a few taxa (Altizer et al. 2011; Satterfield et al. 2015). In a 10-year study, Altizer et al. (2011) studied monarch butterflies (*Danaus plexippus*) and a protozoan parasite (*Ophryocystis elekroscirhha*) to understand the effects of seasonal migration on host–parasite dynamics. Longer-distance migratory populations of monarchs were infected with less virulent isolates, suggesting that longer migration distances cull monarchs infected with virulent genotypes. Similarly, lesser black-backed gulls (*Larus fuscus*) following the long distance migration strategy exhibited higher levels of natural antibodies but lower seroprevalence of the avian influenza virus, suggesting that migration played a role in disease ecology (Arriero et al. 2015). Despite this theoretical framework, these mechanisms have not been examined in the wild (but see Emmenegger et al. 2018) and we need more studies examining the infection status of wild birds during different stages of the migration cycle.

The phenology of parasites varies across haemosporidian genera despite using avian hosts as an intermediate host for completion of their life cycle. The overlap in phenology of parasites and vectors produces spring relapses and new infections in breeding host populations (e.g., Santiago-Alarcon et al. 2011). The transmission of each parasite genera could easily vary within a region as it is primarily driven by the

emergence of different vector families (see Chaps. 5 and 6). Thus, the absence of gametocytes in the blood during spring season or late emergence of vectors due to environmental conditions could lead to disruption of transmission cycles (migratory mismatch; Hall et al. 2016). In the Nearctic–Neotropical migratory system, Pulgarin-R et al. (2019) explored the extent of parasite dispersal between continents in a long-distance migrant, the gray-cheeked thrush (*Catharus minimus*), across the annual cycle (breeding, migration, and wintering). This study highlighted the presence of parasites in the gametocyte stage in blood during spring migration at stopover sites; however, these lineages were not found in resident tropical birds, suggesting on-route relapses of parasites acquired in the breeding season. In general, haemosporidian prevalence is lineage-specific and varies significantly across the migration cycle – bimodal patterns of spring and autumn infection peaks followed by marked decreases in prevalence during winters (Santiago-Alarcon et al. 2011; Hellgren et al. 2013; Pulgarin-R et al. 2019). These findings highlight that different parasite lineages have evolved different transmission strategies, which in turn is influenced by the presence of compatible vector species. Thus, mismatch in vector emergence and timing at stopover places could also lead to absence of shared parasite lineages between migrant and resident birds (Hall et al. 2016; Pulgarin-R et al. 2019). We need more studies to quantify the prevalence of parasite lineages at different stages of the annual migration cycle (Hellgren et al. 2013; Pulgarin-R et al. 2019).

16.5 Conclusions and Future Directions

Both model research and empirical research have shown that migration influences fitness costs, increases predation risk, and is physiologically demanding. From an evolutionary perspective, migrant hosts have evolved to cope with diverse parasite assemblages; however, the level of parasite host-specificity varies across communities. It will be interesting to compare the patterns in innate and adaptive immunity and response to specific pathogens across migratory and nonmigratory populations. Host generalist parasites exploit a broad range of species and are successful at establishing in novel host communities. Such communities with fluctuating population densities tend to favor parasites with low phylogenetic specificity (Poulin et al. 2011). Therefore, migratory hosts harbor more generalist parasites with more opportunities for cross-species transmission in wintering grounds, whereas dense host aggregations facilitate transmission of specialist pathogens (Altizer et al. 2011). From this perspective, it is important to incorporate data on both host and vector abundance in the models to understand the ecology and evolution of host–parasite systems. In the context of avian haemosporidians, the influence of host density/abundance on disease dynamics and changes in parasite prevalence and parasitemia across space and time will shed more light on the mechanisms that help in predicting future changes in host–parasite associations and disease spread (e.g., Ventim et al. 2012). Similarly, climate variability and habitat heterogeneity

influence diversity and abundance in mosquito species (e.g., Abella-Medrano et al. 2015; van Hoesel et al. 2019) – a combination of landscape ecology and population genetics would help us to understand the demographic history and fluctuations in effective population size in ecologically isolated populations. For vector-borne parasites, it is not clear how change in virulence influences host breeding phenology – how infection status influences movement and physiological costs of migration still needs to be considered for future work.

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Chapter 17
Experimental Parasitology and Ecoimmunology: Concepts and Opportunities in Avian Haemosporidian Studies

Vaidas Palinauskas, Josué Martínez-de la Puente, Sandra Rocío Hernández-Soto, and Alfonso Marzal

Abstract Broadening the field of classical parasitology research by integrating it with ecoimmunology has allowed us a better understanding of the effect of haemosporidians and to identify the most relevant factors that affect the health of birds. Despite the recent advances in avian malaria studies, the lack of experimentation remains the main obstacle for a proper characterization of the natural history of parasites and the functioning of the immune system of birds. It is worth considering the complement of both classical and new immunological methodologies to establish reference information and to assess the reliability of previous studies. The new molecular methodologies represent an advantage for distinguishing the involved genes in the immune response on birds when facing haemosporidian infections, avoiding the underestimation of the real prevalence of hemoparasites (e.g., coinfections), and setting up a starting point for new researchers interested in specializing in this area of study. Here, we present important and recent approaches on experimental parasitology demonstrating the negative effects of avian haemosporidians on their avian hosts. Also, we summarize the main advances in this field in avian malaria studies in the neotropics, as well as pinpointing knowledge gaps and future research opportunities. Additionally, we will recapitulate the main contributions and

V. Palinauskas
Nature Research Centre, Vilnius, Lithuania

J. M.- de la Puente
Estación Biológica de Doñana (EBD-CSIC), Seville, Spain

CIBER Epidemiología y Salud Pública (CIBERESP), Seville, Spain

S. R. Hernández-Soto
Laboratorio de Ecología de Vertebrados e Interacciones Parasitarias – LEVIP, Red de Biología y Conservación de Vertebrados, Instituto de Ecología, Xalapa, Veracruz, Mexico

A. Marzal (✉)
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain
e-mail: amarzal@unex.es

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tools used by ecoimmunologists to study immune defences against avian malaria parasites.

Keywords Antimalarial drugs · Coinfections · Experimental infections · Immune system · Inoculation · Major Histocompatibility Complex · Transcriptomics · Transmission · Virulence

17.1 Introduction

Experimental studies with animals started a long time ago and continues to the present. Sir Ronald Ross, a British officer in the Indian Medical Service, conducted the best-known experimental study using malaria infections, vertebrate hosts, and Diptera vectors. Ross used the assemblage between the avian malaria parasite *Plasmodium relictum* and *Culex* mosquitoes to demonstrate that pathogens causing malaria are transmitted by blood-sucking insects (Cox 2001; Ross 2002; Marzal 2012; see Chap. 1). For his discovery of the mosquito transmission of malaria, Ross was awarded the Nobel Prize in Medicine in 1902. At this point, it is worth mentioning that the first out of the four Nobel Prizes bestowed to malaria researchers was awarded to scientists who worked on avian malaria. Since then, studies on experimental parasitology and ecoimmunology on malaria parasites of birds have played an essential role as a model in malaria studies. For example, most of early experimental studies on avian malaria parasites were used as a model for human malaria investigations. Avian *Plasmodium* spp. were used to extend the techniques for analysis of human malaria parasites by trying to culture them for long-term experiments, and testing different drugs and/or early vaccines (Beaudoin et al. 1974; Davis et al. 1966). As an example, more than 14,000 substances were tested in experimental studies against malaria using bird malaria models in the USA alone (Davey 1951). Some of them were later chosen to test on human *Plasmodium* spp. However, the great majority of these studies were conducted using only a small fraction of avian malaria parasites. In the beginning, *P. relictum* was employed for various purposes, but later were added *Plasmodium gallinaceum*, *Plasmodium lophurae,* and *Plasmodium fallax* (Davey 1951; Coatney et al. 1953; Garnham 1966; Beaudoin et al. 1974; McGhee et al. 1977).

Nowadays, we recognize that bird haemosporidian parasites are among the most pathogenic organisms, being responsible for mass mortality, population declines, and even extinctions of many poultry and wild birds (Valkiūnas 2005). Even though some studies showed subtle but important effects of hematozoan parasites on the life history of their avian hosts (Korpimäki et al. 1993, 1995; Rätti et al. 1993; Allander and Bennett 1994; Dufva 1996), some researchers did not find negative effects of these parasites (Fallis and Desser 1977; Dufva and Allander 1995; Dawson and Bortolotti 2000). Therefore, avian haemosporidian parasites were originally considered as low-pathogenic organisms. The main problem of most of these studies is the lack of experimentation. The demonstration of negative effects of malaria parasites requires an empirical approach, where experimental manipulation of

natural blood parasite loads may reveal their harmful effects (Keymer and Read 1991; Merino et al. 2000; Marzal et al. 2005; Knowles et al. 2010; Martínez de la Puente et al. 2010). In this line, two methodologies have been widely used in experimental parasitology approaches to test for detrimental effects of avian malarial infection on their hosts: (i) experimental removal of parasites through antimalarial medication and (ii) direct inoculation of a parasite on uninfected individuals. Also, experimental infections are useful to understand the life cycle of haemosporidian parasites, identify the development patterns of both intra-erythrocytic and exoerythrocytic stages and evaluate the specificity of parasites on vertebrate hosts (see Chap. 2 for an introduction to the life cycle of avian haemosporidians). In addition, the experimental modification of parasite infections allows researchers to identify the role of bird immune system and other physiological pathways interacting with these infections (Tomás et al. 2007; Arriero et al. 2018).

In this chapter, we will present important and recent approaches on experimental parasitology to test for the negative effects of avian malaria on their avian hosts. Also, we will recapitulate the main advances in this field in avian malaria studies in the neotropics, as well as identifying knowledge gaps and future research opportunities. Additionally, we will review the contributions and tools used by ecoimmunologists to study immune defences against avian malaria parasites.

