UNIVERSIDADE FEDERAL DE MINAS GERAIS
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DIVERSITY OF AVIAN HAEMOSPORIDIAN PARASITES IN BRAZIL IN TIME AND SPACE

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BELO HORIZONTE – MG
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DIVERSITY OF AVIAN HAEMOSPORIDIAN PARASITES IN BRAZIL FROM LARGE TO SMALL SCALE

PhD Dissertation presented to the Graduate Program in Parasitology of the Department of Parasitology of the Institute of Biological Sciences of the Universidade Federal de Minas Gerais. Area of concentration: Immunoparasitology.

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يَا أَيُّهَا النَّاسُ إِنَّاخَلَقْنَاكُم مِّن ذَ كَرٍ وَأُنثَىٰ وَجَعَلْنَاكُمْ شُعُوبًا وَقَبَائِلَ لِتَعَارَفُوا ۚ إِنَّ أَكْرَمَكُمْ عِندَ اللَّـهِ أَتْقَاكُمْ ۚ

إِنَّ اللَّـهَ خَبِيرٌ عَلِيمٌ ۚ (سورة الحجرات .13:49)

O mankind! We have created you from a male and a female, and made you into nations and tribes, that you may know one another. Verily, the most honorable of you with Allaah is that (believer) who has At-Taqwa. Verily, Allaah is All-Knowing, All-Aware.}

[Surah al-Hujuraat (49): 13]

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Abstract

Avian haemosporidian is a diverse group of protozoan parasites including *Plasmodium* and *Haemoproteus* that can infect birds and use a variety of Diptera vectors for their transmission. It has been known that host traits and annual seasonal cycles may influence infection rates of these parasites in bird communities. However, in the Neotropics such influence remains to be determined. We examined the effect of temporal dynamic and host traits on the prevalence and diversity of avian haemosporidian parasites in the Brazilian avifauna of the Caatinga biome. A total of 933 samples were molecularly screened for the presence of *Plasmodium*/*Haemoproteus* infections. We found high avian haemosporidian prevalence (51.3%; n=481/933) varying according to temporal dynamic with the highest prevalence (61%) observed in the beginning of dry season. Prevalence also varied among the 20 well-sampled host species within a range from 0 to 70%. The host functional traits (feeding behavior, flocking behavior, habitat use and nest type) were associated with infection rate for *Haemoproteus* (*Haemoproteus*), with high infection rates observed for granivorous, single species flock, forest habitat independent, and open cup nester birds. For *Haemoproteus* (*Parahaemoproteus*) and *Plasmodium* infection, none correlation with host functional traits was observed. After excluding the large sample size of Columbiformes (n=462/933), *Plasmodium* infection rate showed association with feeding behavior, nest type and migratory behavior. Subsequent to parasite screening and prevalence determination, sequencing PCR was performed to determine parasite lineage diversity. Parasite community was composed of 32 distinct lineages, including 7 new lineages, pointing to high haemosporidian diversity in the Caatinga biome when compared to other studies in seasonally dry ecosystems. Interestingly, our results suggest that avian host traits are important determinants of prevalence and diversity of avian haemosporidian parasite in Caatinga biome. We also analyzed the diversity and distribution of avian haemosporidian lineages on a broad scale across a diverse range of avian hosts in seven ecologically distinct habitats in Brazil. *Plasmodium* was the most common parasite group in Brazil, accounting for 67% of the recovered lineages and that pattern was observed in five out of the seven habitats evaluated here. However, *H.* (*Haemoproteus*) was the most common parasite among birds from the Caatinga biome while *H.* (*Parahaemoproteus*) was prevalent in Restinga habitat, Among the 69 well-sampled lineages (lineages detected at minimum of four times), 48 were habitat-generalist (36 *Plasmodium*, seven *H.* (*Parahaemoproteus*), and five *H.* (*Haemoproteus*), occurring in two or more host species or habitats. *Plasmodium* lineages showed a broader host and habitat range when compared to *H.* (*Parahaemoproteus*) and *H.* (*Haemoproteus*). Interestingly, we found 14 *Plasmodium* lineages that were host and habitat specialist. Our results suggest the existence of both host and habitat generalist and specialist lineages in the two main genera, *Plasmodium* and *Haemoproteus*, in Brazil. Further studies are required aiming to understand the factors influencing the way avian haemosporidian parasites colonize bird communities in the Neotropical region.

Key words: Haemosporida, *Plasmodium*, *Haemoproteus*, temporal dynamic, distribution, Neotropical

Resumo

Hemosporídios aviários são um grupo diverso de parasitos protozoários, que incluem os gêneros *Plasmodium* e *Haemoproteus*. Estes parasitos podem infectar aves e usar uma variedade de dípteros como vetores para sua transmissão. Sabe-se que características do hospedeiro e ciclos sazonais anuais podem influenciar a taxa de infecção desses organismos. Entretanto, tais efeitos em hemosporídeos de aves neotropicais ainda precisam ser determinados. Examinamos o efeito da dinâmica temporal e características do hospedeiro na prevalência e diversidade de hemosporídeos aviários na avifauna brasileira do bioma Caatinga. Um total de 933 amostras foram testadas quanto à presença de infecções por *Plasmodium* / *Haemoproteus*. Encontramos alta prevalência de hemosporídeos nas aves (51.25%; n = 481/933) variando de acordo com a dinâmica temporal. A maior prevalência (61%) foi observada no início da estação seca. A prevalência também variou entre as 20 espécies hospedeiras bem amostradas variando de 0 a 70%. A história de vida do hospedeiro (comportamento alimentar, comportamento de flocagem, uso de habitat e tipo de ninho) também foram associadas à taxa de infecção por *Haemoproteus* (*Haemoproteus*). Altas taxas de infecção foram observadas para aves granívoras, bandos compostos por uma única espécie, com habitats independentes de florestas e com ninhos do tipo aberto. Para a infecção por *Haemoproteus* (*Parahaemoproteus*) e *Plasmodium*, não foi observada nenhuma correlação com as características da história de vida do hospedeiro. Após excluir amostras de Columbiformes (n = 462/933), a taxa de infecção por *Plasmodium* mostrou associação com comportamento alimentar, tipo de ninho e comportamento migratório. Após o diagnostico fizemos uma PCR de sequenciamento para verificar a diversidade de linhagens de parasitos. A comunidade de parasitos foi composta por 32 linhagens distintas, sendo que dessas 7 são novas. Este resultado demonstra uma alta diversidade de hemosporídeos na Caatinga quando comparada a outras fitofisionomias brasileiras em ecossistemas sazonalmente secos. Também analisamos a diversidade e distribuição de linhagens haemosporidianas de aves em larga escala de ampla gama de hospedeiros aviários em sete habitats ecologicamente distintos no Brasil. *Plasmodium* foi o grupo parasitário mais comum no Brasil, representando 67% das linhagens recuperadas e esse padrão foi observado em cinco dos sete habitats aqui avaliados. Entretanto, o *H.* (*Haemoproteus*) foi o parasito mais comum entre as aves do bioma Caatinga, enquanto o *H.* (*Parahaemoproteus*) foi prevalente no habitat de Restinga. Entre as 69 linhagens amostradas (linhagens detectadas no mínimo quatro vezes), 48 eram de habitat geralista (36 *Plasmodium*, sete *H.* (*Parahaemoproteus*), e cinco *H.* (*Haemoproteus*), ocorrendo em duas ou mais espécies hospedeiras ou habitats. As linhagens de *Plasmodium* mostraram mais ampla gama de hospedeiro e habitats quando comparadas com *H.* (*Parahaemoproteus*) e *H.* (*Haemoproteus*). Encontramos 14 linhagens de *Plasmodium* que foram hospedeiras especialistas em habitats especializados. Os resultados sugerem a existência de linhagens hospedeiras generalistas de habitat especializados nos dois principais gêneros, *Plasmodium* e H*aemoproteus*, no Brasil. Mais estudos são necessários para entender os fatores que influenciam a forma como os parasitos haemosporidianos colonizam as comunidades de aves na região Neotropical.

Palavras chave: Haemosporida, *Plasmodium*, *Haemoproteus*, dinâmica temporal, distribuição,

Contents

1. General Introduction

Vector-borne diseases are infections transmitted by vectors to other organisms. Malaria, yellow fever, Dengue fever, Chikungunya, Leishmaniasis, Chagas disease are some examples of diseases transmitted by vectors. Many vector-borne diseases are transmitted by mosquito to human and other animals, although tick, bugs and fleas can also be other vectors. Traditionally, vector-borne diseases had been considered a problem in tropical and subtropical countries, but in the past twenty years the spread of many vectors to new geographical areas of the world poses a serious threat to global public health (WHO, 2014; Singh et al., 2016). Besides that, the shifting of parasites to new hosts can have serious implications for human health as well as to the management of wild and domesticated animal populations (Ricklefs and Fallon, 2002). Vector-borne diseases can exert negative effects on host populations by reducing growth and fitness, such as lower reproductive rates of the infected individuals and can cause mortality episodes in wild animals (van Riper et al., 1986; Marzal et al., 2005; Donovan et al., 2008; Norte et al., 2009).

1.1. Avian haemosporidian parasites

Haemosporidian parasites (Sporozoa: Haemosporida) constitute a diverse group of protozoan including genera *Plasmodium*, *Haemoproteus, Leucocytozoon* and *Fallisia* (Valkiūnas, 2005). *Plasmodium* is associated with human malaria, causing severe health problems and high mortality rates in tropical and subtropical countries (WHO, 2010). These genera can infect squamate reptiles, turtles, birds, and mammals, using a variety of dipterans vector for their transmission (Valkiūnas, 2005).

Avian haemosporidians have long been used as a model organism to understand human malaria. However, due to the use of rodent malaria as a biological model and the success of in vitro cultivation of the main human malaria parasite, *Plasmodium falciparum*, research interests declined in this group at the end of the 20th century (Pigeault et al., 2015). Interestingly, in the past twenty years, avian malaria parasites have gained attention of the researchers due to the recognition of their significance as an important ecological and evolutionary model for studies of host-parasite systems (Braga et al., 2011). More than 200 species of genera *Plasmodium*, *Haemoproteus and Leucocytozoon* have been described from a variety of birds and approximately 20 genera of insect vectors had been identified throughout the world (Braga et al., 2011; Santiago Alarcon, 2012). Moreover, recent

cytochrome *b* gene sequence studies revealed a much higher diversity in those genera than previously thought (Bensch et al., 2004; Ricklefs et al., 2004; Waldenström et al., 2004; Santiago-Alarcon et al., 2012).

1.1.1. Diversity and phylogeny of avian haemosporidian parasites

The terminology "malaria parasite" has been in constant debate among researchers and evolutionary biologists due to the insufficient knowledge about the phylogeny of such parasites (Pérez-Tris et al., 2005; Valkiūnas, 2005). Traditionally, *Plasmodium* species were considered the true malaria parasites, and the terms "malaria parasite" and "avian malaria" have been used widely to distinguish *Plasmodium* species infecting mammalians and birds, respectively, and haemosporidiosis is used to describe infections by *Haemoproteus* (Valkiūnas, 2005; Lapointe et al., 2012). These two genera use different dipteran insects as vectors but have similar lifecycles in their invertebrate hosts. *Plasmodium* use Culicidae mosquitoes belonging to different genera, *Haemoproteus* (*Parahaemoproteus*) are known to be transmitted by biting midges, mostly of the genus *Culicoides* (Ceratopogonidae), whereas *H.* (*Haemoproteus*) is transmitted by louse flies (Hippoboscidae) (Santiago-Alarcon, 2012). Based on genetic evidence and difference in biology, researchers have proposed to assign the rank of separate genera to the two subgenera of *Haemoproteus* (*Haemoproteus* and *Parahaemoproteus*). In a recent study from cranes (Gruidae), Bertram et al., (2017) also found genetic evidence that *Haemoproteus antigonis*, represents a novel clade, which is paraphyletic to other known *Haemosporida*, emphasizing the need to rank as a separate genus.

For a century, researchers have classified haemosporidian parasites on basis of their morphology, life cycle, and vertebrate and insect host taxa. Using morphological and biological characteristics, approximately 40 species of the genus *Plasmodium*, 130 species of the genus *Haemoproteus* and 30 species of the genus *Leucocytozoon* have been described (Valkiūnas, 2005). However, the development of the first molecular protocols for detecting and characterizing the avian malaria lineages in the field (Bensch et al., 2000) signaled the start of a new era in avian malaria research. Molecular studies have recovered over 650 cyt-*b* avian haemosporidian lineages, that can be accessed at a coordinated database (Mal-Avi, http://mbio-serv2.mbioekol.lu.se/Malavi) being possible to record the distributions of lineages and facilitate the investigation of global patterns (Bensch et al., 2009).

It is worth mentioning that there is some discrepancy about the relative importance of morphological characteristics and cyt-*b* sequence differences for establishing species limits in avian malaria (Outlaw and Ricklefs, 2014), while several lines of evidence suggest that most of the cyt-*b* lineages are reproductively isolated units (Bensch et al., 2009).

Molecular studies using single gene phylogeny (Perkins and Schall, 2002; Ricklefs et al., 2004) reveled relatedness proximity of birds and reptile *Plasmodium* and *Haemoproteus* rather than other mammalian *Plasmodium* species. Based on the knowledge of cyt-*b* sequencing, some authors include other genera especially the *Haemoproteus* in the group of malaria parasite (Pérez-Tris et al., 2005). However, another study using four genes of the parasites' three genomes - two mitochondrial genes: cyt-*b* and cytochrome oxidase I, one nuclear gene (adenylosuccinatelyase) and one plastid gene (caseinolytic protease), show that the genus *Plasmodium* is monophyletic with respect to *Haemoproteus* (Martinsen et al., 2008). Using both Bayesian and maximum parsimony analyses, the authors concluded that the genus *Plasmodium* is paraphyletic with respect to *Hepatocystis*, a group of species with a different life cycle and morphology, and that *Plasmodium* of birds and squamate reptiles all fall within a single clade, with evidence for repeated switching between birds and squamate hosts. In contrast, Borner et al. (2016) analyzed a set of 21 nuclear genes, and the resulting phylogeny showed *Plasmodium* as monophyletic and *Leucocytozoon* in a basal position to the rest of the Haemosporida. In addition the author created a data set by combining his own data with two previous published data (Martinsen et al.,2008 and Schaer et al., 2013) resulting in a dataset of 26 genes from 103 haemosporidian parasites. The result of the combined dataset was congruent with the author result of 21-gene dataset results (Figure.1). All these results suggest that the phylogeny of haemosporidian parasites is still a topic of debates for researchers.

Figure1. Majority-rule consensus tree based on a MrBayes analysis of the combined nucleotide dataset comprising the sequence data from Martinsen et al., (2008), Schaer et al,. (2013) and Borner et al., (2016). Splits with a posterior probability 0.95 are indicated by a hollow dot, splits with 1.00 posterior probability are indicated by a filled dot. Taxa included in the nuclear dataset are highlighted in bold (adopted from Borner et al., 2016)

1.1.2. Brief description of the life cycle of *Haemoproteus* and *Plasmodium* (Haemosporida) parasites in birds

Life cycle of *Plasmodium* spp.