17.2 Experimental Removal of Avian Malaria Parasites

Important advances on chemotherapy of malaria were primarily carried out with birds, and resulted in the discovery of new antimalarial drugs such as plasmochin, atebrin, the sulphadiazine group of drugs and amino-quinolines (Fairley 1947; Sweeney 2000). In consequence, antimalarial drugs as chemotherapy have been widely used on poultry and captive individuals in zoos and rehabilitation centres. For example, it has been demonstrated that the oral administration of chloroquine cleared *P. relictum* and *P. elongatum* infection from the blood of captive African black-footed penguins (*Spheniscus demersus*) and prevented their mortality (Stoskopf and Beier 1979). Likewise, Sohsuebngarm et al. (2014) evaluated the efficacy of five antimalarial drugs (artesunate, chloroquine, doxycycline, primaquine and a combination of artesunate and primaquine) on *P. gallinaceum* infecting broiler chickens. But experimental removal of haemosporidian parasites through drug medication has also been employed to provide empirical evidence of the impact of haemosporidian infection on reproductive success, survival, and other fitness components of their bird hosts (Merino et al. 2000; Marzal et al. 2005; Martínez de la Puente et al. 2010). Moreover, this approach has been used to determine the preferences of haemosporidian vectors for uninfected hosts (Tomás et al. 2008; Martínez-de la Puente et al. 2009) and on the effects of *Plasmodium* infection on survival probability of their mosquito vectors (Gutiérrez-López et al. 2019).

Merino et al. (2000) were among the first researchers to use this procedure to test for the fitness consequences of avian malarial infection. They medicated blue tits (*Cyanistes caeruleus*) to experimentally reduce the intensity of infection by

Haemoproteus majoris and the prevalence of infection by *Leucocytozoon majoris*. Their outcomes revealed that medicated females allocated more resources to parental care and, consequently, enhanced their reproductive success. Some years later, Marzal et al. (2005) employed a similar methodology (inoculation of primaquine, an antimalarial drug) to analyze whether clutch size and other reproductive parameters could also be affected by haemosporidian parasites. With this aim, they experimentally reduced prevalence and parasitemia of *Haemoproteus* spp. infection on migratory house martin (*Delichon urbica*) at the beginning of the breeding season. As a result of the experimental reduction of haemosporidian parasites, clutch size was on average 18% larger in medicated than in control (non-medicated) birds, and these differences rose up to 39% at hatching and 42% at fledging. Overall, these findings demonstrated that haemosporidian parasites have harmful effects on reproductive success of their wild hosts, potentially influencing the evolutionary dynamics of heavily infected populations of birds. Additionally, Tomás et al. (2005) explored the role of hemoparasites as a potential source of physiological stress for wild blue tits. By the experimental reduction of the prevalence of infection by *L. majoris* and the parasitemia by *H. majoris* in female birds, they revealed an increase in stress proteins (heat shock proteins) in control females in comparison to medicated ones, suggesting that the stress response may allow birds to keep haemosporidian infections under control during reproductive stress. Further research using experimental reduction in parasite infections allowed authors to identify the link between haemosporidian infections, bird immune response and parental effort (Tomás et al. 2007). For example, Arriero et al. (2018) showed that parasitemia could modulate trade-offs between different components of the anti-parasite defence strategy. They also revealed variation in immune parameters over the course of infection, and individual consistency in how birds respond to a malaria infection.

The initial absence of evidence supporting the negative effects of haemosporidian parasites in studies in wildlife can be attributed to several reasons. By capturing birds in nature, it is possible to define composition and prevalence of haemosporidian parasites in different bird species, but evaluation of parasite prevalence using mist nets should be taken with caution as trapping probability of infected birds depends on parasite impacts on birds' health and host activity, especially during primary parasitemia (Mukhin et al. 2016). In this sense, because parasitemia varies during infection, the dynamics of haemosporidian infection could have been the cause of difficulties for detecting their fitness effects in wild populations of birds (see Chap. 2). During the short acute phase of a malaria infection, parasites usually appear in the blood at high densities and hosts can suffer striking mortality (Atkinson and van Riper 1991; Valkiūnas 2005). However, in individuals that survive this acute stage, long-term chronic infections develop, and parasites persist at lower abundance and are presumably controlled by host adaptive immunity (Atkinson and van Riper 1991; see Chap. 2).

Studies based on antimalarial treatments have revealed how these chronic, low-intensity parasite infections can reduce host fitness through negative impacts on reproduction, survival, and body condition. For example, Martínez de la Puente et al. (2010) experimentally showed long-term direct survival costs of chronic

Haemoproteus infection in blue tits. The medication with primaquine reduced parasitemia in females and led to an increase in local surviving until the next breeding season compared to control birds. However, the medication did not reduce parasitemia in male blue tits, showing a sex-specific effect of medication on *Haemoproteus* intensity, probably due to sex effects on drug kinetics. Furthermore, Knowles et al. (2010) demonstrated that chronic avian malarial infections can have significant effects on reproductive success and may constitute an important selection pressure in wild bird populations. They orally administrated the antimalarial drug Malarone™ to experimentally reduce chronic *Plasmodium* parasitemia in blue tits, leading to a higher hatching success, provisioning rates, and fledging success. More recently, Schoenle et al. (2017) evaluated the physiological consequences of chronic haemosporidian infection in adult male red-winged blackbirds *Agelaius phoeniceus*, showing that the treatment with an antimalarial drug reduced *Plasmodium* parasitemia and increased hemoglobin and hematocrit of medicated males.

However, some other experimental studies failed to identify any harmful effect of haemosporidian parasites on the life-history traits of their avian hosts. For example, Schoenle et al. (2017) detected no effect of experimental reduction of haemosporidian parasitemia on body condition, immune metrics, plasma corticosterone concentrations, total antioxidant capacity, or reactive oxygen metabolites in red-winged blackbirds. This lack of evidence could be due to a real absence of detrimental effects of malaria parasites on these characters, and thus other traits should be considered. Alternatively, the cure with some antimalarial drugs is highly efficient for blood stages of *P. relictum*, but exoerythrocytic stages were unaffected (Palinauskas et al. 2009). Hence, the effect of experimental reduction of malaria burden is only temporal, and medicated individuals can reach normal parasitemia levels some weeks after the treatment (Arriero et al. 2018). Finally, chronic, low-intensity parasite infections normally show few or no measurable harmful effects on their hosts (Valkiūnas 2005; Woodworth et al. 2005; Valkiūnas et al. 2006; Bensch et al. 2007). This is due to observed negative effects of haemosporidians on the phenotype of their hosts that usually occurs when parasitemia reaches high levels (approximately 20 gametocytes per 1000 erythrocytes, or higher), which mainly happens during the short initial primary infection or in relapses (Valkiūnas 2005; see Chaps. 1, 2 and 4). Therefore, the experimental inoculation of blood infected with *Plasmodium* provoking an initial acute parasitemia would be essential to demonstrate the detrimental effects of malaria infection on their bird hosts.

17.3 Experimental Inoculation of Avian Haemosporidian Parasites

Celli and Sanfelice (1891) contributed to the first attempts to infect experimental birds not by mosquito bite, but by using inoculum of infected blood. This experimental set-up opened a whole new era for malaria research both in humans, other mammals, and especially in birds at the beginning of the twentieth century. Early

infection experiments similar to the ones currently conducted were done using inoculation of infected blood either to the blood stream or into the muscles (Celli and Sanfelice 1891; Garnham 1966; Iezhova et al. 2005; Palinauskas et al. 2008; Fig. 17.1). Such studies are feasible only with some haemosporidian parasites like *Plasmodium* as these parasites, unlike *Haemoproteus* or *Leucocytozoon*, undergo erythrocytic merogonic development in the peripheral blood (Garnham 1966; Valkiūnas 2005). This is not a natural way of getting infection and some important developmental stages are missed. Nonetheless, it provides a unique opportunity to study some of the patterns of parasite development and the impact on host health even for those parasite species for which vectors are still unknown. Until now, for the majority of the described avian and lizard *Plasmodium* species, vectors are unknown, but the information about parasites development, exoerythrocytic stages and the impact on host health can be obtained by performing inoculation experiments (Palinauskas et al. 2015, 2016). Additional studies combining passages of parasites between birds, and between birds and mosquitoes, may provide useful tools for studies on the ecology and evolution of bird–*Plasmodium* interactions (Pigeault et al. 2015).

Experimental inoculation of avian malaria parasites from infected donors or exposure to the bite of infected mosquitoes has been widely used by researchers to study the harmful effects of parasitic infection (Fig. 17.1). Following this idea, some experimental studies have documented mortality caused by avian malaria infection. In this sense, Palinauskas et al. (2015) reported anemia and mortality provoked by cerebral paralysis in three species of birds (domestic canary *Serinus canaria*, Eurasian siskin *Carduelis spinus* and common crossbill *Loxia curvirostra*) experimentally infected with the cryptic avian malaria parasite *Plasmodium homocircumflexum.* In another study, Ilgūnas et al. (2016) experimentally infected individuals from three different species (Eurasian siskin, common crossbill, and common starling *Sturnus vulgaris*) by intramuscular sub-inoculation of blood infected with *P. homocircumflexum*. All exposed birds developed malaria infection and died between 30 and 38 days post-inoculation (dpi), with mortality probably attributed to the observed blockage of brain capillaries with phanerozoites, the exoerythrocytic

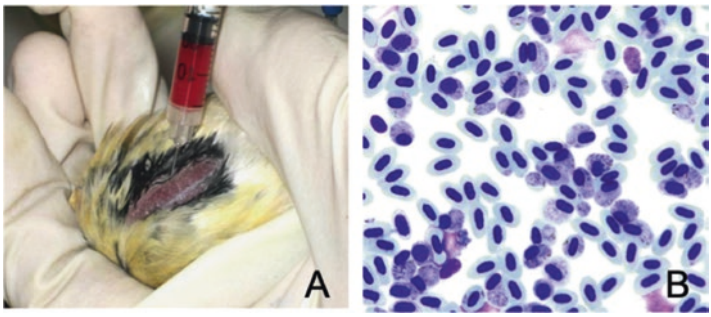

Fig. 17.1 (**a**) Experimental inoculation of infected blood into pectoral muscles of recipient bird; (**b**) High intensity of *Plasmodium relictum* parasitemia after experimental infection

form of malaria, which led to cerebral ischemia and cerebral paralysis. Moreover, Carlson et al. (2016) intravenously inoculated *Plasmodium* spp.—infected blood into five canaries. As a result of infection, three of malaria-inoculated birds were in poor body condition, with minimal coelomic fat reserves, and showed atrophy of the pectoral muscles. Three of the infected canaries died, revealing splenic lesions typical of avian malaria infection. Also, the detrimental effects of malaria infection on fitness components other than survival have been experimentally demonstrated. For example, Coon et al. (2016) showed that feather growth rate was negatively affected by experimental infection of avian malaria in captive house sparrows *Passer domesticus*. In addition, they also showed that malaria infection led to a decrease in hematocrit values.

Some studies have revealed within- and between-species differences in susceptibility and/or virulence of malaria infection. For example, Coatney (1938) performed experimental infections with infected blood to demonstrate complexity of factors that are important for the development of parasites in vertebrate host. It was shown that there might be not only 100% susceptibility or resistance of the vertebrate host, but partial development to some extent can also happen in nature. Moreover, parasites may develop for a short period. For instance, *P. relictum* isolated from pigeon and inoculated to domestic chickens of several days old, increased parasitemias and remained infectious for several days; however, parasites' capability to stay in the blood stream was limited and did not last longer than 11 dpi (Coatney 1938). This is a good example of abortive development in an atypical host for *P. relictum*. Also, this experimental infection demonstrated that parasites can develop and multiply by increasing the level of parasitemia and also form merozoites capable of invading new red blood cells to some extent in a non-typical host. Similar results were previously obtained by Manwell (1933) during experimental studies with *P. relictum*, revealing differences in parasite development and impact on host health depending on isolates. Different isolates were called as *Coatney strain* or *Becker strain* (Becker et al. 1957; Huff et al. 1959; Becker 1961). From nowadays' perspective, it seems that these strains may represent different lineages of morphologically identically looking parasites, like *P. relictum* and its different genetic lineages SGS1, GRW11, and GRW04 (Palinauskas et al. 2007; Valkiūnas et al. 2007, 2018). It also might be the case when the same lineage (e.g., *P. relictum* SGS1) isolated from different donor birds has different virulence (Palinauskas, unpublished data). Such cases are well described in mice malaria parasites, like *Plasmodium chabaudi*, where different isolates of the same species cause different virulence to recipient vertebrates (Taylor et al. 1998; Mackinnon and Read 1999).

Furthermore, experimental studies demonstrated that there might be differences in susceptibility of individuals belonging to the same bird species. For example, Atkinson et al. (2001) exposed captive Omao *Myadestes obscurus* and honeycreepers *Paroreomyza montana* to the bite of *P. relictum*-infected mosquitoes to measure parasitemia and mortality over time. All Omao birds survived for the duration of the experiment, whereas 75% of honeycreepers died, showing gross lesions of acute malaria including enlarged, blackened livers and spleens, and thin and watery heart blood. Also, Palinauskas et al. (2007, 2008) carried out some experimental

inoculations on captive passerines to evaluate the effects of *P. relictum* (lineage SGS1) on five species of birds (chaffinch *Fringilla coelebs*, common crossbill, house sparrow, siskin, and starling). They demonstrated that the same parasite lineage could cause malaria of different severity even in phylogenetically closely related bird species. For instance, parasite developed in birds belonging to Fringillidae and Passeridae, but starlings were resistant and only 50% of experimental house sparrows were susceptible to the infection. Moreover, infection of *P. relictum* led to a significant decrease in hematocrit value and hypertrophy of spleen and liver in infected common crossbills and siskins, but not in other species. Similar results were recently observed by Ilgūnas et al. (2019), where only a fraction of individuals showed infection after inoculation of infected blood with *P. elongatum* (lineage GRW6). They infected common starlings and common crossbills to test for susceptibility and virulence of this widespread avian malaria parasite. None of the experimental starlings developed the infection. In addition, they found a significant decrease in average hematocrit value, but not in body mass, of the experimental group compared to the control group. Furthermore, Palinauskas et al. (2009) experimentally infected greenfinches *Carduelis chloris*, showing that these birds developed light parasitemia and showed no significant effect of infection on their body mass. In addition, the infection did not cause mortality or morbidity of greenfinches, only provoking a slight decrease in hematocrit values. These differences represent the complexity of factors playing a role in host susceptibility, including genetic background of the host, age, sex, and physiological condition. This information obtained from experimental infections is crucial for a better understanding of adaptation processes and important mechanisms involved in the maintenance of parasitemia in vertebrate hosts, which sheds light on the study of host–parasite interactions. Additional experimental studies on avian *Plasmodium* parasites are needed to get a better understanding of the mechanisms that cause these differences, further considering the role of hosts' immune system.

Other haemosporidian parasites belonging to the genera *Haemoproteus* and *Leucocytozoon* did not get that much of attention compared to *Plasmodium* for several reasons. First, pathogens belonging to these two genera do not infect humans, so they have received less attention. Second, experimental infections with these genera require a vector since *Haemoproteus* and *Leucocytozoon* spp. do not have erythrocytic merogony, which hinders experimental infections using infected blood inoculation to recipients (Garnham 1966; Valkiūnas 2005). Several experimental studies with *Leucocytozoon* spp. were highlighted because of their importance for domestic birds and economic losses. For example, some empirical studies showed that *Leucocytozoon smithi*, *Leucocytozoon simondi*, and *Leucocytozoon caulleryi* cause rapid morbidity and mortality in young turkeys, ducks, and chickens, respectively (Desser 1967; Akiba et al. 1971; Steele and Noblet 1992; Valkiūnas 2005). *Haemoproteus* parasites were considered as relatively benign for a long time (Garnham 1966; Bennett et al. 1993), thus considerable little information was gained from experimental infections. However, several studies showed that this assumption was not correct as *Haemoproteus* causes mortality in birds maintained

in captivity and wild birds (Atkinson et al. 1988; Cardona et al. 2002; Martínez de la Puente et al. 2010; Olias et al. 2011; Ortiz-Catedral et al. 2019).

Atkinson et al. (1988) experimentally proved the harmful effects of *Haemoproteus* infection in birds. They induced an experimental infection with sporozoites of *Haemoproteus meleagridis* by an intraperitoneal inoculation of separate pools of *Culicoides edeni* that had taken blood meals from domestic poultry infected with *H. meleagridis*, showing the pathological effects of such infection in domestic turkeys. Some years later, Garvin et al. (2003) analyzed the pathogenicity of *Haemoproteus danilewsky* in captive blue jays *Cyanocitta cristata*. They inoculated intraperitoneally 3.000–4.000 sporozoites of *H. danilewsky* obtained from *C. edeni*, and revealed sub-lethal pathologic changes in the liver, lung, and spleen of birds. Also, Valkiūnas et al. (2006) infected nestling blackcaps (*Sylvia atricapilla*) by inoculation in their pectoral muscle with 45 sporozoites of *Haemoproteus belopolskyi* developed in the experimentally infected biting midge *Culicoides impunctatus*. Their findings showed that experimentally infected birds suffered from a significant weight loss, demonstrating a short-term influence of the infection on the birds' body mass.

But not all experimental studies have successfully revealed pathogenic effects of haemosporidian infections. For example, Hahn et al. (2018) tested whether haemosporidian infections may influence aerobic performance in migratory great reed warblers (*Acrocephalus arundinaceus*). They examined metabolic rates and exercise endurance in birds experimentally infected with *P. relictum* GRW04, and found no effect of infections on resting metabolic rate, maximum metabolic rate or exercise endurance. Given the diversity and complexity of avian haemosporidian assemblages, and the variety of host components that can be affected by the parasite infection, additional experimental studies are necessary to fully understand the impact of haemosporidians on their avian hosts.

17.4 Evidence of Pathogenicity of Avian Haemosporidian Coinfections

Traditional microscopic studies examining blood smears have shown that parasitized birds frequently harbor several different parasites, thus constituting coinfections (also named mixed infections) (Valkiūnas et al. 2003a; Palinauskas et al. 2005). Moreover, advances in methods of genotyping have revealed that the number of haemosporidian species infecting birds is much higher than that can be distinguished by traditional methods (Bensch et al. 2004), indicating that multiple infections with haemosporidian parasites are common in wildlife (Hellgren 2005; Pérez-Tris and Bensch 2005; Palinauskas et al. 2015), with a high between-species variation in prevalence. For example, Valkiūnas et al. (2003a) estimated a frequency of coinfections over 80% in some European bird populations. In addition, Balkaya et al. (2016) observed that 85.7% of sparrowhawks *Accipiter nisus* carried

coinfections. Also, Valkiūnas et al. (2006) analyzed haemosporidian infection in 16 bird species by combining molecular and traditional methods, showing coinfections in more than 43% of the examined individuals. More recently, Van Hemert et al. (2019) observed that more than 30% of haemosporidian-infected Northwestern crows (*Corvus caurinus*) harbored a coinfection. Coinfections were detected in 11.5% of birds sampled in Chile (Merino et al. 2008). On the contrary, some other studies found a prevalence of coinfections lower than 10% in investigated birds (Asghar et al. 2011; Podmokla et al. 2014; Dubiec et al. 2016; Marroquin-Flores et al. 2017). But besides their abundance, haemosporidian mixed infections are frequently underestimated in avian malaria studies. For example, from 72 published scientific contributions between 2007 and 2017 on avian haemosporidians showing evidence of coinfections, coinfections were excluded from analyses and/or not reported in the results in 85% of these studies (Marzal, unpublished data).