The development of *Plasmodium* species in avian hosts can be divided into exoerythrocytic merogony, erythrocytic merogony and gametocyte formation. The exoerythrocytic merogony is divided into primary merogony (pre-erythrocytic) and secondary merogony (post-erythrocytic). Cryptozoites and metacryptozoites are the meronts of primary exoerythrocytic merogony, while secondary exoerythrocytic merogony consists of many generations of meronts, named phanerozoites. The vector injects sporozoites into the host, along with saliva during blood feeding. Sporozoites invade the reticular cells of many organs and tissues and develop into primary exoerythrocytic meronts (cryptozoites). Merozoite formation occurs there, which activate the formation of the second generation of primary exoerythrocytic meronts (metacryptozoites) inside the macrophage. Cryptozoites and metacryptozoites are similar but the later contains greater number of merozoites. The period from the inoculation of sporozoites until the maturation of first generation of metacryptozoites is named the prepatent phase of development(Valkiūnas, 2005; Figure 2).

Some of the newly formed merozoites activate the production of the next generation of metacryptozoites and phanerozoites while some invade the erythrocyte, giving rise to agamic stages and gametocytes. The merozoites invade the erythrocyte and after development it changes into trophozoite and later into erythrocytic meronts. The phase in which the parasite is detectable in high number in the blood stream is named the acute phase followed by the crisis phase in which the parasitemia reaches to its peak. Some merozoites formed during this process develop into gametocytes while the rest of them invade the endothelial cells of various organs where secondary exoerythrocytic merogony (phanerozoite) occurs. Parasitemia is low during the chronic or latent phase due to the host's immune response, causing little or no clinical signs. This chronic infection can be maintained for many years (Valkiūnas, 2005).

The disease is transmitted during blood feeding of female mosquitoes (Diptera: Culiciadae). Majority of the vectors species belongs to the genera *Culex, Aedes, Culiseta* and *Anopheles.* The vector ingests the gametocytes during a blood meal while feeding on an infected bird. Inside the midgut, gametocytes escape from the erythrocyte; gametogenesis and sexual reproduction occur being the ookinete promptly formed. Ookinete migrates towards the

epithelial cells of midgut and transforms into oocysts in the basal lamina. Many sporozoites (infective forms) are produced by the process of sporogony inside the oocysts. After maturation, the oocysts rupture, releasing many sporozoites which invade the salivary glands of competent vectors (Valkiūnas, 2005).

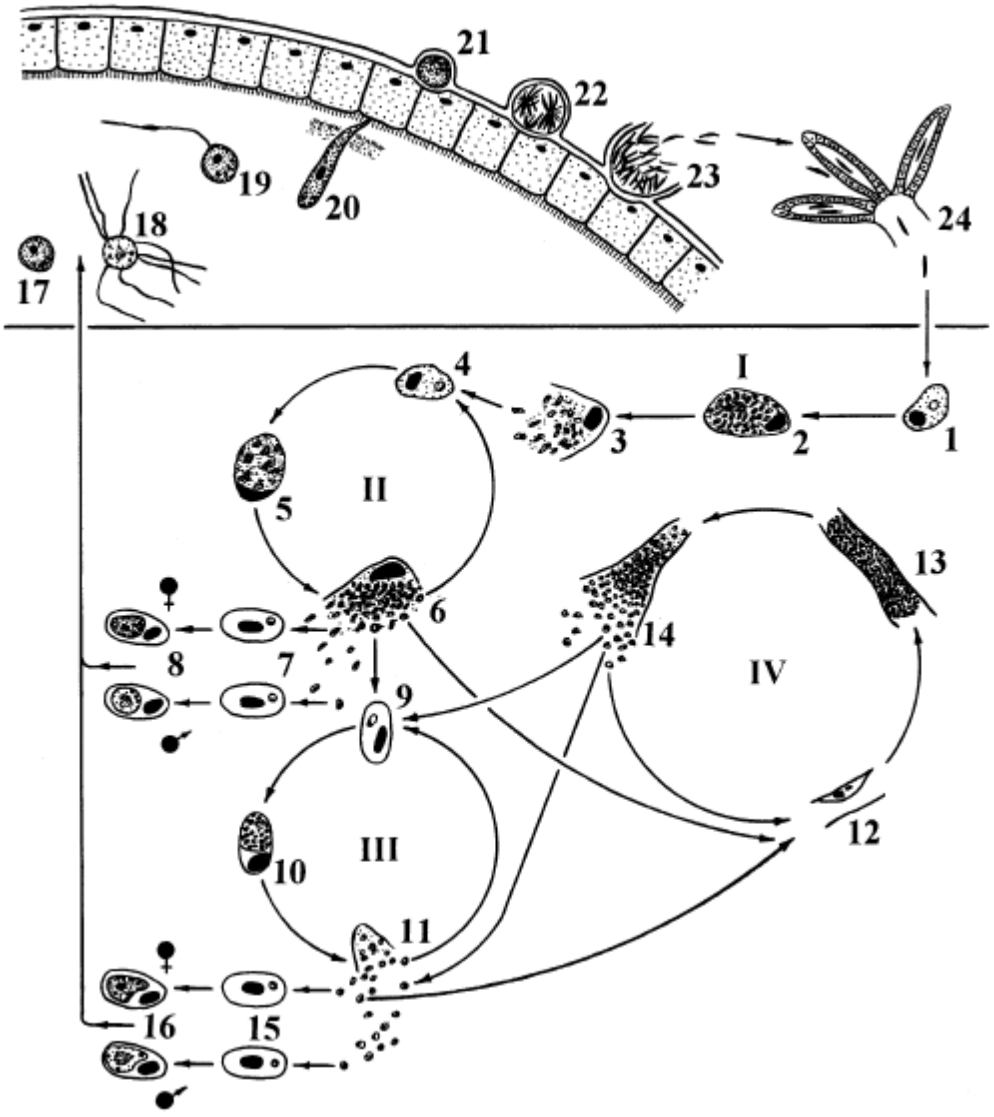

Figure2. Diagrammatic representation of the life cycle of bird malaria parasites (*Plasmodium relictum*as an example) Upper part, in vector; lower part, in bird: I, II – primary exoerythrocytic merogony; III – erythrocytic merogony; IV – secondary exoerythrocytic merogony; *1* – sporozoite in reticuloendothelial cell; *2*, *3* – cryptozoites; *4* – merozoite in macrophage; *5*, *6* metacryptozoites; *7* – merozoites in erythrocytes; *8* – gametocytes; *9* – merozoite in erythrocyte; *10*, *11* – erythrocytic meronts; *12* – merozoite in endothelial cell of capillaries; *13*, *14* – phanerozoites; *15* – merozoites in erythrocytes; *16* – gametocytes; *17* – macrogamete; *18* – exflagellation of microgametes; *19* – fertilization of macrogamete; *20* – ookinete penetrating the peritrophic

membrane; *21* – young oocysts; *22*, *23* – sporogony; *24* – sporozoites in the salivary glands of vector (extracted from Valkiūnas, 2005).

Life cycle of *Haemoproteus* spp. with respect to birds

The life cycle of *Haemoproteus* does not include an erythrocytic merogony as described for *Plasmodium* species. The parasite is transmitted by a variety of vectors including biting midges (Ceratopogonadie) and hippoboscid flies (Hippoboscidae). Sporozoites (infective stage) enter the blood circulation by a vector bite. Sporozoites invade cells of various organs such as lungs, heart, liver, spleen, kidney and other organs and develop into exoerythrocytic meronts evidencing various sizes and shapes at least for two generations. The second generation meronts contain many roundish merozoites which then invade the red blood cell and become infectious gametocytes, which are ingested by the vector during blood feeding. Inside the vector the gametocyte undergoes sexual and asexual reproduction and produces a considerable number of sporozoites. These forms invade cells of the salivary glands and are transmitted to a new host by the vector (Valkiūnas, 2005; Figure 3).

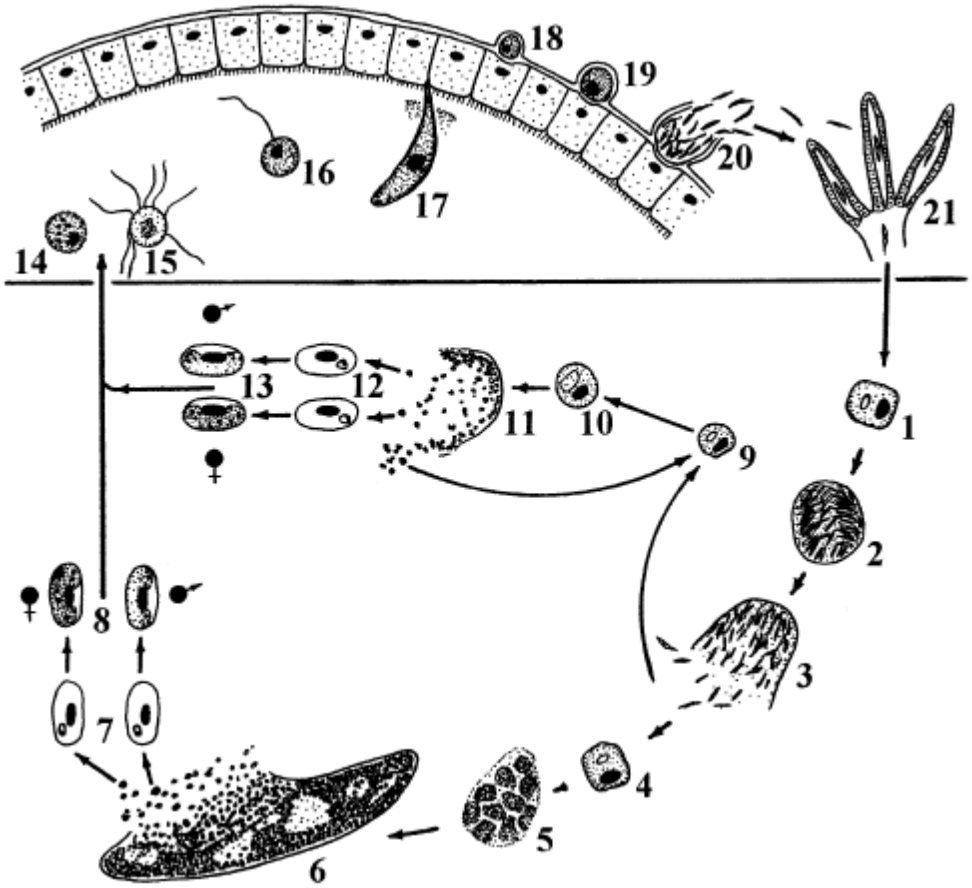

Figure3. Diagrammatic representation of the life cycle of bird haemoproteids (*Haemoproteus mansoni* as an example) Upper part, in vector; lower part, in bird: *1* – sporozoite in endothelial cell; *2*, *3* – exoerythrocytic meronts of the first generation with elongated merozoites; *4* – merozoite in endothelial cell; *5*, *6* – growing and mature megalomeronts in skeletal muscles, respectively; *7* – merozoites in erythrocytes; *8* – mature gametocytes; *9* – merozoite in reticuloendothelial cell in spleen; *10*, *11* – growing and mature meronts in spleen, respectively; *12* – merozoites in erythrocytes; *13* – mature gametocytes; *14* – macrogamete; *15* – exflagellation of microgametes; *16* – fertilization of macrogamete; *17* – ookinete penetrating the peritrophic membrane; *18* – young oocysts; *19*, *20* – sporogony; *21* – sporozoites in the salivary glands of vector (extracted fromValkiūnas 2005).

1.1.3. Effect of haemosporidian infection on avian hosts

Avian haemosporidians had been considered non-pathogenic to their hosts in the natural habitat for a long time as infected animals seemed to be free of clinical disease (Valkiūnas, 2005). However, recent studies have demonstrated negative impacts on host populations with effects that range from growth and fitness reductions, decrease in life span, reproductive success and quality of offspring to mortality episodes of infected birds (Asghar et al., 2015; Marzal et al., 2005; van Riper et al., 1986). The introduction of *Plasmodium relictum* to native Hawaiian forest bird species caused the reduction and extinction of native bird

populations and species, representing a striking example of the impact of a specific disease on a local bird community (Atkinson and Samuel, 2010). Other studies have showed that *Plasmodium* and *Haemoproteus* infections cause an impact on host survival and fitness resulting in significant changes in the humoral immune responses of infected hosts (Ortego et al., 2008; Knowles et al., 2010). However, it is worth mentioning that individuals with chronic infection play a key role by acting as parasite reservoirs, ensuring further transmission to susceptible hosts. Chronically infected host may experience episodes of high parasitemia during reproductive seasons if the host immunity is weakened (Atkinson and Iii, 1991; Schoener et al., 2014).

Most data showing negative impact of avian malaria arise from experimental studies by either inoculation of parasite directly to uninfected birds or by removing the parasite by medication (Atkinson and van Riper III, 1991; Valkiūnas, 2005; Marzal et al., 2008). Marzal et al., (2005) investigated the effect of *Haemoproteus* spp. on the reproductive success of migratory house martin (*Delichonurbica*) by treating birds with antimalarial drug (primaquine). The treated birds showed 18% larger clutch size and an increase in hatching and fledging success by 39% and 42% respectively. In a similar study, Knowles et al., (2010) tested the effect of chronic malaria infection for fitness effect in blue tits (*Cyanistes caeruleus*) by treating them with an antimalarial drug (Malarone^{TM}). They found increase hatching and fledging success in treated blue tits (*Cyanistes caeruleus*). In another study, Marzal et al., (2008) have demonstrated that haemosporidian infection was responsible for a decrease survival in house martin (*Delichonurbica*); more specifically, birds with a double infection presented lower survival rates when compared to single species infection.

Infected birds with high rate of infection are weak and less active in their natural populations; most of the studies of avian haemosporidian parasites used mist-netting method for capturing of wild birds, which is considered a bias method of sampling, as birds captured through this method are often active, healthy and with no sign of disease (Valkiūnas, 2005). Hence, it is possible that the available data showing prevalence in wild can be much higher than the already reported.

1.1.4. Avian malaria and temporal dynamic

Seasonal changes in temperature, precipitation and in any component of the environment exert a substantial impact on the dynamics of diseases (Altizer et al., 2006). Vectors, pathogens and hosts are all dependent on certain optimal environmental condition for

reproduction and survival, and alteration in these conditions can cause considerable effects on the dynamic of disease transmission (Patz et al., 2003; Altizer et al., 2006). A complete knowledge of transmission dynamics of parasites in nature requires understanding how temporal dynamic can impact the elements of a specific biological system such as host biology and susceptibility, immune responses, infection duration, breeding biology and transmission risk (Altizer et al., 2006). However, very few studies have been conducted in order to estimate the effect of temporal dynamic on avian haemosporidian parasites in tropical areas.