This huge variability in the frequency of coinfections among bird species could be due to a differential virulence or mortality of mixed infections in birds belonging to different species. Alternatively, this variability can also be attributed to methodological limitations, such as differences in accuracy and sensitivity of the methods used to identify coinfections. For example, polymerase chain reaction (PCR)-based detection methods are widely used in wildlife haemosporidian studies, but it has been documented that these methods often are not sensitive enough in identifying mixed haemosporidian infections (Martínez et al. 2009; Zehtindjiev et al. 2012). This could be probably due to differences in the affinity of different primers in the detection of haemosporidian parasites belonging to different genera during coinfections (Bernotienė et al. 2016; see Chap. 4 for an introduction to the use of molecular methods in avian haemosporidian research); for example, due to differences in parasitemia by two related parasites (e.g., *Plasmodium* and *Haemoproteus*). To resolve this methodological problem, some studies have proposed new methodologies to increase the accuracy of coinfection identification. First, the use of both PCR-based methods and microscopic examination in parallel would markedly increase the detectability and identification of coinfections in wild bird studies (Dimitrov et al. 2014, 2015). Second, it has been shown that a combination of at least three PCR assays increase the sensitivity in reading most of mixed infections (Bernotienė et al. 2016). Third, recently a new multiple PCR assay has been shown to be highly effective in identifying both single and multiple infections from all three genera of avian haemosporidian parasites (Ciloglu et al. 2019). And fourth, Next Generation Sequencing technologies have been proposed as an efficient molecular approach to detect *Plasmodium* multiple infections (Zhong et al. 2018; see Chap. 4). However, this latter technology is currently applied in human malaria and still has some boundaries, such as limited targeting on partial genomic regions, which may underrepresent complete polymorphism in coinfections.

In addition, several factors, such as environmental variation between study sites, differences in the parasite composition of mixed infections or between-host dissimilarities in susceptibility to haemosporidian infections, may explain the variation in the frequency of observed coinfections (Dimitrov et al. 2015). However, the

frequency observed of coinfections is higher than expected by chance, thus suggesting that some facilitation process may occur (but see Santiago-Alarcon et al. 2011 for an example of a suggestive negative interaction between different lineages of the *H. belopolskyi* parasite). On the one hand, haemosporidian infection may restrict some movement and behavior of birds such as preening for removing ectoparasites and vectors (Yorinks and Atkinson 2000), thus increasing the likelihood to become infected by a second haemosporidian parasite. In addition, the infection with one parasite can suppress the immune response of the host against a subsequent infection by other parasite (Kamya et al. 2006), hence enhancing the probabilities to get a coinfection. Although antagonistic effects between parasites infecting simultaneously have been documented (Fenwick 1980; Juhl and Permin 2002; Santiago-Alarcon et al. 2011), several authors have found synergetic effects between two malaria parasites in coinfections (Taylor et al. 1998; Zehtindjiev et al. 2008). In this regard, theory predicts that multiple infections could be especially injurious for hosts, affecting plumage coloration and leading to anemia, loss of body mass, and reduced survival (Graham et al. 2005; Davidar and Morton 2006; del Cerro et al. 2010). However, information about the interactions between the parasites in coinfections and their effects on host fitness has been poorly investigated and the reported results are inconclusive.

For example, Evans and Otter (1998) showed a lethal coinfection with *Haemoproteus* and *Leucocytozoon* in juvenile snowy owls *Nyctea scandiaca*, although both parasite species on their own were considered to be harmless. In addition, Marzal et al. (2008) explored the effects of single and double malaria infections on survival, body condition and reproductive success in house martins. They showed that coinfected birds showed higher mortality rates, reduced body condition and higher ectoparasite load. Moreover, Marzal et al. (2013) also revealed that house martins harboring a double infection had the lowest feather growth rate compared to single infected and non-infected, which may lead to a lower reproductive success. In this line, Lewis (2016) showed a decrease in reproductive success of blue tits and great tits (*Parus major*) simultaneously infected with several haemosporidians. Surprisingly, Marzal et al. (2008) reported that house martins with coinfections had higher reproductive success, despite being in poor physical condition. Also, some other investigations found inconclusive results when exploring the association between haemosporidian coinfections and host behavior. In this regard, Dunn et al. (2011) reported that male great tits infected with two different haemosporidian parasites showed better problem-solving performance than uninfected or single infected males, and females with mixed infections were more exploratory than uninfected or females infected by only one haemosporidian species. Also, Marinov et al. (2017) reported an association between haemosporidian coinfections and higher fearfulness in yellow wagtails (*Motacilla flava*). Furthermore, other studies did not find any effect of coinfections in different fitness traits of their avian hosts. For example, Sanz et al. (2001) found no relationship between primary reproductive parameters and the number of blood parasite species infecting female pied flycatchers *Ficedula hypoleuca*. Moreover, no significant effect of haemosporidian coinfection on the body condition and feather quality of house martins (van Rooyen

et al. 2014; Marzal et al. 2013), as well as the absence of effect of malaria coinfections on body condition and male carotenoid ornament expression in the scarlet rosefinch *Carpodacus erythrinus* have been reported (Synek et al. 2013).

The main problem of the above investigations is that they are based on observational studies. In fact, only about 7% of investigations analyzing coinfections in birds are based on experimental studies (Marzal, unpublished data). These studies are very important because they provide empirical evidence allowing us to reach firm conclusions about the interaction between the parasites in coinfections and their impact on host fitness. For example, Palinauskas et al. (2011) explored the effect of coinfections on experimentally infected passerine birds with *P. relictum* and *Plasmodium ashfordi*, showing that these mixed infections decreased haematocrit levels, and are highly virulent and act synergistically during primary infections in some but not all passerine birds. Also, Dimitrov et al. (2015) performed an experimental study in 16 species of common wild European birds to test for the effects of *Plasmodium* spp. parasites. They reported that birds harboring coinfections showed higher parasitemias. In addition, they showed several cases of severe pathology and even mortality in coinfected birds with haemosporidians, highlighting that mortality of birds during coinfections was tenfold higher than during single malaria infections.

In early studies with some malaria parasites, it was shown that some species such as *Plasmodium cathemerium* and *P. relictum* confer effective immunity against homologous parasites when reciprocal crosses are made, or when crossed with *Plasmodium circumflexum* and *P. elongatum* (Manwell 1938). But at the same time, these parasites exhibit differences when crossed with species belonging to subgenus *Novyella*. Several observational and experimental studies also revealed that the interaction between the parasites vary depending on parasite and host species, thus cannot be generalized to all other species (Palinauskas et al. 2011, 2018). Moreover, Dimitrov et al. (2015) found differences in the susceptibility of birds to coinfections of two *Plasmodium* species after the inoculation of infected blood, revealing that these differences were not only among different parasite species, but also among different cytochrome b (cyt b) lineages of the same morphospecies and even different isolates of the same cyt b lineage. For these reasons, more cross experiments are needed to better understand the interaction and effects of haemosporidian coinfections.

17.5 Avian Hematology, Bird Immune System, and Haemosporidian Infections

The avian immune system plays a central role in controlling infections by haemosporidian parasites. Avian malaria parasites and related haemosporidians are widespread parasites infecting birds in all continents except Antarctica (Valkiūnas 2005). However, interspecific differences in the susceptibility to parasite infections have been largely reported, with some avian groups usually showing extremely low

prevalence or, even, total absence of infection by blood parasites, including tropical and subtropical species (Martínez-de la Puente et al. 2017; Campioni et al. 2018; Masello et al. 2018). Ecological and evolutionary factors may largely determine the prevalence of infections by haemosporidians in wild birds (Tella et al. 1999; Arriero and Møller 2008; Quillfeldt et al. 2011). These differences could be explained by factors including a differential exposure to vector attacks, in terms of a differential preference for competent vectors of avian malaria parasites to feed on susceptible avian species (Rizzoli et al. 2015), or a differential habitat use potentially affecting their exposure to blood parasite infections by bird species (Piersma 1997; Figuerola and Green 2000). However, host-related factors may also determine the observed patterns, with differences in the immunological responses of birds to blood parasites playing a central role (Martínez-Abrain et al. 2004). Due to its importance on the ecology and evolution of bird species, many authors have measured a diversity of components of bird immune system (e.g., innate and acquired immunity). Among others, these studies include measures of the inflammatory response after inoculation of antigens such as phytohemagglutinin (PHA) (Navarro et al. 2003; Morales et al. 2006; Martínez-de la Puente et al. 2013), measures of the levels of different molecules (e.g., total immunoglobulin level, Tomás et al. 2007; haptoglobin level, Lobato et al. 2017; natural antibody levels and complement activity, Arriero et al. 2015) or white blood cells counts (e.g., Ricklefs and Sheldon 2007), many of them being studied in the context of blood parasite infections.

In addition, the quantification and characterization of the blood tissue and its components (e.g., polychromatophils in blood smears; or measurement of hematocrit and hemoglobin, total and differential count on avian leucocytes) allow establishing ranges and parameters used to assess the health status of birds (Campbell 2015). Thus, studies on avian hematology provide reliable estimates in the assessment of the immune capacity in birds, allowing detection of anemia and morphological changes in blood cells related to parasite infection (Clark et al. 2009). For example, several studies have shown that some anthropic disturbance, such as urbanization (Ruiz et al. 2002; Fokidis et al. 2008; Chávez-Zichinelli et al. 2013) or some agriculture practices (Kilgas et al. 2006; Deikumah et al. 2015) have a negative effect on birds' health, as revealed by the low body condition, increased number of leucocytes and higher heterophil lymphocyte index of birds living in disturbed areas. All these hematological changes are related to an increased stress caused by habitat fragmentation and reduced resource availability, which could make birds more susceptible to acquiring infections and developing diseases (Delgado-Velez 2015; Santiago-Alarcon et al. 2020).

Despite a gradual increase in the number of studies in ecoimmunology of wild and domestic species under different environmental scenarios during the last decade in the Neotropical region (Lobato et al. 2011; Martin et al. 2011; Copete-Sierra 2013; Maceda-Veiga et al. 2015), these studies are still scarce. This could be in part due to the difficulty in processing hematological data in the field, where limitations make it necessary to use portable technologies designed for "point of care" clinical examinations (known by its acronym as POC) (Hernández-Soto et al. 2019). For the most part, these studies report the heterophil lymphocyte index as the most

convenient parameter to show the stress effect, a measure used to estimate the state of health of organisms (Maxwell 1993; Ruiz et al. 2002; Davis et al. 2008; Fokidis et al. 2008; Zylberberg et al. 2013; Nurwahyuni et al. 2016; Santiago-Alarcon et al. 2019).