Owing to the dependence of vectors to the environmental conditions, temporal dynamic acts as an important factor in the spreading of vector-borne diseases (Hess et al., 2001). Most of the annual changes associated with vector-borne diseases are linked to the rainfall (Altizer et al., 2006). The larval stages of mosquitoes are dependent on water and studies reported a rise in cases of vector-borne diseases following a heavy rainfall (Hoshen and Morse, 2004). On the other hand, severe drought may eliminate breeding site in water deficient areas, while the low rainfall can convert flowing water (not suitable for breeding) into an isolated pool (suitable for breeding) and can boost the transmission (Landesman et al., 2007).

Besides precipitation, temperature also exert important effect on the survival of pathogens and vectors causing diseases (Patz et al., 2003). Low temperature can limit the growth and survival of vectors, while increase in temperature up to certain upper range can accelerate growth and development that can lead to earlier sexual maturity, facilitating increase in parasite transmission (Rogers and Randolph 1988; Altizer et al., 2006). Paaijmans et al., (2010) reported that daily changes in temperature affect rates of parasite infection, development and biology of mosquitoes, determining the intensity of parasite transmission. The development of parasite inside the vector is also dependent on temperature (Altizer et al., 2006). Hence, temperature is an important factor influencing transmission and continuous presence of haemosporidians in a host population (LaPointe et al., 2005; Gonzalez-Quevedo et al., 2014).

Studies have described an increase in prevalence of haemosporidian parasites according to high temperatures and rainfall, as both factors may favor growth and development of dipteran vectors (Hay et al., 2000; Cosgrove et al., 2008). In contrast, a study performed by Ishtiaq et al., (2017) in Himalayan foothill have not find any effect of temporal dynamic on avian malaria parasite prevalence. To better elaborate and understand the effect of temporal

dynamics on the prevalence of haemosporidian parasite further studies are required since rapid modifications of current patterns of temporal dynamic may become a major challenge for future ecological studies due to global climatic changes (Altizer et al., 2006).

1.1.5. Avian haemosporidian studies in Brazil

Brazil is a large tropical country and possess a great diversity of ecosystems supporting one of the most biologically diverse avifauna populations in the world (Marini and Garcia 2005). It is composed of several unique biomes, such as the Pantanal, the world's largest freshwater wetland; the Amazon, the largest tropical forest; the Cerrado, a unique type of savanna; the semi-arid Caatinga; the endangered Atlantic Forest; and the Pampas, highly productive grassland. Several studies addressed various aspects of avian haemosporidians parasites in different parts of Brazil (Ribeiro et al., 2005; Sebaio et al., 2010; Belo et al., 2011; Fecchio et al., 2011, 2013, 2017, 2018; Lacorte et al., 2013; Ferreira et al., 2016; Pinheiro et al., 2016; Ferreira Junior et al., 2017). Following, some studies reporting variable prevalence and diversity of avian haemosporidian parasites in different biomes are briefly discussed. Ribeiro et al., (2005) found a high prevalence of *Plasmodium* (39.6%) in passerine birds from Atlantic Forest. A relatively low parasite prevalence of *Plasmodium spp* (< 10%) was recorded in wild birds from the Brazilian Atlantic Rainforest and Cerrado in two different studies (Sebaio et al., 2010). Three Brazilian habitats within the Cerrado biome (intact Cerrado, disturbed Cerrado and transition area Amazonian rainforest-Cerrado) have been also evaluated for the presence of *Plasmodium* and *Haemoproteus* (Belo et al., 2011). However, neither prevalence nor diversity of infections by haemosporidians differed significantly among those three habitats. Lacorte and collaborators (2013) reported a high prevalence and lineage diversity of haemosporidian parasites in southeast Brazil and most of these lineages were reported for the first time. Ferreira et al., (2016) investigated the effect of habitat modification and temporal dynamic on the distribution of avian haemosporidian parasites in southeastern Brazil and found that avian communities inhabiting in advanced successional stages are associated with lower prevalence of avian haemosporidian parasites. Moreover, they also found effect of temporal dynamic on parasite prevalence and relative abundance of specific parasite lineages. Recently, a study performed by Fecchio et al., (2017) reveals a high diversity of haemosporidian parasites among Amazonian birds. Beside this great diversity of haemosporidian parasites in different region of Brazil, still there is a lack of information about avian malaria parasites in many regions such as Brazilian Caatinga biome.

1.2. Brazilian Caatinga

Caatinga is a semi-arid Brazilian biome that occupies 844,453 km^2 of the country's northeastern region, representing 54.53% of this geographical region of Brazil (IBGE, 2005). It is considered a semi-arid ecosystem due to its high solar radiation, high annual average temperature (26 to 28ºC), low cloudiness, and low relative humidity, with variation in annual rainfall ranging from 240 to 900 mm (Silva and Leal 2003). Inappropriately, for a long time this biome had been considered a low diversity region in terms of endemism and species richness (Vanzolini et al., 1980, Leal et al., 2005). However, recent studies suggest that biodiversity in Caatinga is much greater than already estimated, as its home to more than 500 bird species with 22 endemic to Caatinga.

A total of 932 vascular plant species, 240 fish species, 167 species of reptiles and amphibians, 62 families and 510 species of birds and 148 species of mammals so far has been recorded in this region (Leal et al., 2005). The plant composition is dominated by cacti, bromeliads, grasses, shrub and trees of low or medium height (3-7 meter) that shed their leaves seasonally, having deep and thick roots (Prado et al., 2003). In addition, the extremely irregular rains from year to year, results in severe periodic droughts (Leal et al., 2005). Despite this great diversity of avifauna, very little is known about vector-borne diseases in wild birds from this biome. However in a rescent study based on microscopic detection of blood smear, *Haemoproteus columbae* has been reported from a Columbiformes bird of the species *Columbina talpacoti* from the satate of Paraiba, northeastren Brazil (Lugarini et al., 2018). Moreover, due to irregular distribution of rainfall and long period of severe drought, it is important to know how this pattern of temporal dynamic affects the distribution of avian haemosporidian parasite in this particular set of environmental condition.

2. Relevance of the study

Haemosporidian is a diverse group of blood parasites that can infect a wide range of avian species around the world using arthropod vectors for its transmission (Valkiūnas, 2005). Presence of competent vectors, susceptible hosts and favorable environmental conditions are necessary for a successful transmission of parasite (Sehgal, 2015). Haemosporidian parasites in vertebrate host populations have contributed to the knowledge of many features of host-parasite ecology and evolution (Bensch et al., 2000). However, there is a lack of data on the influence of environmental condition on the transmission of avian haemosporidian parasites, where contradictory results have been described.

Brazil is a diverse country in term of ecosystems and avian species richness. Several studies have been conducted by our research group (Ribeiro et al., 2005; Belo et al., 2009; Sebaio et al., 2010; Lacorte et al., 2013; Motta et al., 2013; Ferreira et al., 2016; Pinheiro et al., 2016; Ferreira Junior et al., 2017; Fecchio et al., 2018) aiming to understand the distribution and diversity of avian haemosporidian parasites in different Brazilian biomes. Association between *Plasmodium* prevalence and habitat type and biological characteristics of birds (nest type, age, sex, feeding behavior, and species assemblage) in passerine birds of the Atlantic Forest has already been demonstrated (Ribeiro et al., 2005). Other study have evidenced a high diversity and similar lineage composition of haemosporidian parasites between Cerrado and the dry seasonal tropical forest but a different lineage composition in Atlantic rainforest (Lacorte et al., 2013). Previous studies addressing seasonal aspects of parasitism have shown variations year around such as a study conducted in a Seasonal Dry Forest (Mata Seca), depicting an increase parasite prevalence at the middle of the dry season, until the beginning of the following rainy season (Ferreira Junior et al., 2017). However, further studies are required to fully understand the influence of temporal dynamic in different biomes on the host-parasite systems. It is the case of Caatinga, an endemic Brazilian biome consisting of semi-arid lands, with low humidity and low rainfall. As the dry and rainy seasons are clearly differentiated, Caatinga is an ideal study site to investigate the effect of temporal dynamic on the diversity of avian haemosporidian parasites. Another peculiar characteristic of Caatinga is its high degree of endemism. It is worth mentioning that endemic species are facing greater risk of extinction in their natural habitat in many regions of the world mainly due to habitat loss and human intervention. Thus, here we explore the diversity and lineage composition of avian haemosporidian parasites among endemic birds from the Brazilian Caatinga and then

compare this data with the other Brazilian habitats aiming to explore the diversity and specificity of avian malaria lineages on a broad scale. We believe that our study will add important information that can be useful for avian species conservation strategies not only in Caatinga but throughout Brazil. This is the first study exploring the prevalence and diversity of avian haemosporidian parasites from the northeastern Brazilian semiarid Caatinga. Our study may add important and new information about the effect of temporal dynamic on the local haemosporidian-bird system that can be useful for the avian species conservation plans in Caatinga, as well as similar semiarid region across the world.

3. Objective and main tasks

3.1. Objective

The overall objective of the current work is to evaluate the prevalence and diversity of avian haemosporidian parasites in wild birds of the Brazilian Caatinga biome and to compare it with other Brazilian habitats.

3.2. Main tasks

- Determine the prevalence and diversity of avian haemosporidian parasites in Seridó Ecological Station using molecular tools.
- Investigate the effect of temporal dynamic on the distribution of avian malaria parasites in Brazilian Caatinga.
- Compare the prevalence and diversity of avian haemosporidians found in Caatinga with such features in other Brazilian biomes.

Chapter I

PREVALENCE, DIVERSITY, EFFECT OF TEMPORAL DYNAMIC AND HOST FUNCTIONAL TRAITS ON AVIAN HAEMOSPORIDIAN PARASITES IN THE BRAZILIAN CAATINGA BIOME

Introduction

Temporal dynamics acts as an important factor in the spreading of vector-borne diseases. Vectors, pathogens and hosts, are dependent on certain optimal abiotic conditions for reproduction and survival, and modification of such conditions may cause considerable effects on the transmission of several diseases (Hess, et al., 2001; Patz et al., 2003; Altizer et al., 2006). Low temperatures can limit the growth and development of blood parasites inside their vectors (Valkiūnas, 2005; LaPointe et al., 2010). On the other hand, high temperature can accelerate growth and earlier sexual maturity of parasites (Altizer et al., 2006). Similarly, studies reported a rise in vector borne disease after heavy rainfall and decrease in the prevalence after severe drought (Hoshen and Morse, 2004; Landesman et al., 2007). In order to understand the impact of temporal dynamic on avian haemosporidian parasites, extensive studies are required, as rapid modification in the pattern of temporal dynamic may become a major challenge to future ecological studies due to global climate change (Altizer et al., 2006).

Vector-borne parasitic diseases prevalence, diversity and distribution are largely associated with a number of biological and ecological traits (Poulin, 1997; Garamszegi, 2011; Wilkinson et al., 2016). For example, individual biological traits such as sex, plumage color, embryonic development and body condition are related with differences in blood parasite infection rates (Ricklefs, 1992; Norris, 2000; Wood et al., 2007). For example, a negative effect of haemosporidian on body condition and breeding performance was detected in Great Tit *Parus major* (Norte et al., 2009). Similarly, species level traits, such as habitat selection, feeding behavior, nest type, geographic distribution, flocking behavior and migratory behavior have all been implied as predictors of parasite prevalence (Ricklefs, 2005; Loiseau et al., 2010, 2012; Kamiya et al., 2014 Lutz et al., 2015). Prevalence of *Plasmodium* was positively associated with nest type; high infection rate in birds that form open cup and close cup nest (González et al., 2014).

Avian haemosporidian is a diverse group of protozoan parasites that include genera *Plasmodium* and *Haemoproteus* that infecting birds and use a variety of Diptera vectors for their transmission throughout the world. Recent studies have demonstrated negative impacts on host populations with effects ranging from growth and fitness reductions, decrease in life span, reproductive success and quality of offspring to mortality episodes of infected birds (Valkiūnas, 2005). Few studies addressed temporal dynamic of avian haemosporidian parasites in tropical environments with contradictory results. Ferreira Junior et al., (2017) found association of temporal dynamic with parasite prevalence and relative abundance of specific lineages in Brazil while Fallon et al., (2004) and Valkiūnas et al., (2004) in Caribbean region, found no association with temporal dynamic and haemosporidian prevalence. Thus, it is worth mentioning that additional studies in areas with a clear pattern of temporal dynamic are required to understand the haemosporidian temporal dynamics transmission and its determinants.

Caatinga is a semi-arid biome located in northeastern Brazil, covering an area of approximately 844,453 km^2. The climate in the region is classified as semi-arid according to Koöppen's classification (Alvares et al., 2013), characterized by a long dry season with irregular distribution of rainfall. Inappropriately, for a long time this biome had been considered a low diversity region in terms of endemism and species richness (Vanzolini et al., 1980, Leal et al., 2005). However, recent studies suggested high biodiversity in Caatinga. It homes more than 500 birds species with 22 endemic to Caatinga. It is formed by seasonally dry tropical forests (SDTF) whose physiognomy and floristic composition vary considerably, from open, shrub-like covers, to closed-canopy forest patches. SDTFs are one of the world's most threatened ecosystems, and are at risk for major biodiversity loss due to habitat fragmentation, deforestation for agriculture use, climate change and dry season fire (Murphy and Lugo 1986; Janzen 1988; Sánchez-Azofeifa et al., 2005, 2009; Espírito-Santo et al., 2009). SDTF have received relatively little attention from conservationists and ecologists in comparison to tropical rain forests (Pennington and Ratter, 2006). Thus, the aim of the study was to investigate the temporal dynamic of avian haemosporidian parasite in a Brazilian SDTF in Caatinga biome and to understand how host functional traits can influence parasite diversity and distribution in this environment. We hypothesized that: (1) host functional traits such as feeding behavior, flocking behavior, migration, nest type and habitat use would influence the prevalence of haemosporidian parasites infection in the Brazilian Caatinga; (2) Prevalence and diversity of haemosporidian parasites would vary in relation to seasons.

2. Material and Methods

2.1. Study area

The study was carried out at the Seridó Ecological Station - ESEC Seridó - (06 ° 34 '36.2 S and 37 ° 15'20.7 W), located in the municipality of Serra Negra do Norte, state of Rio Grande do Norte, encompassing an area of 1,163 ha (Figure 1). The climate of the region is semiarid, hot and dry (reaching up to 10 dry months) with irregular distribution of rainfall (Velloso, et al., 2002). In the Seridó Ecological Station, mean annual precipitation varies between 500 and 800 mm/year and mean annual temperature varies between 28°C and 30°C, with highest temperatures reaching 40°C and lower between 17°C and 20°C (Varela-Freire, 2004).

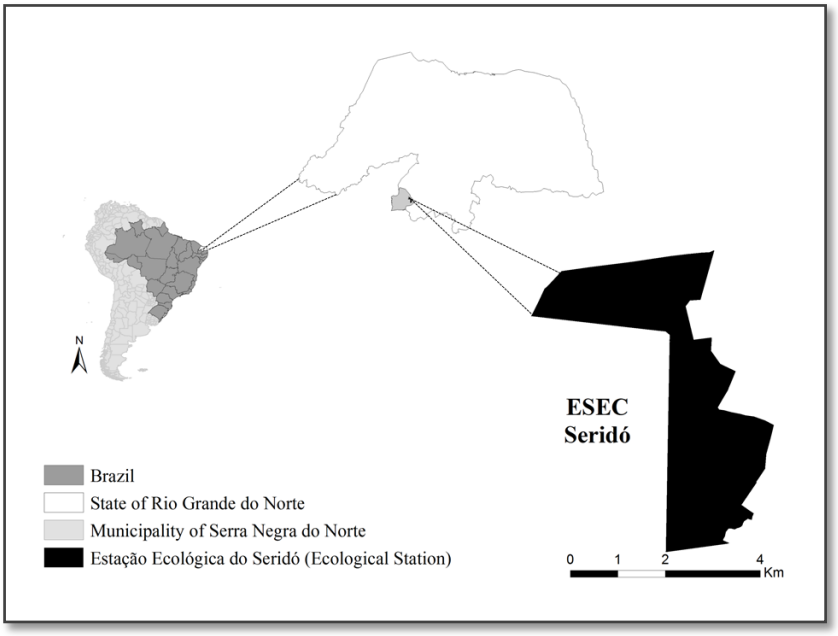

Figure1. Map of Seridó ecological station (ESEC) Rio Grande do Norte, Brazil

The vegetation of the Seridó region is classified as arboreal-shrub Caatinga, hyperxerophilous small trees with a height of less than 7 m, presenting a sparse distribution and a lower number of species in relation to other types of Caatinga biomes. Grasses are the predominant vegetation that cover the soil during several months of the year (Duque, 2004). According to Bezerra et al., (2012) the Seridó region has a very rich avifauna diversity, with 178 species representing 52 families, of which the Tyrannidae was the best represented family, with 23 species. Two species (*Rhea Americana* Linnaeus, 1758 and *Picumnus fulvescens* Stager, 1961) are native from the Caatinga biome and are considered near threatened species according to the International Union for Conservation of Nature (IUCN, 2016).

2.2. Birds samples collection

Wild birds were captured in four field campaigns, each one consisting of seven days of samplings in four different sampling periods (June 2013: “end rainy”, January 2014: “mid rainy”, July 2014: “beginning dry”, and December 2014 “end dry”). We selected an area of 350m by 250m at Seridó Ecological Station for sampling. Twenty-four ornithological mist nets of 18m length, 3m height and 19mm mesh, supported by aluminum stakes were used during field work. Nets were operated before dawn (between 4:30 am and 5:00 am), checked after every 30min and were closed before the high temperatures of the day (between 9: 30 am and 10: 00 am), adding an effort of 181,440 m^2 hour, (area of net (18 ×3) × (4 campaign ×7 days ×5 hours) ×24 nets) effort calculation based on Straube and Bianconi, 2002). Data regarding temperature and precipitation during the sampling were collected from the nearest ecological station (Figure 2).

Captured birds were identified, ringed and weighed; presence of ectoparasites (ticks, mites and lices) and incubation patch (brood patch) were examined. Sex and age (young and adults) were determined using plumage characteristic. Blood was collected from the brachial vein using insulin needles and stored on filter paper and captured birds were subsequently released in the same locality from where they were captured. All samples were collected under permit no.38647-2 provided by Sistema de Autorização e Informação em Biodiversidade (SISBIO).

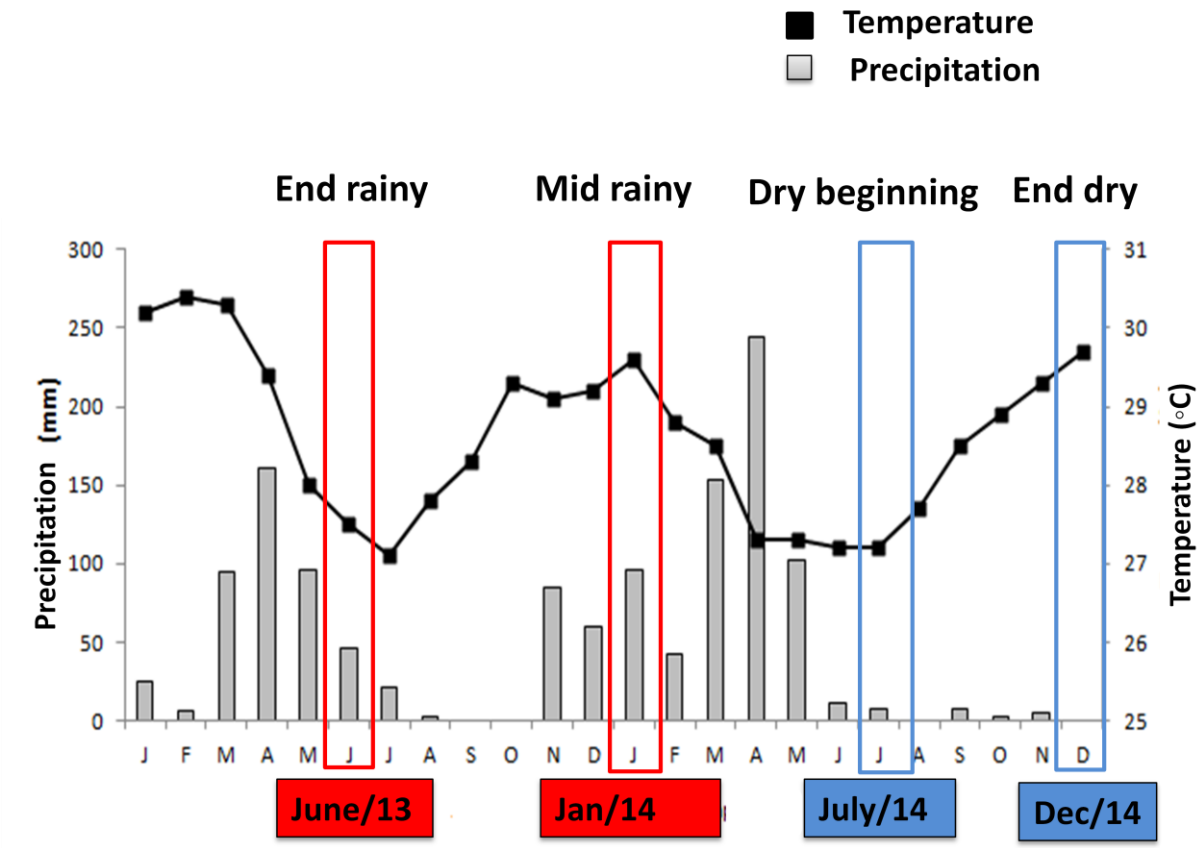

Figure2. Climatogram showing monthly temperature and precipitation during the four sampling periods.

2.3. Molecular characterization of haemosporidians

2.3.1. Genomic DNA extraction

The genomic DNA was extracted from the collected blood using phenol-chloroform technique followed by precipitation with isopropanol. Small pieces of the filter paper containing blood samples were placed in 1.5 mL microtubes, which received 250 µL of lysis buffer (50 mM NaCl, 50 mM Tris-HCl pH = 7.4, 10 mM EDTA, 1% (v / V) of Triton X-100, 200µg / mL of Proteinase-K), being incubated at 55°C 18-24 hours. Subsequently, 125 µL of buffered phenol and 125 µL of pure chloroform were added to the microtubes which were centrifuged at 12900 g for five minutes. The upper phase of the mixture was transferred to new 1.5 mL microtubes and 300 µL of chloroform-isoamyl alcohol solution was added at a ratio of 24: 1 being centrifuged at 12900 g for five minutes. In the next step, the lower phase of the mixture was carefully collected with micropipette and discarded. To the remaining liquid, 300 µL of pure chloroform was added and the material was centrifuged at 12900 g for five minutes. The lower phase was discarded and 600 µL of pure isopropanol at -20°C were

added to the microtubes. The microtubes were gently stirred until the observation of the precipitate and were subsequently centrifuged at 12900 g for 20 minutes. The liquid was discarded by inverting the microtubes and 600 μL of 70% ethanol at -20°C were added. After manual stirring, the material was again centrifuged at 12900 g for 20 minutes, ethanol was discarded, and the material was kept at 37°C until complete dried. The DNA was diluted in 1X TE solution (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and pH 8.0) at room temperature for 18 h or at 55°C for one hour and stored at -20°C.

DNA quantification for all the samples was performed using the NanoDrop™ Lite Spectrophotometer (Thermo Scientific®) according to the manufacturer's instructions**.**

2.3.2. Molecular detection of *Plasmodium / Haemoproteuss*

For the molecular detection of haemosporidiosis, PCR was performed for amplification of a highly conserved region of the mitochondrial SSU and LSU rRNA gene according to Fallon et al., (2003). This PCR can amplify both *Plasmodium* and *Haemoproteus* genera in the same reaction. The primers used were:
Forward primer 343F: 5'-GCTCACGCATCGCTTCT-3'
Reverse primer 496R:5'-GACCGGTCATTTTCTTTG-3'

In the amplification reaction, approximately 100 ng of the template DNA was used in 15 μL of reaction volume containing 1X buffer 10 (Phoneutria®); 3 mM MgCl2; 0.16 μM dNTP; 1 U Taq DNA polymerase (Phoneutria®); 0.2mM of each primer and ultra-pure sterile water.

The thermo cycler amplification program (SimpliAmp™ Thermal Cycler, USA Life Technologies) consisted of 30 cycles of denaturation at 94°C for 1 minute, followed by annealing at 62°C for 1 minute and extension at 72°C for 1 minute and 10 seconds. Initial denaturation occurred at 94°C for 2 min, and final extension at 72°C for 3 min, ending at 4°C. Positive controls were derived from genomic DNA of experimentally infected chicks with *P. gallinaceum*, kindly provided by the Medical Entomology Laboratory of the René Rachou Research Center - CPqRR, Belo Horizonte. Sterile ultrapure water was used as a negative control.

PCR-products were electrophoresed on non-denaturing 6% polyacrylamide gel in 1X TBE buffer. The gel was fixed in 10% ethyl alcohol solution and 0.5% acetic acid, stained in silver nitrate solution and the DNA fragments evidenced in a solution of sodium hydroxide and formaldehyde (Sanguinetti et al., 1994).

2.3.3. Nested-PCR for sequencing portion of cyt b gene of avian haemosporidians

Bird's samples that were positive in the screening PCR were subjected to Nested-PCR, described by Hellgren et al., (2004), which amplifies a fragment of 478 bp of the mitochondrial cytochrome b gene (cyt-*b*). The primers used were:

HaemNFI→ 5'-CATATATTAAGAGAAITATGGAG-3 '
HaemNR3→ 5'-ATAGAAAGATAAGAAATACCATTC-3 '
HaemF→ 5'-ATGGTGCTTTCGATATATGCATG-3 '
HaemR2→ 5'-GCATTATCTGGATGTGATAATGGT-3 '

For the first amplification reaction, about 100 ng of template DNA was used in 25 μL of reaction volume containing 1X buffer 10 (Phoneutria®); 3 mM MgCl 2; 0.125 mM dNTP; 1U Taq DNA polymerase (Phoneutria®); 0.4 mM of the HaemNFI and HaemNR3 primers and sterile ultrapure water. The first reaction program consisted of 25 cycles of denaturation at 94°C for 30 seconds, followed by annealing at 50°C for 30 seconds and extension at 72°C for 45 seconds. The initial denaturation occurred at 94°C for 3 minutes and the final extension at 72°C for 10 minutes, ending at a temperature of 4°C. Subsequently, 1 μL of the pre-amplified product was mixed with 24 μL of the buffer of the second reaction which is similar to the buffer of the first one, except that the primers used were HaemF and HaemR2, at the same concentration. The second reaction consisted of 30 cycles of denaturation at 94°C for 30 seconds, followed by annealing at 50°C for 30 seconds and extension at 72°C for 45 seconds. The initial denaturation occurred at 94°C for 3 minutes and the final extension at 72°C for 10 minutes, ending at a temperature of 4°C. The use of the positive and negative controls and electrophoresis of amplified products followed the same criteria of the PCR described above, to ensure the quality of the experiment.

2.3.4. Purification of Nested-PCR products

The purification of the amplified DNA was performed following the protocol described by Green and Sambrook (2012)with minor modifications. To the products of the two Nested-PCR (45μL), an equal volume of a 20% solution of PEG 8000 was added. After vortexing for 15 seconds, the mixture was incubated for 15 minutes at 37 °C and then centrifuged for 15 minutes at 12900 g. The supernatant was carefully removed and discarded. The pellet was washed by adding 125 μL of 80% ethanol and subsequent centrifugation. Washing was

repeated and the supernatant was discarded by inversion; the tube was then incubated at 37°C for at least 40 minutes to evaporate the ethanol. After that, 12 μL of sterile ultrapure water was added and the pellet was suspended by pipetting. Part of the purified DNA (1 μL) was electrophoresed using polyacrylamide gel electrophoresis technique and the remainder was stored at -20°C until use.

2.3.5. Sequencing and precipitation reaction of PCR products

The purified DNA was sequenced by the dideoxynucleotide method in ABI 3100® capillary automated sequencer (Perkin Elmer, USA) using the Big Dye Terminator Mix kit (Applied Biosystems, USA) according to the reaction and reading conditions indicated by the manufacturer. Approximately 2 μL of the purified PCR product were used in each reaction by adding 1 μL of HaemF and HaemR2 primers at 10 pmol concentrations in separate microtubes, 1 μL of Big Dye, 1.5 μL of *Save Money* buffer (Applied Biosystems, USA) and sterile ultrapure water to complete 10 μL. This reaction was performed in a thermo cycler (SimpliAmp™ Thermal Cycler, USA Life Technologies) using the following cycle: denaturation at 96°C for 15 seconds, annealing at 50°C for 15 seconds, extension at 60°C for 4 minutes, for 30 cycles. Then the product of the sequencing reaction was purified by precipitation using isopropanol and ethanol and homogenized in form amide. The products were sequenced using the facility of the Laboratory of Cellular and Molecular Parasitology, Center of Research René Rachou/ FIOCRUZ Minas Gerais (Belo Horizonte, Brazil).

2.3.6. Editing genetic sequences and phylogenetic analyses

Sequences obtained were aligned and edited using Chromas Pro (Technelysium Pty Ltd, Helensvale, Australia); presence of mixed infections was also checked (presence of double peaks in the eletrochromatograms). Sequences obtained were compared to those already deposited in free access databases such as GenBank (http://www4.ncbi.nlm.nih.gov) and MalAvi (Bensch et al., 2009 - http: // mbio-serv2., which is a cured database, which increases the reliability of the obtained data. Sequences with a minimum of one base difference were considered separate cytochrome b lineages, and those with no database record were considered novel lineages.