The interpretations of the above-mentioned measurements of components of avian immune system have been traditionally considered as potentially reflecting bird immune competence to fight off infections, or immune activation to face an active infection (Sheldon and Verhulst 1996; Ricklefs and Sheldon 2007; Biard et al. 2015). For example, a recent study analyzed haemosporidian infection and avian hematology in Neotropical birds from the Turdidae family (Thrushes) in environmental gradients (altitudinal and anthropic disturbance) (Hernández-Soto 2019). In the altitudinal gradient, the birds showed greater susceptibility to infection in the upper and lower elevations of the gradient (0 and 3200 m above sea level, respectively). Total leucocyte count also increased at these altitudes, which would indicate an active response by the immune system. Furthermore, birds living in habitats with a higher degree of anthropogenic disturbance (cattle fields) showed the highest prevalence of blood parasite infections. Birds from these cattle fields also showed higher values of the heterophil lymphocyte index, and lower values of leucocytes and thrombocytes, which could indicate immunosuppression due to the chronic increase in glucocorticoid levels (Oppliger et al. 1998; Sapolsky 2000; Fokidis et al. 2008).

With the aim to explore different components involved in the immune responses of wild birds against haemosporidian infections, new molecular approaches have been developed during the last decades (see below).

17.6 Major Histocompatibility Complex and Blood Parasite Infections

The major histocompatibility complex (MHC) drives important immunological mechanisms mediating avian–haemosporidian parasite interactions. Different studies have found support for the relationship between the MHC diversity, or the presence of specific alleles, and the resistance, tolerance, or susceptibility to infections by blood parasites. To our knowledge, the first study on this topic was performed by Westerdahl et al. (2005), who investigated the relationships between MHC class I heterozygosity (measured as the number of different alleles) and the presence of specific alleles in relation to the infection status by three avian malaria and malaria-like parasite lineages. In particular, authors found a higher MHC heterozygosity in great reed warblers infected by the lineage GRW2, corresponding to *P. ashfordi*, than in uninfected individuals. Similarly, the presence of the allele B4b was positively associated with the infection with this parasite lineage. However, non-significant associations were found for the case of other parasite lineages found in the population, including the lineages GRW1 and GRW4 belonging to *Haemoproteus*

nucleocondensus and *P. relictum*, respectively. Further studies in this blood parasite–bird model allowed authors to identify that, although a higher prevalence by *P. ashfordi* was found in birds with the B4b allele, individuals with this allele also exhibited a lower parasitemia (Westerdahl et al. 2012). Therefore, parasite prevalence and parasitemia should be considered as two different faces of the same trait (parasite infections), supporting the necessity to incorporate these two variables in studies on this topic. In this respect, a study in blue tits from central Spain found further support for the associations between different MHC alleles (namely UA104, UA108, UA114, UA117) and the malaria-like parasite *Leucocytozoon* (Rivero-de-Aguilar et al. 2016). Authors found both positive and negative associations between alleles and the *Leucocytozoon* parasitemia, which in some cases differed between age classes (nestlings and adult birds). In addition, contrary to the case of a Swedish population of blue tits (Westerdahl et al. 2013), Rivero-de-Aguilar et al. (2016) found that none of the studied MHC-I alleles (UA104, UA108 and UA117) were associated with parasitemia by *Haemoproteus* parasites. Interestingly, these studies support the fact that contradictory results could be obtained according to the identity of the parasite studied (lineage/species/genus) and the host characteristics (e.g., bird age, species, population). In addition, the results by Rivero-de-Aguilar et al. (2016) suggest that the interaction between different MHC alleles may also play a role in bird–blood parasite interactions. However, these studies may be especially difficult to perform in species with a high diversity of MHC alleles, as in the case of common coots *Fulica atra* (Alcaide et al. 2014), due to the high number of combinations of MHC alleles potentially tested.

17.7 Using Next-Generation Sequencing Technologies to Understand Avian Malarial Infections

During the last years, recently developed molecular approaches have been used to identify the immunological responses of birds against blood parasite infections. This is the case of next-generation sequencing technologies, which have facilitated the study of whole transcriptomes in avian species (see Chap. 4). For example, Videvall et al. (2015) used a transcriptomic approach to identify the differential responses of Eurasian siskins experimentally infected with the avian malaria parasite *P. ashfordi* (lineage GRW2) compared to uninfected birds. In addition, authors tested for potential differences during the course of infections, including samples from individuals prior to the experimental treatment, during the peak of parasitemia after 21 dpi, and 31 dpi when birds showed a reduced parasitemia. Interestingly, authors identified the functions of the differentially expressed genes and found 28 genes related to immunological response being overrepresented in birds at 21 dpi. Among the potential functions of these genes, those involved in the activation and differentiation of white blood cells, including both lymphocytes and leucocytes, were identified. In addition, 10 days later only 13 genes were overrepresented in

infected birds, supporting the idea of a modulation of the immunological responses against parasites during the course of infections. Furthermore, in addition to those genes related to immunological responses to infections, authors identified a number of overrepresented genes related to the regulation of cell death and stress responses, further supporting the link between stress and immune responses and haemosporidian infections in birds (Merino et al. 2006; Morales et al. 2006; Knowles et al. 2009). In addition to this malaria–bird assemblage studied, other authors have investigated the role of experimental infections by different pathogens on the gene expression of different tissues of their avian hosts. For instance, Newhouse et al. (2017) infected captive zebra finches *Taeniopygia guttata* with West Nile Virus (WNV) and found support for the activation of components of both the adaptive and innate immune pathways in infected birds compared to uninfected ones. However, it is important to consider the potential differences found in this study according to the different tissues analyzed from birds. In this respect, only a fraction of the overrepresented genes may be shared between different tissues of the same individual, probably based on their different physiological functions (e.g., hypothalamic, spleen, liver, or red blood cells) (Watson et al. 2017; Scalf et al. 2019). This is especially relevant for the case of avian malaria parasites, as the life cycle of these parasites include different phases in internal organs and peripheral blood of the bird hosts (Valkiūnas 2005). However, further studies should be conducted to test for differences in gene expression under natural conditions, considering the diversity of bird–parasite assemblages found under natural conditions, especially in the tropics. Moreover, additional factors potentially regulating these responses, such as habitat alteration (e.g., urbanization processes), should be considered (Fig. 17.2; Capilla-Lasheras et al. 2017; Watson et al. 2017). These studies should also consider the potential effect of coinfections by haemosporidians as well as the potential effect of a previous infection with related parasites, which could modulate the responses of birds to parasites in following exposures. Studies combining medication experiments with experimental infections may provide further evidence for the role of different components of the avian immune system to fight off infections.

One potential limitation of studies using next-generation sequencing technologies is the relative elevated economic costs of these analyses. To partially solve this limitation, authors have identified candidate genes involved in the bird immune responses against parasite infections. Using qPCRs authors are able to identify potential differences in the expressions of these specific genes between and within individuals. Following this rationale, Pauli et al. (2015) identified by qPCR the differential expression level of a subset of genes, including those involved in immunological responses, between feather samples of common buzzards *Buteo buteo* with different color morphs (dark, light and intermediate individuals). These approaches may be very useful to identify the genetic and immunological mechanisms reflecting the differential blood parasite prevalence found in raptors with different color morphs, such as Eleonora's falcons *Falco eleonorae* (Gangoso et al. 2016). However, the conclusions obtained could vary with respect to the candidate genes selected. For instance, Capilla-Lasheras et al. (2017) did not find evidence for a differential expression level of genes involved in antiparasitic responses (i.e., TLR4, LY86, and GATA3 genes) in malaria-infected and uninfected blue tit nestlings.

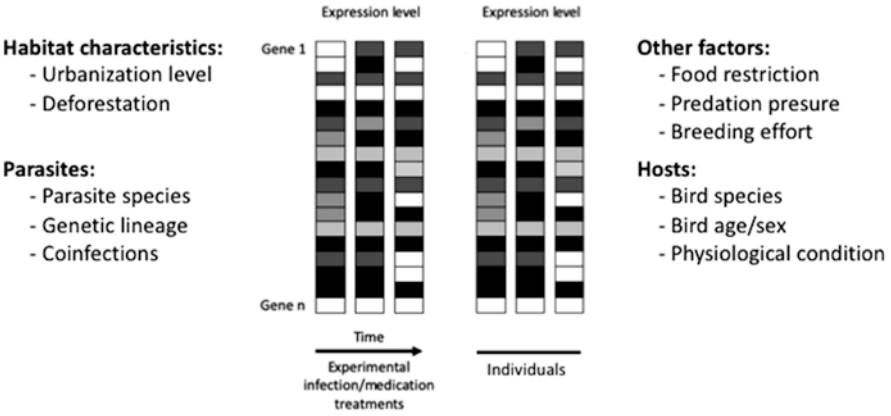

Fig. 17.2 Different approaches could be used to identify those genes over/underrepresented (shown with different colors) in birds infected by haemosporidians. Here, we present two different scenarios simulating the gene expression level of individuals after suffering the experimental manipulation of the parasite load (e.g., experimental infections/medication treatments to reduce parasitemia) and comparing the gene expression of different individuals (e.g., infected vs. uninfected birds or birds with different parasite loads). Factors potentially affecting the immunocompetence of birds and their responses to infections are identified including external factors (e.g., habitat characteristics and other potential sources of stress) and biotic factors such as the host–parasite assemblage studied

17.8 Research in the Tropics

Looking back to the rise in the field of malariology, in general, the largest numbers of experimental studies were conducted in Europe and North America, particularly in the USA. Studies in the Neotropical region, as most of tropics and Australia, are underrepresented. It might also be that some of those studies being in a different language were less accessible for the international community.