The phylogenetic inference used was the Bayesian phylogenetic maximum likelihood implemented by the MrBayes 3.2.2 program (Ronquist and Huelsenbeck, 2003) using the molecular evolution model GTR + I+ G, as recommended by Model Test (Posada and

Crandall, 1998) which selects the best-fit nucleotide substitution model for a set of genetic sequences. Two Markov chains were run simultaneously for 5 million generations in total that were sampled every 1000 generations. The first 1250 trees (25%) were discarded as a burn-in step and the remaining trees were used to calculate the posterior probabilities of each estimated node in the final consensus tree. *Leucocytozoon schoutedeni* was used as an out-group to root the genetic tree.

2.4. Statistical analysis

We used chi square test to check the prevalence and abundance of bird families and species along the different sampling periods. For further analysis we used only well-sampled bird species (species sampled 10 or more times), to eliminate the rare species, because species sampled only a few times can give a false idea of prevalence. We used a generalized linear model (GLM) to check the effect of each ecological functional traits (feeding behavior, flocking behavior, migratory behavior, nest type and habitat use) on prevalence as well as on relative prevalence of genus *Plasmodium* and genus *Haemoproteus* (*H. (Haemoproteus)* and *H. (Parahaemoproteus*) using sampling season as a replicate. It is worth mentioning that *H.* (*Haemoproteus*) subgenus infects only birds of the order Columbiformes and some seabirds (Valkiūnas, 2005, Levin et al., 2011). Thus, in order to check whether this large sample size is affecting the relative prevalence of *Plasmodium*/*Haemoproteus*, we did perform the analysis excluding Columbiformes. All the statistical analysis were done using R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/.Mix infection detected in the study were excluded from relative prevalence analysis but included in parasite diversity data.

3. Results

3.1. Bird Composition of Seridó Ecological station

A total of 933 birds were sampled from four different sampling periods (June 2013: "end rainy" n= 238, January 2014: "mid Rainy" n= 213, July 2014: "beginning dry" n=324, December 2014 "end dry" n=158; Table1), representing seven orders (Caprimulgiformes, Columbiformes, Cuculiformes, Galbuliformes, Passeriformes, Piciformes and Strigiformes), 20 families and 56 species. Families with the highest number of species sampled were Tyrannidae (n=15), Columbiformes (n=7), Thraupidae (n=6), Picidae (n=4) and Caprimulgidae (n=3), while individual species with the highest number of samples collected were *Columbina minuta* (n=401), *Coryphospingus pileatus* (n=78), *Phaeomyias murina* (n=41), *Myiarchus tyrannulus* (n=39), *Columbina picui* (n=36), *Myiodynastes maculatus* (n=26) *Volatinia jacarina* (n=20), *Ammodramus humeralis* (n=17), *Hemitriccus margaritaceiventer* (n=17), *Veniliornis passerinus* (n=16), *Pachyramphus polychopterus* (n=14), *Casiornis fuscus* (n=14), *Elaenia chilensis* (n=14), *Myiarchus swainsoni* (n=14), *Empidonomus varius* (n=13), *Tyrannus melancholicus* (n=11), *Coccyzus melacoryphus* (n=10), *Leptotila verreauxi* (n=10), *Polioptila plumbea* (n=10), and *Zenaida auriculata* (n=10) respectively (Table S1).

Twenty bird species from12 families were sampled 10 or more times; thus, we considered these birds as well-sampled species and families. Among the 20 "well-sampled" bird species, *Columbina minuta, Columbina picui, Hemitriccus margaritaceiventer, Coryphospingus pileatus, Leptotila verreauxi, Myiarchus tyrannulus, Myiodynastes maculatus, Nystalus maculatus, Polioptila plumbea, Veniliornis passerinus, Volatinia jacarina* and *Zenaida auriculata,* were sampled in the four periods of collection. *Ammodramus humeralis, Casiornis fuscus, Turdus amaurochalinus* were sampled in "end rainy", "mid rainy" as well as in "end dry" periods (no samples in dry beginning), while *Hydropsalis parvulus, Myiarchus swainsoni, Phaeomyias murina* and *Tyrannus melancholicus* were sampled in "end rainy", "mid rainy" and "dry beginning" (no samples in end dry period). The *Coccyzus melacoryphus, Empidonomus varius* and *Pachyramphus polychopterus* were sampled only in the "mid rainy" and "end rainy" periods while *Elaenia chilensis* was sampled only in the first "end rainy" period (Table S1).

3.2. General parasite prevalence

A total of 481 birds out of 933 samples (51.5 %) were infected by genera *Plasmodium/Haemoproteus* representing four orders, 16 families and 41 species. Prevalence varied among the well-sampled host families ($\chi 2 = 85.32$, df= 11, $P< 0.001$; Figure 3A) and varied from 0% (Cuculidae, n=10 and Caprimulgidae, n=10) to 61.8% (Thraupidae, n=11). Prevalence also varied in the 20 well-sampled species within a range of 0% (*Hemitriccus margaritaceiventer* n=15, and *Coccyzus melacoryphus* n=10) to 70% (*Volatinia jacarina* n=20) and (*Zenaida auriculata* n=10); while 62.1% prevalence was observed in the highly sampled (*Columbina minuta* n=401; Figure 3B). The prevalence of infection by haemosporidian parasites was also different among seasons ($\chi 2 = 17.204$, df= 3, $p< 0.001$). The highest prevalence (60.8%) was observed in July 2014 during the "beginning dry" period (Figure 4). However, this high prevalence was due to the large number of Columbiformes birds captured in this month as will be discussed later.

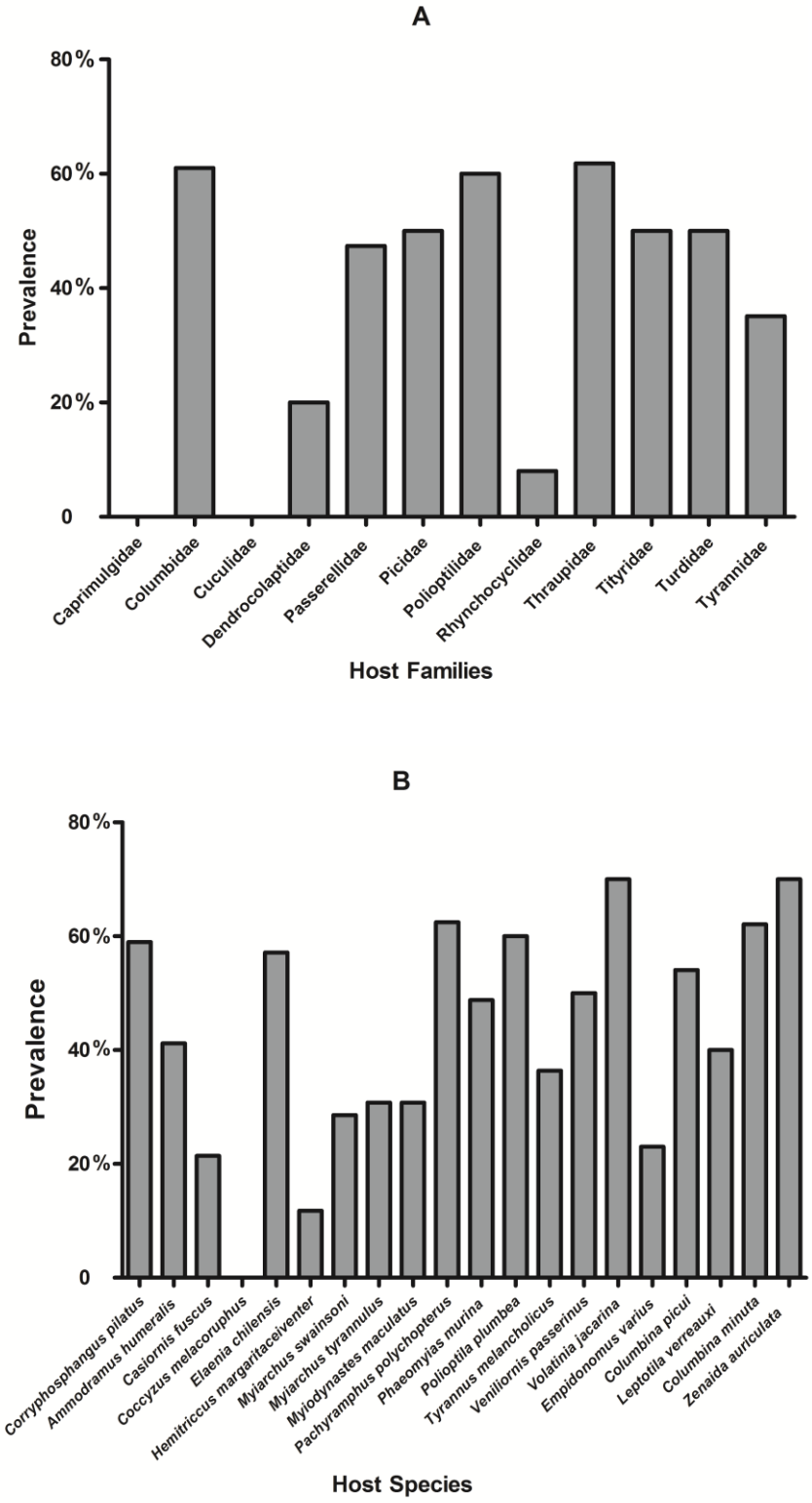

Figure3. Prevalence of haemosporidia infection in selected avian host families and species from the Brazilian Caatinga. A. Prevalence was also significantly different among the 12 well sampled families. ($\chi 2 = 71.63$, df= 19, $P < 0.001$). B. Prevalence was significantly different among the 20 well sampled species ($\chi 2 = 85.32$, df= 11, $P < 0.001$).

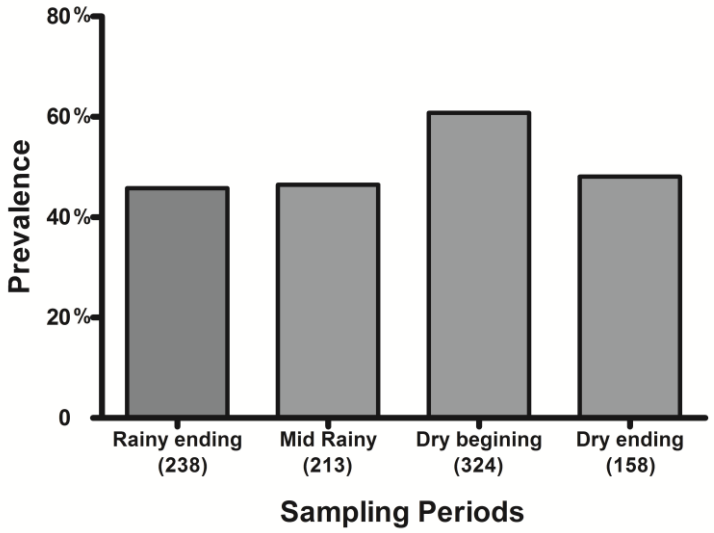

Figure4. Prevalence of haemosporidian infections among the four different sampling periods. ($\chi 2$ = 17.204, df= 3, P< 0.001). Numbers in parenthesis represent the total of birds analysed per sampling period.

3.3. Relative prevalence and host functional traits

We checked the prevalence of each host trait on general relative prevalence (Table 1). Parasite prevalence varied among the three different feeding guilds (F=13.46, df=06, *P* =0.006). Prevalence was higher in granivouros birds (347/572, 60.66%) when compared to omnivorous (08/14, 57.4%) and insectivores (79/225, 35.11%; Figure 5A). We did not find associations between parasite prevalence and any of other functional traits tested, i.e. flocking behavior (F=0.47, df=09, *P*=0.63), migratory behavior (F=0.47, df=09 P=0.63), nest type (F=1.17, df=09, *P*=0.35) and habitat use (F=0.47, df=09, *P*=0.63).

We checked the effect of each host trait on the relative prevalence of *Plasmodium* and both *Haemoproteu*s subgenera (Table 1). The relative prevalence of *H.* (*Haemoproteus*) was higher in granivorous birds (89/90, 98.8%; F=9.34, df=06, *P*=0.01), in species with single specie flocks (89/90, 98.8%; F=7.33, df=09, *P*=0.01), in species using open cup nests (89/90, 98.8%; F=7.1, df=0, *P*=0.01) and in birds independent of forest habitat (89/90, 98.8%: F=64.268, df=09, *P*<0.001; Figure 5 C-E). There were no differences in infection rates for *Plasmodium* and *H.* (*Parahaemoproteus*) in relation to host species traits.

Table.1 The results of a generalized linear model relating five avian functional traits to the general relative prevalence and to the prevalence of each parasites group in avian hosts.