One of the most important discoveries in malariology about exoerythrocytic stages of haemosporidian parasites was made in Brazil by Aragao in 1891 (Garnham 1966). W. Lobato Paraense, in early experimental studies during the World War II, worked with *Plasmodium juxtanucleare* performing experimental infections from chicken to different species of mosquitoes (*Aedes aegypti, Ae. lepidus, Culex quinquefasciatus*) and then aimed to infect uninfected chickens with salivary glands (Paraense 1944). When exoerythrocytic stages of *P. gallinaceum* were revealed (around 1940), W. Lobato Paraense started to work with this parasite. He conducted experimental infections in Rio de Janeiro using chicken–*P. gallinaceum*–*Ae. aegypti* model system, and together with professor S. Americano Freire they verified the curative and prophylactic activity of a chemotherapeutic sulphadiazine (Freire and Paraense 1944). Later, their studies focused on defining the patterns of development of *P. nucleophilum* and *P. circumflexum*, presenting detailed descriptions of blood and exoerythrocytic stages of *P. circumflexum* in experimentally infected domestic

canaries (Paraense 1952). By using experimental infections of chicks, he also contributed to the knowledge on the development of *Plasmodium lophurae* in different hosts (Paraense 1948). Finally, he demonstrated that exoerythrocytic stages that multiply in endothelium of the encephalic capillaries cause death of vertebrate hosts (Paraense 1947).

Descriptive studies analyzing the prevalence of avian haemosporidian parasites from South America became more popular after revealing the high species richness of these parasites (Renjifo et al. 1952; Bennett and De Souza 1980; Bennett et al. 1991; see Chap. 1). During these studies, authors collected birds and analyzed spatiotemporal prevalence of haemoparasites mostly belonging to the genera *Plasmodium, Haemoproteus, Leucocytozoon*, and *Trypanosoma* (Bennett and De Souza 1980; Adriano and Cordeiro 2001; Rodríguez and Matta 2001; Valkiūnas et al. 2003b; Londoño et al. 2007; Rodríguez et al. 2009). Most of these studies revealed new host–parasite associations. When new molecular methods became available, new studies analyzing prevalence, parasite richness and composition of genetic lineages appeared across some uninvestigated Neotropical regions (Ribeiro et al. 2005; Durrant et al. 2006; Lacorte et al. 2013; Harrigan et al. 2014; Marzal et al. 2015; Tostes et al. 2015; Moens et al. 2016). These studies included the analysis of some life-history traits and ecological factors that are important to explain the prevalence of haemosporidians in wild birds (Fecchio et al. 2011; see Chaps. 2, 6, 10, and 11), as well as analyses evaluating connections of distant parasite faunas of migrant and resident birds (Ricklefs et al. 2017; see Chap. 16 for a summary on avian migration and parasitism). Many studies analyzed blood parasites obtained from captive birds in zoos and rehabilitation centres in Brazil (Belo et al. 2009; Chagas et al. 2013, 2016, 2017), with special focus on Humboldt (*Spheniscus humboldti*) and Magellanic penguins (*Spheniscus magellanicus*) (Bueno et al. 2010; Silveira et al. 2013; Vanstreels et al. 2014; Sallaberry-Pincheira et al. 2015). After obtaining hundreds of genetic lineages and some new morphologically distinct parasites, the number of novel bird–haemosporidian associations is rapidly increasing across tropical regions (Mantilla et al. 2013a, b, 2016; Matta et al. 2014a, b; Lotta et al. 2015; Walther et al. 2016).

The experimental research across tropical regions is developing slowly, having its main focus on *Plasmodium* parasites (e.g., *Plasmodium juxtanucleare* and *P. gallinaceum*). Studies with *P. juxtanucleare* started in Brazil with the aim of investigating the development, morphological, and morphometric features of this parasite in experimentally infected chicken (Elisei et al. 2001), followed by other studies, which included molecular examination of obtained *P. juxtanucleare* lineages and aimed to determine hematological changes of infected birds and testing antimalarial compounds (Silveira et al. 2009a, b). Authors experimentally demonstrated that the antimalarial drug routine did not affect the parasitemia of *P. juxtanucleare*, but chloroquine kept it at a low level, which makes this drug useful for acute parasitemia treatment. In addition, experiments with *Aedes fluviatilis–P. gallinaceum–Gallus gallus domesticus* demonstrated that the saliva of mosquitoes is important for better infectivity of the parasite; but, at the same time, chickens exposed to saliva prior to infection develop lower parasitemias compared to non-exposed individuals (da

Rocha et al. 2004). It seems that components of mosquitos' saliva are important for infection balance in vertebrate hosts. In a similar model system, using *P. gallinaceum* and *Aedes* vectors, Araujo et al. (2011) investigated ecdysteroid levels in hemolymph and expression patterns of genes involved in immune response and vitellogenesis. Recently, Ferreira-Junior et al. (2018) described the first cases on how the common parasite of domestic chickens *P. juxtanucleare* naturally infects wild passerine birds, and raised the issue that domestic birds can be important sources or reservoirs of malaria parasites in agricultural areas.

17.9 Knowledge Gaps and Research Opportunities

Even though *Plasmodium* parasites are among the best-studied pathogens, there is still a gap in understanding the diversity, specificity, virulence, and development of these pathogens in vertebrate hosts. There is even less knowledge about other haemosporidians belonging to *Haemoproteus* and *Leucocytozoon* genera. Molecular studies reveal that the richness of haemosporidian parasites is hugely underestimated in tropical regions. According to Clark et al. (2014), the highest diversity in the world of avian malarial lineages (including *Haemoproteus* spp. lineages) should be in South America. If that is the case, the research on these parasites, desirably including various experimental studies, should be the most active in this continent.

Information about possible vectors is very fragmented in general (Valkiūnas 2005; Santiago-Alarcon et al. 2012), but defining vectors of pathogens should be one of the biggest priorities because it is the corner stone for better understanding the biology of the parasite, host–parasite interactions and ecological niche in ecosystems (see Chaps. 5 and 6). Experimental studies are of great help, both for defining possible vectors and for evaluation of parasite's specificity and virulence on vertebrate host and insect vectors. Some bird species like domestic canary, ducks, chickens and siskins seem to be susceptible to a variety of *Plasmodium* parasites (Ilgūnas et al. 2013; Dimitrov et al. 2015; Palinauskas et al. 2015, 2016). Thus, using these already available experimental methodologies or applying them to specific bird species may help answering to what extent parasites are dangerous for endemic and other bird populations, especially during rapid climate change. These methodologies are particularly applicable to studies in tropical regions, as these ecosystems are vulnerable due to anthropogenic activities and climate change.

By catching birds in nature, it is possible to define composition and prevalence of haemosporidian parasites in different bird species, despite known biases of catching mostly birds that have successfully overcome the acute phase of infection. From collected material, it is possible to define parasite species and identify molecular lineages using microscopy and molecular tools. Also, big data of collected samples in the wild at some point can help understanding specificity and host range. However, this information may limit our knowledge on other factors including the biology of the parasite, the development patterns of erythrocytic and exoerythrocytic stages and virulence for the host. Even the evaluation of parasite prevalence using mist

nets should be taken with caution as trapping probability of infected birds depends on parasite impact on birds' health and host activity, especially during primary parasitemia (Mukhin et al. 2016).

Experimental infections with avian malaria parasites are useful in understanding some general processes of infectious diseases, for instance defining if chronic infectious diseases as malaria have an impact on telomere shortening. A recent study by Asghar et al. (2016) showed in siskins that birds experimentally infected with *P. ashfordi* (lineage GRW2) for longer periods of time experienced faster telomere attrition compared to controls. After this study, analysis of human patients infected with a single *P. falciparum* revealed similar patterns (Asghar et al. 2018). These findings have broad implications in understanding the mechanisms behind infectious diseases and their impact on host ageing and lifespan.

New technologies, such as next-generation sequencing, were developed which brought new insights into whole new genomic research of various pathogens (see Chap. 4). These methods enhance the characterization of both host and parasite molecular responses by pinpointing separate genes and their activities, or getting the whole picture of the transcriptomes (Lauron et al. 2014; Videvall et al. 2015, 2017; Weinberg et al. 2018). These methods also boost potential discoveries of unique genes expressed by the organisms. During experimental infections and analysis of closely and distantly related pathogens, it is possible to follow the changes in gene response and find the evolutionary patterns. Birds are particularly suitable for such analyses, as they contain a huge variety of haemosporidian parasites, which undergo natural selection without influence of antimalarial treatments and are available in all continents. In addition to studies on birds, the use of transcriptomics to avian malaria parasites revealed that expressed genes did not vary much between infection status but was significantly different between bird individuals (Videvall et al. 2017), thus showing that parasites are flexible in adjusting to different environments (e.g., different host birds) or a between-individual difference in responding to infections. A study by Lauron et al. (2014) using *P. gallinaceum* transcriptome defined positive diversifying selection of intra-erythrocytic and erythrocyte invasion genes. Recently, transcriptomes of two more species *P. homocircumflexum* and *Plasmodium delichoni* were sequenced (Weinberg et al. 2018). Also, attempts to obtain more genomic material of *Haemoproteus columbae* for a deeper phylogenetic analysis was successful after obtaining infected blood from *Columba livia domestica*, which was caught in Bogotá (Colombia) (Toscani Field et al. 2018). In this regard, in experimentally infected birds, parasites develop higher primary infections, allowing to use the material for various analyses including single-cell microdissection techniques with the aim to study either single parasite cells (Palinauskas et al. 2010) or total genomes (Lutz et al. 2016). This information serves for a more complete picture about differences between single cells of parasites, and helps understanding interactions between the parasites. Precisely planned experiments are essential for providing material to analyze different aspects of infections at individual host level within a single species and comparative analyses between different organisms.

Another part of haemosporidians' life cycle that lacks information and needs clarification is the exoerythrocytic development of haemosporidian parasites. Already in the mid-twentieth century, authors admitted that studies about exoerythrocytic stages of haemosporidians are much more complicated to conduct compared to those working with blood stages or even sporogonic stages in vectors (Huff et al. 1959). Controlled experimental infections are crucial to get information about exoerythrocytic stages of these parasites. Recently, a molecular chromogenic *in situ* hybridization method was developed for analysis of exoerythrocytic stages of avian malaria parasites (Dinhopl et al. 2011) and was successfully applied for experimentally infected birds with *P. homocircumflexum* (Ilgūnas et al. 2016). Experimental infections and analysis of internal organs using *in situ* hybridization should be used in future studies in all geographical regions.