Response variable	Explicative variable	F	df	*P*
Total Prevalence	**Feeding behavior**	**13.46**	**6**	**0.006**
	Migration	0.47	9	0.63
	Flocking behavior	0.47	9	0.63
	Habitat use	0.47	9	0.63
	Nest type	1.17	9	0.35
***Haemoproteus* (*Haemoproteus*)**	**Feeding behavior**	**0.34**	**6**	**0.01**
Prevalence	Migration	2.39	9	0.14
	Flocking behavior	**7.3**	**9**	**0.01**
	Habitat use	**64.26**	**9**	**0.001**
	Nest type	**7.1**	**9**	**0.01**
***Haemoproteus* (*Parahaemoproteus*)**	Feeding behavior	0.75	6	0.5
Prevalence	Migration	1.76	9	0.22
	Flocking behavior	0.61	9	0.5
	Habitat use	0.19	9	0.8
	Nest type	0.72	9	0.5
***Plasmodium* Prevalence**	Feeding behavior	3.02	6	0.12
	Migration	0.11	9	0.89
	Flocking behavior	0.11	9	0.89
	Habitat use	0.11	9	0.89
	Nest type	0.47	9	0.6

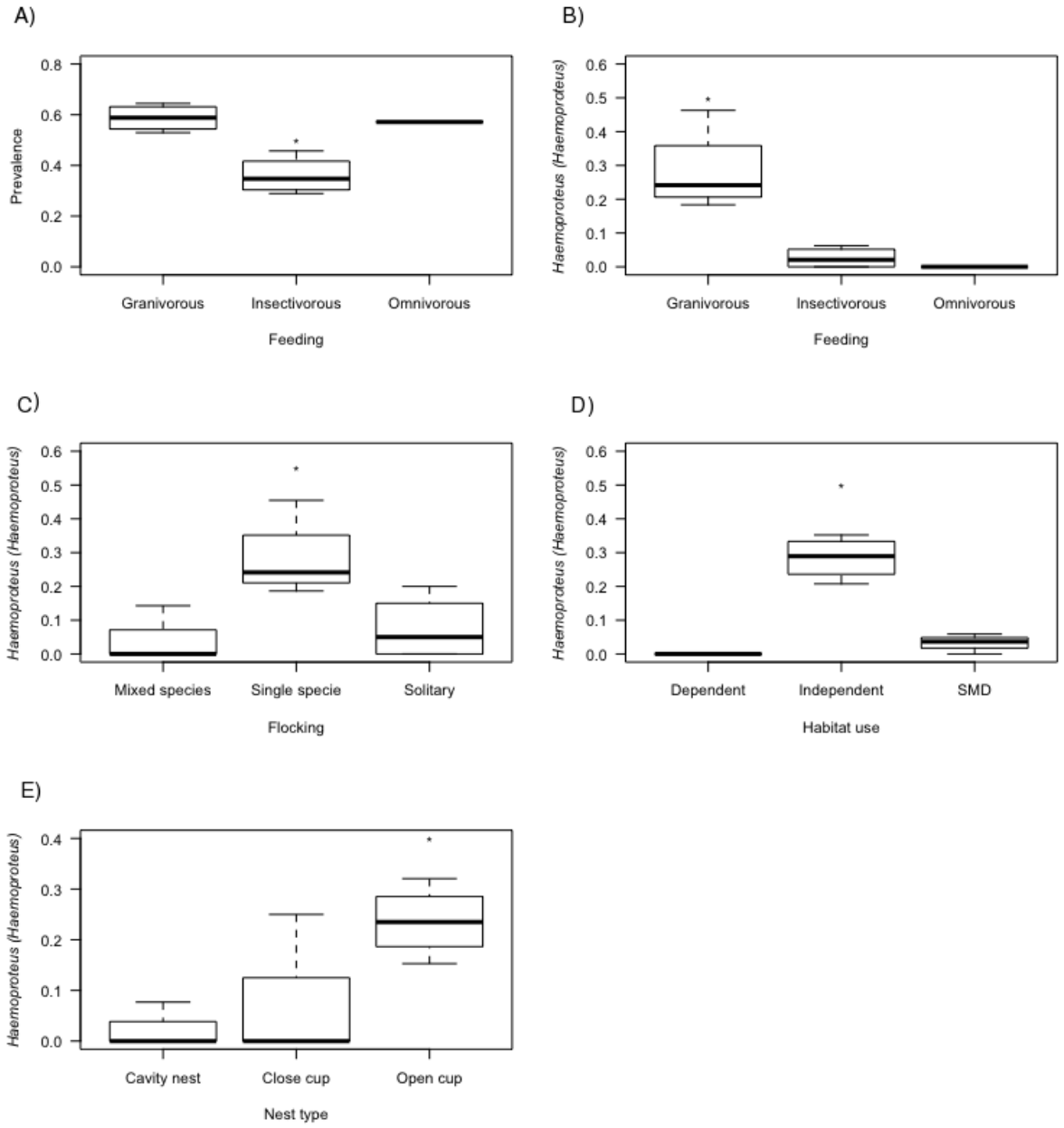

Figure5. Relative prevalence of Haemosporidian parasites in birds in respect to different functional traits. (A) Relative prevalence of all three parasites. (B-E) Relative prevalence of *Haemoproteus (Haemoproteus)* in relation to feeding behavior, flocking behavior, habitat dependence and nest type.

3.4. Relative prevalence and host functional traits without Columbiformes

In the present study, almost half of the bird 462/933 (49.5%) sampled during the four sampling periods belongs to family Columbidae (Table 2). It is worth mentioning that *H.* (*Haemoproteus*) subgenus infects only birds of the order Columbiformes and some seabirds (Valkiūnas, 2005, Levin et al., 2011). Thus, in order to check whether this large sample size is affecting the relative prevalence of *Plasmodium*/*Haemoproteus*, we did perform the analysis excluding Columbiformes. With that, we removed almost all of the *H.* (*Haemoproteus*) infections. *H.* (*Parahaemoproteus*) and *Plasmodium* mainly infected non-Columbiformes bird. We found association of feeding behavior (F=8.52, df=06, p=0.01) and flocking behavior (F=4.2, df=09, p=0.04) with prevalence (Figure 6A-B). *Plasmodium* prevalence was high in granivorous birds and mix species flocks while no effect on *H.* (*Parahaemoproteus*) was observed. Interestingly relative prevalence of *Plasmodium* was high in granivorous 39/59, 66.1%), open cup nester 51/59, 86.44%) and resident birds 48/59, 81.3, Figure 6C-E).

Table.2 Summary information of sampling seasons, including infection data

Sampling Year	Sampling season	Positive	Negative	Total	Prevalence
Overall					
June 2013	End rainy	109	129	238	45.79%
January 2014	Mid rainy	99	114	213	46.47%
July 2014	Beginning dry	197	127	324	60.80%
December 2014	End dry	76	82	158	45.79%
Total		**481**	**452**	**933**	**51.55%**
Without Columbiformes					
June 2013	End rainy	70	89	159	44.02%
January 2014	Mid rainy	59	91	150	39.33%
July 2014	Beginning dry	25	29	54	46.29%
December 2014	End dry	45	63	108	41.66%
Total		**199**	**272**	**471**	**42.255**

3.5. Parasite diversity

All 481 samples positive in the screening PCR were subjected to the cyt-*b* PCR and gene sequencing. However, we were able to sequence only 191 individual infections: 68 *Plasmodium* infections from 22 species, 90 *Haemoproteus* (*Haemoproteus*) infections from four species, and 19 *Haemoproteus* (*Parahaemoproteus*) infections from 10 species (Table 3). We detected 14 mixed infections in 10 bird species. The parasites community was

composed of 32 distinct lineages (*Plasmodium*=17; *Haemoproteus*=05 and *Parahaemoproteus*=10). Seven of these lineages were obtained for the first time in the present study (Table 3 and Figure 7). *Haemoproteus* (*Haemoproteus*) was the most common subgenus (50.8%; n=90) as compared to *Plasmodium* (38.4%; n=68) and *Haemoproteus* (*Parahaemoproteus*) (10.8%; n=19) ($\chi 2$ = 50.059, df= 2, P< 0.001; Figure8). Relative prevalence also varied among the four sampling periods ($\chi 2$ = 33.9, df= 6, P< 0.001). *Plasmodium* was the most prevalent in the "mid" (45.7%) and at the "end" (40%) of the rainy period (40%). *H.* (*Haemoproteus*) was more common in the "beginning" (76.7%) and at the "end" (50%) of dry period, while *H.* (*Parahaemoproteus*) was the low prevalent in all four sampling periods (Figure 9).

We observed a significant difference among host range profile according to the parasite lineages recovered. *H.* (*Haemoproteus*) mainly infected Columbiformes (89/90, four species), with one sequence detected in *Pachyramphus polychopterus* (Tityridae). This parasite subgenus was represented by five genetic lineages: SocH3 (n=69), SocH2 (n=3), COPIC01 (n=16) and two new lineages ZENAUR01 (n=1) and ZENAUR02 (n=2; (Table 3). Lineage SocH3 was obtained in all four sampling periods.

Table.3 Number of sequences of haemosporidian parasites obtained from Columbiformes and non-Columbiformes birds.

Birds	*Plasmodium*	*H.* (*Haemoproteus*)	*H.* (*Parahaemoproteus*)	Total
Columbiformes	8	89	2	99
Non-Columbiformes	60	01	17	78
Overall birds	68	90	19	177

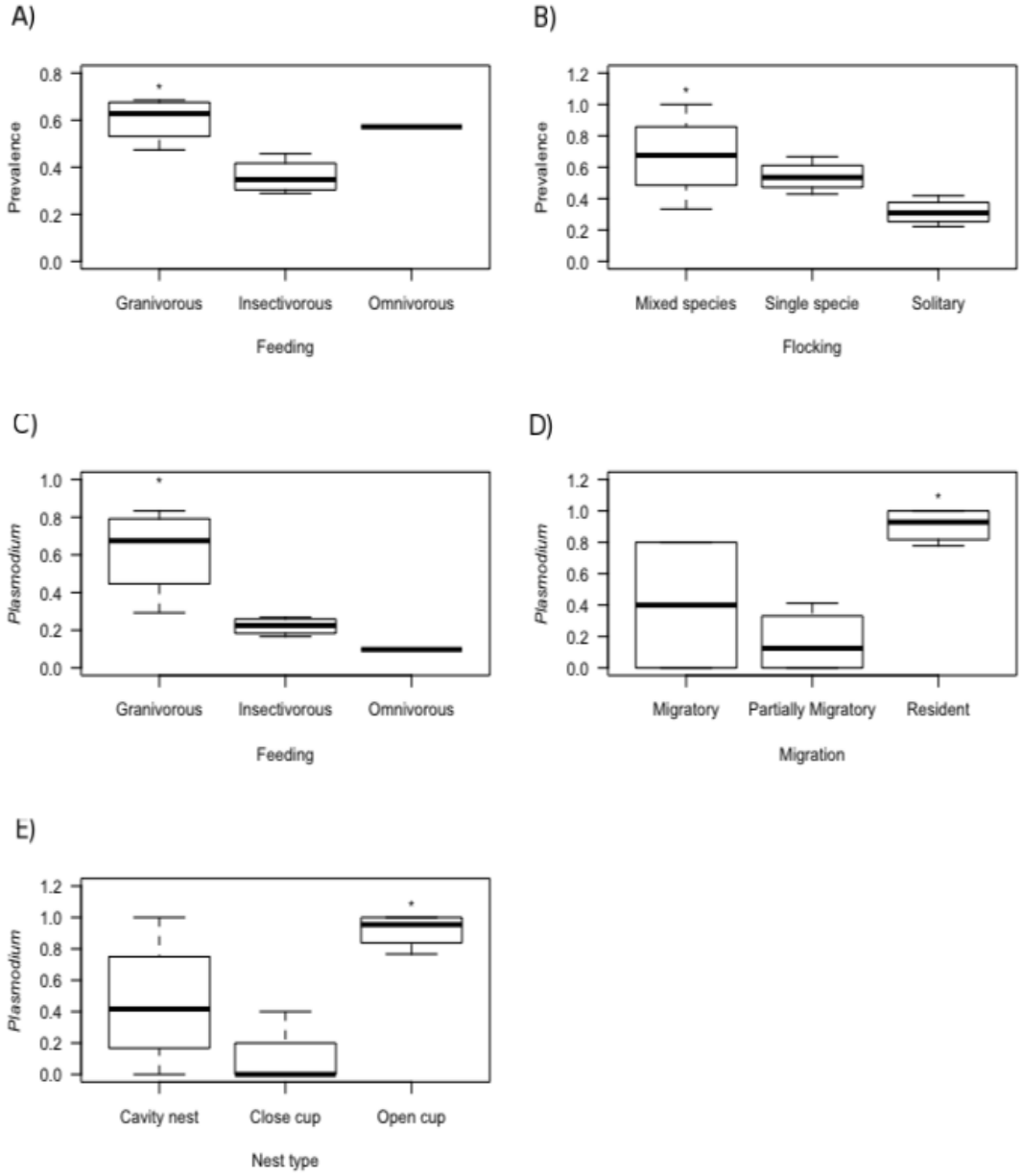

Figure6. Relative prevalence of Haemosporidian parasites in birds with respect to different functional traits excluding Columbiformes.(A-B) Relative prevalence of all three parasites to feeding guild and flocking behavior. (C-E) Relative prevalence of *Plasmodium* in relation to feeding guild, nest type and habitat dependence.

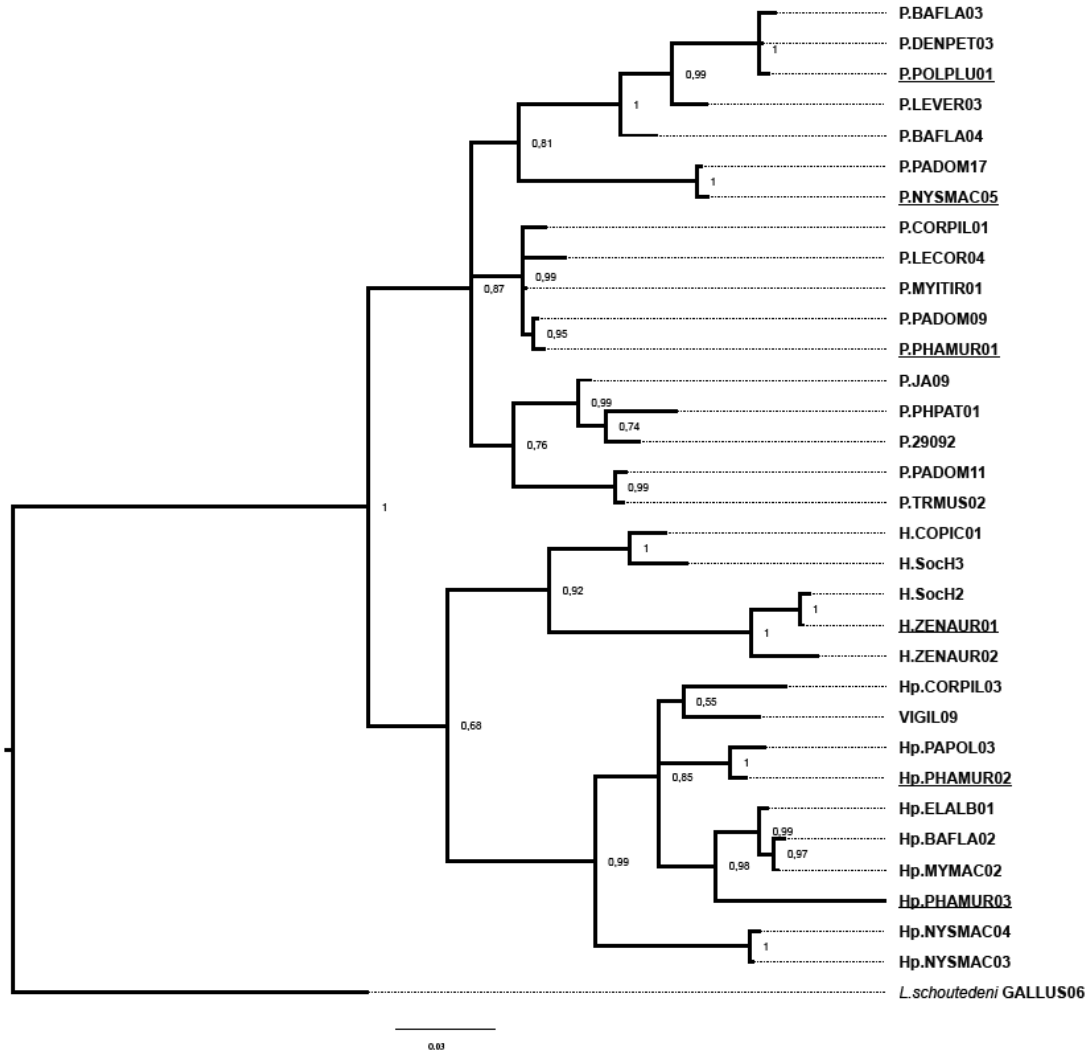

Figure7. Bayesian phylogenetic tree showing lineages detected in Brazilian Caatinga. Posterior probabilities and nucleotide changes (scale bar) are shown. *Leucocytozoon schoutedeni* represents the outgroup. New lineages reported for the first time are underlined.