It is worth mentioning and emphasizing that all experimental data are valuable. There are always some studies with negative results when parasites do not develop in one or another vector, or when pathogens do not develop in vertebrate hosts after inoculation experiments (Dimitrov et al. 2015; Palinauskas et al. 2015; Žiegytė, unpublished data; Bukauskaitė, unpublished data). In most of these cases, such studies are difficult to publish, but they are of great interest as these results are crucial for understanding the transmission of haemosporidian parasites and for further progress in this field.

17.10 Concluding Remarks

Experimental studies have been routinely incorporated into the research on avian malaria–bird interactions. Different approaches have been used including the experimental infection by different parasite lineages or species and the experimental manipulation of the parasite load through antimalarial drugs. These studies have revealed the deleterious effects of infections in both domestic and wild birds, including the adverse consequences of coinfections by different parasites. In addition, birds have been traditionally used as study models in ecoimmunology to identify host responses to infections by haemosporidian parasites in the wild; novel molecular approaches have opened new doors to the study of the different components involved in the responses of birds against infections. In spite of that, important limitations have been identified, with most studies focusing on a handful of model species of both parasites and hosts. This is especially the case of the avian malaria-like parasites of the genera *Haemoproteus* and *Leucocytozoon*. It is important to keep in mind the possibility of integrating classical and modern methodologies in the study of the immune response in birds. Beyond the availability of resources or conditions to use certain laboratory techniques, it would be ideal for generating reference information that would be used as a starting point for future studies in the tropical regions. In addition, future studies should consider the role of insect vectors in the interaction between birds and parasites (see Chap. 6). A lack of information exists

for competent vector species from tropical regions, thus further research should be conducted in this biogeographical areas.

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Chapter 18
Concluding Remarks

Diego Santiago-Alarcon and Alfonso Marzal

Abstract In 1884, the Russian protistologist Vassily Danilewsky described intraerythrocytic parasites in the blood of birds, highlighting that these parasites resembled the human malaria parasites described by Laveran few years before. Since then, bird haemosporidians have represented an excellent model for the study of host–parasite interactions. For many years, important advances in medical parasitology and malaria research, as well as other ecological, evolutionary, genetic, and immunological investigations, have been possible thanks to avian haemosporidian models. The field has exponentially grown during the past 20 years with the development of molecular methods, showing that genetic diversity of these parasites is greater than their morphological diversity, thus providing new insights into the taxonomy and biology of these pathogens. However, far from losing validity, investigations using traditional microscopy methodologies are still essential for the correct diagnoses and for understanding life history characteristics and biodiversity of this group. Although bird haemosporidians have been intensively studied in many temperate countries, the tropical and subtropical zoogeographical regions have considerably received less attention, thus constituting a significant gap in our knowledge and a priority for research. This is the origin and starting point of this volume. In these concluding remarks, we will recapitulate and point out the main items discussed across the synthesis and conceptual chapters presented in this book.

Keywords Avian haemosporidians · Birds · *Haemoproteus* · *Leucocytozoon* · *Plasmodium* · Tropical ornithology · Tropical parasitology

D. Santiago-Alarcon (✉)
Red de Biología y Conservación de Vertebrados, Instituto de Ecología, Xalapa, Mexico

A. Marzal
Department of Anatomy, Cellular Biology and Zoology, University of Extremadura, Badajoz, Spain

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18.1 Introduction

Our field of study started about 140 years ago, when Charles Louis Alphonse Laveran discovered in 1880 gametocytes in the peripheral blood of humans. In the particular case of avian haemosporidian research, it started in 1884, when Vassily Danilewsky discovered the common infection of wild birds by intracellular malaria-like parasites. Danilewsky developed the first investigations to determine the pathological effects of such blood parasites on birds and showed the similarities with human malaria parasites, as well as the seasonality patterns of infection. Subsequently, in 1897 William MacCallum discovered the sexual stages of haemosporidians using the columbid parasite *Haemoproteus columbae*; that same year Sir Ronald Ross proved the involvement of mosquitoes (Diptera) in the transmission of parasites of the genus *Plasmodium* in birds (see Valkiūnas 2005; Cox 2010; Marzal 2012, for a historical perspective on haemosporidian research). Since those initial studies that set the foundation of the field, we have come a long way to broaden our knowledge on ecological and evolutionary aspects of avian haemosporidians. Of particular relevance are the molecular studies conducted mostly during the past 20 years (see Chap. 4) and the increase of research on the Diptera insect vectors during the past 15 years, especially from an experimental framework (see Santiago-Alarcon et al. 2012; Chaps. 6 and 17).

Wildlife haemosporidian research during the past century was mainly characterized by studies determining prevalence and parasitemias across the globe, as well as by the taxonomic description of new morphological species (see Valkiūnas 2005; Chap. 1). Fortunately, the field has exponentially grown since approximately 20 years ago with the advent of molecular methods, which opened the field to researchers with interests beyond parasitology and veterinary science (e.g., ecology, evolution) and whom did not necessarily have the required skills for microscopy work. Thanks to the molecular methodologies, we now have a much better understanding of the systematics of the group (Chaps. 2, 3 and 4), we are making progress on the ecological dynamics of these systems and their response to anthropic impacts (Chaps. 8, 10, 11, 13, and 14), and we are deciphering their evolutionary diversification (Chap. 12). However, such benefit has come at a cost given that most researchers (particularly young ones) are unable to taxonomically identify and to describe these parasites; thus, the field currently has just a handful of taxonomists across the world. Thus, here is one important gap that needs to be filled: we urgently need more taxonomists for the Order Haemosporida.

The wildlife haemosporidian research community has become a vibrant entity composed of motivated individuals that regularly meet every 2 years since about 10 years ago, when Robert E. Ricklefs established the wildlife malaria RCN network via a NSF grant (http://malariarcn.org; FB: https://www.facebook.com/malariarcn/). The community has grown since then mostly out of a small set of research groups: Gediminas Valkiūnas in Lithuania, Robert E. Ricklefs, Patricia G. Parker, Carter T. Atkinson, and Susan L. Perkins in the USA, Staffan Bensch in Sweden, and Santiago Merino in Spain. Now their students are helping extend the field across

the world, in particular across tropical regions where we still need many more researchers, particularly working with Diptera vectors (see Chaps. 1, 5 and 6). The last meeting was held in Beijing, China, during the first 5 days of November 2018, where we could share some exciting developments: (1) the community has grown now to include researchers across all biogeographical regions, (2) the coming of the first transcriptomes and genomes allowing more detailed genetic and phylogeographic studies (e.g., avian malaria *Plasmodium relictum*), as well as more nuclear markers for more in-depth biodiversity studies, (3) new methodologies for experimental work aimed to understand the life cycle of haemosporidians both within vertebrate and Diptera hosts, and (4) more researchers working with Diptera vectors across different habitats and seasons (see also Sehgal 2019; Fig. 18.1).

18.2 This Book

In the present volume, we have collected and recapitulated all the different topics that are currently under study, providing a synthesis and future directions within each chapter. Because now the research field is expanding to include different disciplines across biology, many interested people from different fields could feel at a lost and probably would lose interest. Thus, in order to make our field of research more attractive and to avoid losing promising talents, we prepared this book not just as a synthesis of what is currently known, but rather also as a textbook or a primer. Readers will find many chapters that are not directly related with avian haemosporidian parasites, but they are rather conceptual chapters aimed at constructing the foundational knowledge needed by students to understand current topics (e.g., ecological niche modeling, macroecology, ecological networks, urban and landscape ecology, Diptera taxonomy, and systematics), and as a way of gently getting embedded in the subject matter. We have made sure that experts write each conceptual chapter and that at the same time they would frame each specific field for applications to vector-borne parasites.

This book emphasizes avian haemosporidian research in tropical regions across the world, given that such biogeographical areas have received the least attention; also there are already authoritative treatments for non-tropical regions and other relevant topics that should be consulted along with this book (see Valkiūnas 2005; Okwa 2012; Carlton et al. 2013). Following, we provide specific bullet points highlighting important issues coming out from both the synthesis and conceptual chapters presented in this book.

1. Across tropical countries surveyed during the past century, parasites of the genus *Haemoproteus* were the most prevalent at each studied location.
2. Avian malaria and related haemosporidian parasites are still understudied or no studied at all for most tropical countries (e.g., Bolivia, Paraguay, Nicaragua, Benin, Angola, Ethiopia, Laos, Sri Lanka).

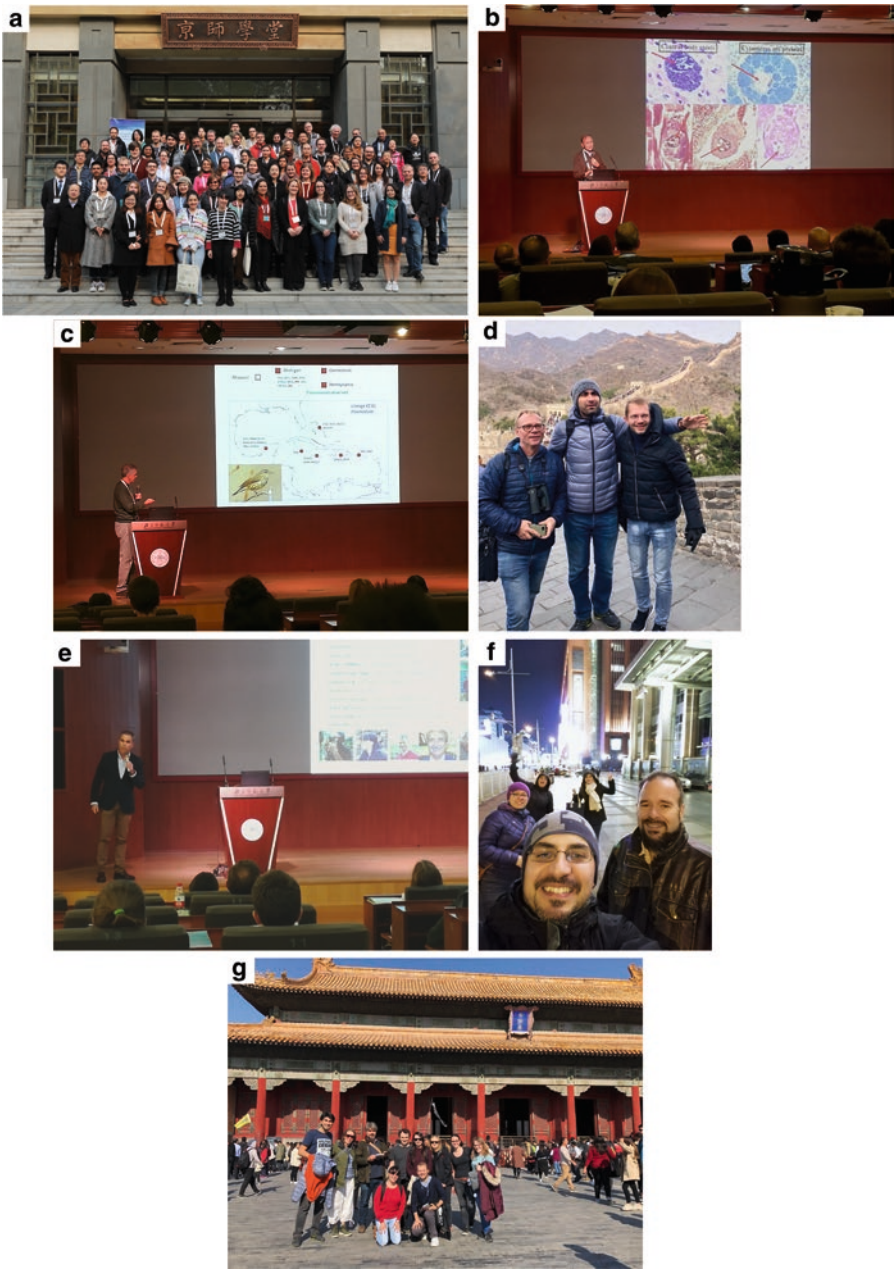

Fig. 18.1 Pictures from the Beijing conference held in China during November 2018. (**a**) Official group photo of the participants in the 4th International Conference on Malaria and Related Haemosporidian Parasites of Wildlife, held Nov. 1–5, 2018 in Beijing China. (**b**) Prof. Gediminas Valkiūnas giving a plenary lecture. (**c**) Prof. Robert E. Ricklefs giving a plenary lecture. (**d**) From left to right: Prof. Staffan Bensch, Dr. Arif Ciloglu, Dr. Vaidas Palinauskas. (**e**) Dr. Alfonso Marzal giving a keynote lecture. (**f**) From front to back: Dr. Diego Santiago-Alarcon, Dr. Leonardo Chapa-Vargas, Dr. Carolina Hernández-Lara, Dr. I. Karina Monzalvo Santos, M.Sc. Yeraldi G. Guillén Rodríguez. (**g**) Group photo of the participants during their visit to the Forbidden City in Beijing

3. There is a dearth of taxonomic expertise, so we urgently need researchers trained in classical microscopy work around the world.
4. Recent ecological studies suggest that avian haemosporidian biodiversity is greater in tropical regions, but for most detected novel molecular lineages, there are still knowledge gaps regarding their life cycles, vectors, pathogenicity, and genetics.
5. Systematics and phylogenetics have become an indispensable tool for many biological disciplines. Thus, researchers and students are encouraged to learn such methods, having a nice introduction in this book in Chap. 3.
6. Each study must make efforts toward using both microscopy and molecular methods (at least the mtDNA cyt b MalAvi region to identify genetic lineages). Sometimes funds are limited, in which case it can be justifiable the lack of identification of genetic lineages, but this should be avoided as much as possible.
7. It is important to consider the temporal aspect of parasitemia when estimating prevalence, given that low parasitemias can lead to more false negatives.
8. Abortive development of infections in new hosts is ecologically and evolutionarily important as they represent the first stages of host range expansion, because the parasite has achieved some degree of successful establishment and replication in a novel host.
9. Transcriptomes may eventually be helpful to develop molecular markers specific for different parasite developmental stages. In addition, genomes and developing a common set of nuclear markers will aid in obtaining a robust phylogeny for all haemosporidians, as well as to determine the population genetic structure of these parasites.
10. The Diptera is one of the most diverse insect orders, and it is particularly rich in tropical regions. Its taxonomy and systematics are complex, even for the four main families transmitting haemosporidian parasites. Thus, we provide a conceptual chapter (Chap. 5) introducing these topics for the novice, so he/she will be able to identify insects belonging to blood sucking families relevant for haemosporidian research.
11. PCR methods have helped the identification of putative Diptera vectors in the wild, but caution must be exercised as PCR can detect abortive infections. These methods have helped to conduct field studies of Diptera assemblages across tropical regions, which have been understudied in comparison with temperate areas.
12. Experimental infections have clearly demonstrated that haemosporidians have negative effects on bloodsucking Diptera, causing high mortality rates sometimes even in competent insect species.
13. Studies, mostly from human vector-borne parasites, have shown that temperature and precipitation influence reproduction rates of parasites and their vectors, affecting transmission dynamics. Despite this knowledge, very few studies have taken advantage of ecological niche models and species distribution models to tackle important ecological issues of haemosporidian parasites, such as

describing environmental relationships at different spatiotemporal scales, and niche shifts and specialization under climate change.

14. Although ecological niche models are a relatively recent addition to the geography of parasites, these methods have plenty to offer to the field of animal health. For example, mapping geographic patterns of disease transmission risk and the identification of risk factors. Thus, Chap. 7 introduces this topic for the novice.
15. Although a lot of avian haemosporidian research efforts have been put in archipelagos (mainly the Antilles, Galapagos, and Hawaii), the availability of genetic lineages is still not enough to build a solid island biogeography for these parasites. However, there are hints indicating that larger islands and those closer to the mainland have a richer parasite assemblage. Also, there seems to be processes indicating taxon cycles and small island effects for haemosporidian parasites that need further investigation.
16. Macroecological and interaction network methods can help elucidate distribution patterns of parasites across environmental gradients. The limited information on antagonistic interactions suggests that the structure of antagonistic networks does not change with latitude or some other climatic gradient, but this is still premature, and more research is needed. Beta diversity of antagonistic interactions, on the other hand, seems to be associated with geographic and climatic gradients. Chap. 9 provides an introduction to macroecology and interaction networks.
17. At the local scale, there are environmental effects on the ecological dynamics of avian haemosporidian infections, but the effects are highly dependent on context (i.e., the identity of the interacting species and the gradient under study). For instance, latitudinal gradients of diversity may be lost depending on the parasite group; pollution gradients may interact with other environmental factors complicating predictions; and bird life history traits may determine prevalence and parasitemia.
18. There are at least three relevant barriers to haemosporidians dispersal: geographic, environmental, and phylogenetic (i.e., species identity). In addition, there are two filters that avian parasites need to overcome in order for a successful dispersion, an encounter filter, and a compatibility filter.
19. Community level processes dominate the diversification of haemosporidians, and the speciation of these vector-borne parasites cannot be adequately accounted under the classical allopatric or sympatric models.
20. Codivergence in haemosporidians is only observed at taxonomic levels above the species (e.g., family). Considering that many lineages can successfully infect multiple hosts (i.e., low host specificity) suggests that haemosporidians present a good degree of phenotypic plasticity.
21. In addition to identifying host switches, it is necessary to investigate parasite fitness on multiple hosts along with the demographic histories of vertebrate hosts, insect vectors, and parasites. For example, by studying well-characterized parasite clades.

22. Landscapes are highly modified to fulfill human needs, which is drastically impacting the world at different levels. Given the high rate of urbanization across the world, to study the direct and indirect effects that the urbanization process and other associated anthropic impacts are having on host–vector–parasite interactions are a current priority for global health. This research is particularly urgent in order to determine hazards and risks of infection and of novel emergent diseases.
23. Current research indicates that urbanization, agricultural development, and habitat destruction decrease biodiversity, leading to a homogenization of hosts, vectors, and parasites. More frequent contacts between native and introduced species provide opportunities for host switches and the emergence of novel diseases. Because not all species respond in the same way to the same environmental disturbances, the responses and predictions to such disturbances have to be gauged in a system-by-system scenario, particularly at the local scale.
24. The synergistic effect of globalization and environmental disturbances are augmenting the probability of biological invasions worldwide, where invasive parasites represent one among many risks to local biodiversity, economy, and human and animal health. In the specific case of haemosporidians, researchers should aim to identify the most invasive parasites/genetic variants and their vectors along with the local environmental factors responsible for promoting or deterring the invasion process.
25. Migratory bird species have evolved to cope with a diverse assemblage of parasites, and some parasite species (particularly generalists) have used migratory birds as vehicles to reach distant geographical locations.
26. Experimental approaches, either by parasite inoculation or by decreasing parasite loads with antimalarial drugs, have demonstrated the deleterious effects of haemosporidian parasites on different life history traits of their avian hosts.
27. Molecular advances in genotyping have revealed that many birds frequently harbor several different haemosporidian parasites, thus constituting mixed infections (also named coinfections). But besides their abundance, haemosporidian coinfections are frequently underestimated in avian malaria studies, and the negative consequences of these coinfections are mostly unknown.
28. Birds have been traditionally used as study models to assess immune capacity in the fight against infections by haemosporidian parasites. Recently, new molecular approaches (e.g., characterization of major histocompatibility complex alleles, next-generation sequencing technologies, qPCR) have been developed to identify the different components involved in the responses of birds against infections.

We do hope that this volume becomes a usable tool and an inspirational source for both students and researchers interested in wildlife vector-borne parasites. All of the researchers participating in the creation of this book have put their best efforts to make it accessible and thorough, but at the same time rigorous so it can be an entry port to this exciting field of science.

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