We obtained 68 sequences of genus *Plasmodium* representing 17 lineages with three new descriptions POLPLU01, PHAMUR01 and NYSMAC05 (Table 3). Most common lineages were PADOM11 (15 times from six species), PHPAT01 (13 times from nine species), PADOM09 (10 times from eight species), LECOR04 (six times from two species) and LEVER03 (five times from two species), PADOM11 and LECOR04 were obtained in all four sampling periods, PHPAT01 was detected in the end of rainy and in the middle of the rainy period, while PADOM09 was obtained only in the end of rainy period (Table 4).

Table 4. Lineages detected in birds of Seridó ecological station during the four different sampling periods.

Lineages	Parasite Species	Host Species	Host Family	Sampling Periods				Total	Gene Bank Accession Number
				End Rainy	Mid Rainy	Beginning Dry	End Dry		
SocH3	*H.* (*Haemoproteus*)	*Columbina minuta*:66 *Columbina picui*:02 *Pachyramphuspolychopterus*:01	Columbidae Columbidae Tityridae	10 1 0	9 0 1	41 1 0	6 0 0	66 02 01	
SocH2	*H.* (*Haemoproteus*)	*Zenaidaauriculata*:02 *Columbina picui*:01	Columbidae Columbidae	01 01	0 0	01 0	0 0	02 01	
COPIC01	*H.* (*Haemoproteus*)	*Columbina minuta*:15 *Columbina picui*:01	Columbidae Columbidae	10 01	02 0	03 0	0 0	15 01	
ZENAUR01	*H.* (*Haemoproteus*)	*Zenaidaauriculata*:01	Columbidae	0	0	0	01	01	New Lineage
ZENAUR02	*H.* (*Haemoproteus*)	*Zenaidaauriculata*:01	Columbidae	0	0	0	01	01	New Lineage
PADOM11	*Plasmodium*	*Columbina minuta* :02 *Coryphosphangus pileatus*:08 *Molothrus bonariensis* :01 *Pitangus sulphuratus*:01 *Turdus rufiventris*:01 *Volatinia jacarina* :02	Columbidae Thraupidae Icteridae Turdidae Tyrannidae Thraupidae	0 01 01 0 0 0	01 05 0 01 01 0	01 01 0 0 0 01	0 01 0 0 0 01	02 08 01 01 01 02	
PADOM09	*Plasmodium*	*Ammodramus humeralis*:01 *Casiornis fuscus*:01 *Coryphosphangus pileatus*:01 *Pachyramphus polychopterus*:02 *Empidonomus varius*:01 *Phaeomyias murina* :02 *Tyrannus melancholicus* :01 *Volatinia jacarina* :01	Passerellidae Tyrannidae Thraupidae Tityridae Tyrannidae Tyrannidae Tyrannidae Thraupidae	01 01 01 02 01 02 01 01	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	01 01 01 02 01 02 01 01	
PHPAT01	*Plasmodium*	*Ammodramus humeralis*:02 *Columbinaminuta*:01 *Coryphosphangus pileatus*:02 *Elaenia chilensis* :03	Passerellidae Columbidae Thraupidae Tyrannidae	02 0 01 03	0 01 01 0	0 0 0 0	0 0 0 0	02 01 02 03	

		Myiarchus swainsoni :01 *Myiarchus tyrannulus*:01 *Myiodynastes maculatus*:01 *Paroaria dominicana*:01 *Phaeomyias murina* :01	Tyrannidae Tyrannidae Tyrannidae Thraupidae Tyrannidae	01 0 0 01 0	0 01 01 0 01	0 0 0 0 0	0 0 0 0 0	01 01 01 01 01	
LECOR04	*Plasmodium*	*Volatinia jacarina* :03 *Icterus jamacaii* :03	Thraupidae Icteridae	02 0	0 01	01 0	0 02	03 03	
LEVER03	*Plasmodium*	*Leptotila verreauxi* :02 *Columbina minuta*:03	Columbidae	01 0	01 0	0 0	0 03	02 03	
CORPIL01	*Plasmodium*	*Coryphosphangus pileatus*:04	Thraupidae	02	02	0	0	04	
BAFLA03	*Plasmodium*	*Zonotrichia capensis* :01 *Elaenia chilensis*:01 *Volatinia jacarina* :01	Passerellidae Tyrannidae Thraupidae	01 01 0	0 0 0	0 0 0	0 0 01	01 01 01	
DENPET03	*Plasmodium*	*Ammodramus humeralis*:01 *Volatinia jacarina* :01	Passerellidae Thraupidae	01 0	0 0	0 0	0 01	01 01	
MYITIR01	*Plasmodium*	*Myiarchus tyrannulus*:02	Tyrannidae	01	0	0	01	02	
PADOM17	*Plasmodium*	*Columbina minuta*:01	Columbidae	0	0	01	0	01	
TRMUS02	*Plasmodium*	*Polioptila plumbea* :02	Polioptilidae	01	0	01	0	02	
BAFLA04	*Plasmodium*	*Coryphosphangus pileatus*:01	Thraupidae	0	0	0	01	01	
JA09	*Plasmodium*	*Volatinia jacarina* :01	Thraupidae	0	01	0	0	01	
2092	*Plasmodium*	*Columbina minuta*:01	Columbidae	01	0	0	0	01	
PHAMUR01	*Plasmodium*	*Phaeomyias murina* :01	Tyrannidae	01	0	0	0	01	New Lineage
NYSMAC05	*Plasmodium*	*Nystalus maculatus*:01	Bucconidae	0	0	01	0	01	New Lineage
POLPLU01	*Plasmodium*	*Polioptila plumbea* :01	Polioptilidae	0	0	01	0		New Lineage Mix infection
PAPOL03	*H.* (*Parahaemoproteus*)	*Columbina minuta*:02 *Elaeniachilensis*:01 *Myiarchus swainsoni* :01	Columbidae Tyrannidae Tyrannidae	0 01 01	02 0 0	0 0 0	0 0 0	02 01 01	

		Pachyramphuspolychopterus:04	Tityridae	03	01	0	0	04	
BAFLA02	*H.* (*Parahaemoproteus*)	*Tyrannus melancholicus* :03	Tyrannidae	02	01	0	0	03	
NYSMAC03*	*H.* (*Parahaemoproteus*)	*Nystalus maculatus* :02	Bucconidae						Mix infection
NYSMAC04	*H.* (*Parahaemoproteus*)	*Myiodynastes maculatus* :01	Tyrannidae	01	0	0	0	01	
ELALB01	*H.* (*Parahaemoproteus*)	*Myiarchus swainsoni* :01 *Empidonomusvarius*:01	Tyrannidae Tyrannidae	01 01	0 0	0 0	0 0	01 01	
CORPIL03	*H.* (*Parahaemoproteus*)	*Coryphosphangus pileatus*:01	Thraupidae	01	0	0	0	01	
MYMAC02	*H.* (*Parahaemoproteus*)	*Myiodynastes maculatus* :01	Tyrannidae	01	0	0	0	01	
VIGIL09	*H.* (*Parahaemoproteus*)	*Vireo olivaceus* :01	Vireonidae	01	0	0	0	01	
PHAMUR02	*H.* (*Parahaemoproteus*)	*Phaeomyias murina* :01	Tyrannidae	01	0	0	0	01	New Lineage
PHAMUR03	*H.* (*Parahaemoproteus*)	*Phaeomyias murina* :01	Tyrannidae	01	0	0	0	01	New Lineage
Total (32)	**03**	**26 species**	**10**	**71**	**34**	**53**	**19**	**177**	

In red are the new lineages recovered in this study.

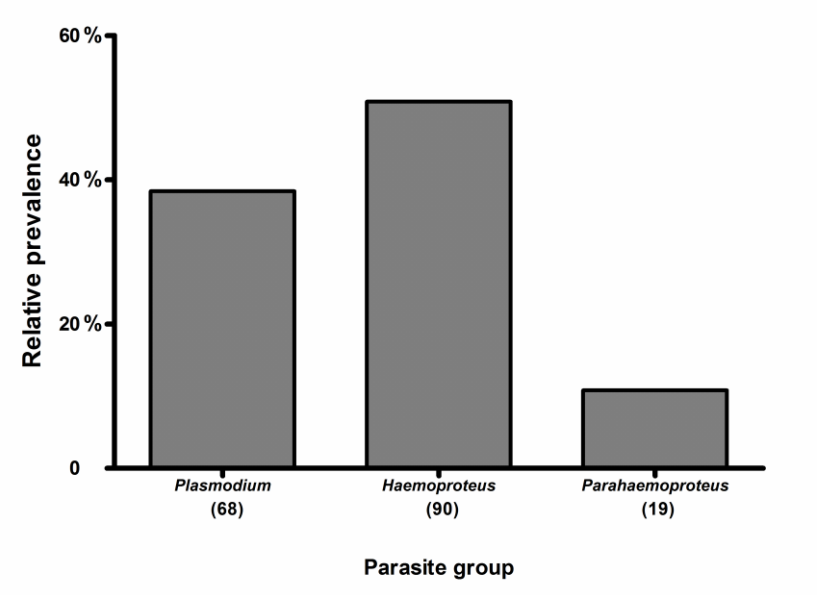

Figure8. Prevalence of the three parasites in avifauna of Seridó ecological station Brazilian Caatinga. Prevalence of the three genera was significantly different. ($\chi 2 = 50.059$, df= 2, $p < 0.001$).

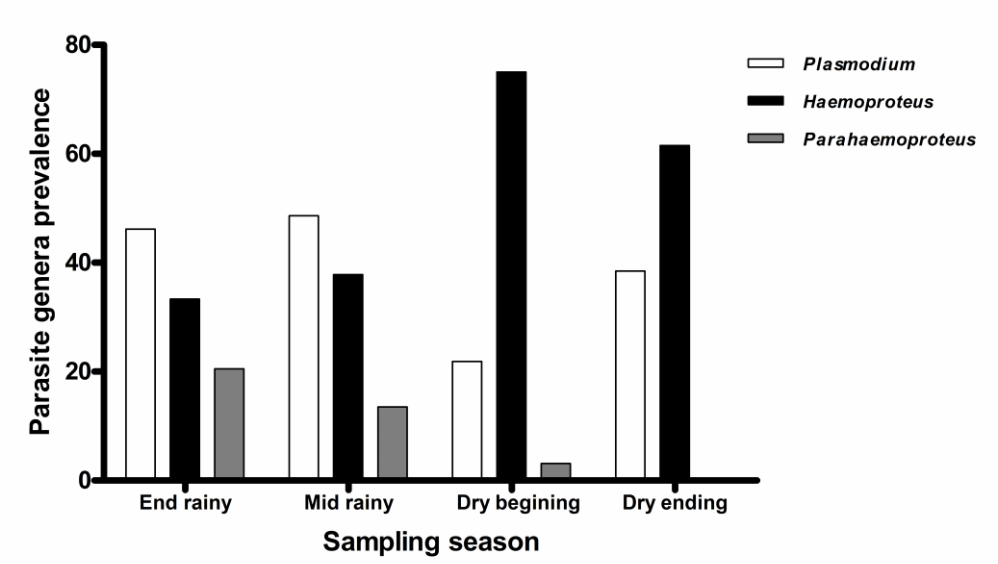

Figure9. Prevalence of haemosporidian genera was significantly different in four different sampling period in birds of Seridó ecological station ($\chi 2 = 33.68$, df= 6, $p < 0.001$).

H. (*Parahaemoproteus*) parasites were recovered 19 times representing 10 lineages infecting 10 bird species (Table 3). Lineage PAPOL03 was obtained eight times from four species, BAFLA02 three times from one specie, ELALB01 two times from two species and the remaining seven lineages (CORPIL03, MYMAC01, VIGLOG01, NYSMAC03, NYSMAC04, PHAMUR02 and PHAMUR03) were obtained only once (Table 3). The

lineage NYSMAC04 was found two times in a mix infection with NYSMAC03. *H.* (*Parahaemoproteus*) parasites were not detected at the end of the rainy season.

3.6. Host-parasite interaction among seasons

We compared the diversity of parasite lineages according to the season and we observed a difference in lineage diversity and host range among the four sampling periods (Figure10). Parasite lineage diversity was higher in both "end rainy" and "end dry" as compared to "beginning dry" and "mid rainy". A total of 24 lineages were recovered from 20 species in the "beginning dry" period as compared to "end dry" where 14 lineages were recovered from seven species. In the "dry beginning" period five lineages were recovered from seven species while 10 lineages were recovered from 12 species in "mid rainy" period.

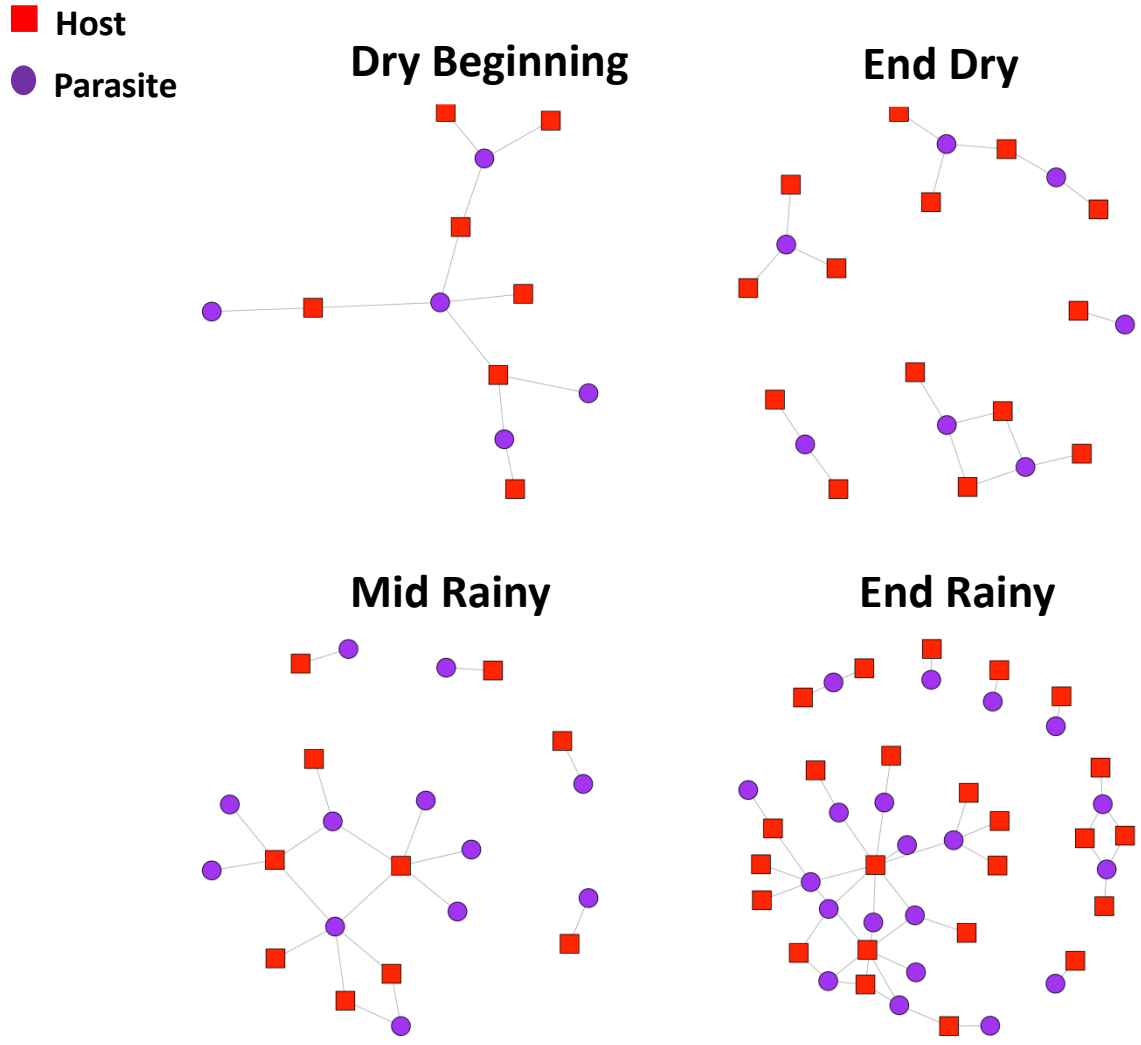

Figure10. Host-parasite lineage interaction with respect to sampling season. The square represents distinct host species and the circle represents parasite lineages.

3.7. Co-infections

We detected 14 co-infections based on the observation of double peaks in chromatogram, representing 6.9% of all infected birds (Table S2). All of them were intra-generic co-infections (seven *H.* (*Haemoproteus*), five *Plasmodium* and two *H.* (*Parahaemoproteus*). All of the co-infections with one double peak were resolved into their respective lineages by considering each peak for one lineage. We were able to identify only 12 lineages (three *H.* (*Haemoproteus*), five *Plasmodium* and four *H.* (*Parahaemoproteus*) from the co-infections detected.

4. Discussion

4.1. General parasite prevalence

We found a high haemosporidian prevalence in Caatinga (52%) when compared to other Brazilian habitats, such as 34% in tropical dry forest, 33% in Atlantic rainforests and 27 % in Brazilian savannah (Lacorte et al., 2013), 20% in Amazon rainforest (Fecchio et al., 2017), 42% in Tropical dry forest (Ferreira et al., 2017) and 24.6% in restinga (Rodrigues, unpublished data). High prevalence (50.9%) was observed for *Haemoproteus* (*Haemoproteus*) contrasting the prevalence detected for *Plasmodium* (38.4%) and *Haemoproteus* (*Parahaemoproteus*) (10.8%). This result is different from other studies that have already been conducted in Brazil which have found higher prevalence of the *Plasmodium* genus as compared to the *Haemoproteus* genus (Lacorte et al., 2013; Fecchio et al., 2017; Junior et al., 2017). This different scenario in parasite prevalence may be explained on the basis of distribution and abundance of different vectors as a consequence of climate factors (temperature, humidity, etc). Indeed, haemosporidian parasites used different dipteran vectors: Culicidae mosquitoes for *Plasmodium*, biting midges (Diptera: Ceratopogonidae) for *H.* (*Parahaemoproteus)* and hippoboscid flies (Hippoboscidae) for *H.* (*Haemoproteus)* (Valkiūnas, 2005). Climatic factors are important determinants of the transmission of vector-borne diseases due to their influence on vector distribution (Parham and Michael, 2010). One possible explanation may be the large sample size of Columbiformes in the present study where a single specie *Columbina minuta* was highly infected 92/401(22.9%); (*H. Haemoproteus n=82, H. Parahaemoproteus n=2 and Plasmodium n=08). High levels of infection among Columbiformes birds by H.* (*Haemoproteus) might be associated with its vector biology since* those flies (Hippoboscidae) nearly spend entire adult life on their host (Levin and Parker, 2014).

Prevalence also varied among seasons, with the highest prevalence in beginning dry. The majority of birds sampled in this period belong to the family Columbidae, which showed high parasite prevalence, and might have influenced this result. This bird group was mainly parasitized by *H.* (*Haemoproteus*), which is transmitted by Hippoboscidae flies which spend almost all of the adult life on its host (Levin and Parker, 2014; Valkiūnas, 2005). Moreover, the detection of *Plasmodium* and both *Haemoproteus* sub genera lineages in all four sampling periods reveals a year around transmission of theses parasite in Caatinga biomes.

4.2. Parasite diversity

A considerable number of lineages (*Plasmodium*=17; *Haemoproteus*=5 and *Parahaemoproteus*=10), including seven newly described ones reveals a high parasite diversity in the Caatinga biome. Both *Plasmodium* and *H.* (*Parahaemoproteus*) lineages infected a considerable number of bird species (22 and 10 species, respectively) as compared to *H.* (*Haemoproteus*) (only four species). Our finding depicting a high lineage diversity of *Plasmodium* as wells as a high lineage specificity of *Haemoproteus* spp. is consistent with two previous studies (Lacorte et al., 2013; Ferreira Junior et al., 2017), suggesting the existence of specific mechanism of host infection ranging from complete generalism to a high level of host specificity in the Brazilian parasite lineages.

The high diversity of haemosporidian parasite in Caatinga biome reveals the importance of studies in areas with a high degree of endemism. Moreover, we detected *H.* (*Haemoproteus*) lineages infecting passerine birds (SocH2 form *Myiarchus tyrannulus* and SocH3 from *Pachyramphus polychopterus*) instead of Columbiformes birds. This is not the first descriptions since, lineages from this subgenus have also been found infecting passerine birds in two others studies conducted in Brazil (Lacorte et al., 2013; Ferreira Junior et al., 2017). These findings highlight the possibility of the subgenus *H.* (*Haemoproteus*) using non-Columbiformes as possible hosts. However, it is worth mentioning that confirmation of a true infection must include the morphological characterization of these parasites to discard abortive infection (Valkiūnas et al., 2009). Unfortunately, we were not able to verify this due to lack of microscopic blood slides.

4.3. Host ecological traits and prevalence

Biological and ecological traits of parasites and their hosts can influence the prevalence and distribution of vector-borne parasitic diseases (Wilkinson et al., 2016). Here, we investigate the association of host functional traits with prevalence and diversity of haemosporidian parasites (*Plasmodium* and *Haemoproteus*) in birds captured in a seasonally dry ecosystem, the Brazilian Caatinga. We analyzed the effect of host life history trait on overall parasite prevalence and found that feeding behavior is a predictor of avian haemosporidian prevalence among our well-sampled species (bird species sampled more than four times). Several studies demonstrated the effect of host ecological traits on the probability of avian haemosporidian infection with varying degree of results in different zoogeographical regions (Fecchio et al., 2011, 2013, 2017; González et al., 2014; Lutz et al., 2015; Matthews et al., 2016).

Contrasting results have been addressed by some studies such as that performed by Laurance and co-workers (2013) in tropical northern Australian rainforest birds reporting similar results as those evidenced in our study, while Fecchio et al., (2017) have found no relationship between feeding behavior and haemosporidian prevalence in Amazonian rainforest birds. Excluding the large sample size of Columbiformes, same pattern for feeding behavior was observed with high prevalence among granivorous birds. Additionally, flocking behavior was also found to be associated with prevalence, with high prevalence in mixed species flock birds. At genus level, granivorous bird species were more likely to carry *Haemoproteus* (*Haemoproteu*s) infections when compared to omnivorous and insectivorous. Interestingly, excluding Columbiformes birds, same pattern was observed for *Plasmodium*. González et al., (2014) also found low prevalence of *Haemoproteus* spp. in insectivorous birds as compared to other diets while in contrast Laurance et al., (2013) found a high infection rate in insectivorous birds. All the above cited works have been done in tropical environments while the present study has been conducted in a seasonally dry ecosystem; thus, such differences might be due to distinct habitat profiles that may modulate the interactions between parasites, vertebrate hosts and their competent vectors (Valkiūnas, 2005).

We found a high prevalence of *H.*(*Haemoproteu*s) in birds that participate in single species flocks, which is consistent with the results of Fecchio et al., (2011), and Lutz et al., (2015). González et al., (2014), however, found frequent infections of *Haemoproteus* spp. in mix species flock. The single species flocking behavior of well-sampled *Columbina minuta* might be the reason for high *Haemoproteus* (*Haemoproteu*s) prevalence in our study. In case of *H.*(*Haemoproteu*s), social behavior (formation of single species flocks) of bird may be the reason that facilitates the vector transfer among the member of the social group, as its vector Hippoboscidae flies spend almost all adult life on its avian host (Valkiūnas, 2005; Levin and Parker, 2014). In contrast, Matthews et al., (2016) did not find any effect of flocking behavior on *Haemoproteus* spp. These differences in results suggest some biome specific effect of flocking behavior on *Haemoproteus* spp. infection pattern that deserves more investigation.

Haemoproteus (*Haemoproteu*s) infection rate was high in birds that form open cup nest. The same pattern was observed for *Plasmodium* when Columbiformes were excluded. Our findings in Caatinga partially match the previous results, consistent with data reported by Lutz et al., (2015), who found low *Haemoproteus* spp. infection in close cup and cavity nest. In contrast, González et al., (2014) and Matthews et al., (2016) did not find any association of nest types on *Haemoproteus* spp. infection. Matthews et al., (2016) found higher

Plasmodium infection in birds that form open cup nest but, Fecchio et al., (2011) and Lutz et al., (2015) found high *Plasmodium* infection in close cup nest. The higher infection of *Plasmodium* in bird that form open cup nest could be associated with the higher vector encounter rates because there is no physical barrier between the birds and vector compared to close cup and cavity nest which provide a natural protection. The conflicting results regarding nest type will require further investigation and comparison of a variety of host species with varying nesting strategies.

We found a high *Haemoproteus* infection in species that were forest habitat independent as compared to forest dependent and partially forest dependent species. This difference may be attributed to difference in vector encounter rate. Forest independent species forage widely for food, which may provide greater opportunity to encounter vector than the forest dependent and partially forest dependent species resulting higher *Haemoproteus* infection. Indeed, habitat generalist species have a high prevalence of blood parasites than species restricted to specific habitat (García-Longoria et al., 2014).

We did not find any association of migratory behavior with general prevalence or genera prevalence; however, excluding Columbiformes, *Plasmodium* prevalence was high in resident bird species compared to migratory and partially migratory species. Migratory species are thought to be highly infected with blood parasites because of their exposition to variety of vectors and parasite during their annual migration (Waldenström et al., 2002). Studies examining the influence of migratory behavior on blood parasites produced mixed results. Fecchio et al., (2013) found no relationship between prevalence and migration in Brazilian Cerrado. Matthews et al., (2016) found low prevalence of *Haemoproteus* spp in Neotropical migrant birds than partially migrants or resident species. Similarly, Slowinski et al., (2018) found a low prevalence of haemosporidian parasites in migratory song birds as compared to resident birds in Virginia. The high *Plasmodium* prevalence in resident birds could be explained by the weak host immune defense of the resident species compared to migratory species. It is hypothesized that migratory species have larger immune defense organs than the resident species due to greater exposure to diverse parasitic fauna (Møller and Erritzøe, 1998).

4.4. Conclusion

In conclusion, the present study showed that Caatinga birds might be infected by diverse blood parasite at the same level detected in other Brazilian habitats. Haemosporidian prevalence detected in birds from our study in Caatinga is higher than other Brazilian habitats. Prevalence varied among the four sampling season with high prevalence in the dry beginning season. *Plasmodium* prevalence was high in the end rainy and mid rainy seasons while *H.* (*Haemoproteus*) was more prevalent in the dry beginning and end dry seasons. Our result of host life history trait showed a diversity of relationship among the three parasites, *H.* (*Haemoproteus*) showed association with feeding behavior, flocking behavior, nest type and habitat use. *Plasmodium* also showed association with feeding behavior, nest type and migratory behavior when the large sample size of Columbiformes was excluded. These differences in association may be attributed to the use of different vectors. We detected seven new lineages in the study; further studies with diverse sampling hosts at multiple locations may reveal diversity and possible endemicity of haemosporidian lineages in Caatinga biome.

Chapter II

GOGRAPHICAL DISTRIBUTION OF AVIAN HAEMOSPORIDIAN PARASITES IN BRAZIL: NEW INSIGHTS FROM MOLECULAR DATA

1. Introduction

Dynamics of infectious disease transmission must be considered in an ecological framework as environmental factors seriously affect ecology and evolution of vector-borne infectious diseases (Patz et al., 2003; Pulgarín al., 2018), probably through their effects on vector and host populations (Roche et al., 2013). However, the tremendous variations of parasites, hosts, vectors across landscape, turn this scenario greatly complex impairing the knowledge of determinants enrolled in the transmission of parasites (Sehgal, 2015). Hence, comparative studies on a broader scale in different habitats are needed to understand mainly factors affecting parasite prevalence and richness at habitat level.

Parasites vary in the mechanism by which they infect their host. Generalist parasites can infect a diverse range of host, while specialist infects only a small subset of hosts (Walker et al., 2017). There are two hypotheses that explain mechanism of the parasite prevalence and richness. The "Niche-breadth" hypothesis that suggests that generalist parasites having the ability to infect a diverse range of host species will colonize different host communities efficiently than the host specialist parasites and thus will have broader range and abundance (Brown, 1984; Krasnov et al., 2004; Hellgren et al., 2009). On the other hand, "Trade-off" hypothesis suggests that specialization allows greater adaptation to a single host defense system and ultimately can achieve high prevalence and local abundance through entire range of that single host specie (Poulin, 1998; Poulin and Mouillot, 2004; Drovetski et al., 2014). Interestingly, both strategies seem to be successful for colonizing new areas and for local abundance (Drovetski et al., 2014). In contrast, another hypothesis ''The Integrative Hypothesis of Parasite Specialization", suggests that the phylogenetic distance of the host species determines host specificity of a parasite rather than the number of host being infected by the parasites (Pinheiro et al., 2016).

Avian haemosporidian is a diverse group of protozoan parasites that include four genera *Plasmodium, Haemoproteus, Leucocytozoon* and *Fallisia* and use a variety of Diptera vectors for their transmission. More than 200 species of these parasites have been described around the world using more than 16 insect genera as vectors (Valkiūnas, 2005; Marzal, 2012).

Studies reported global and heterogeneous distribution of avian haemosporidian parasites across different habitats and hosts (Lacorte et al., 2013; Loiseau et al., 2012; Clark et al., 2014). The high richness of haemosporidian parasites associated to their uneven distribution in wild hosts make them an excellent model for understanding the spatial variation in parasite prevalence and richness among different habitats.

Brazil is a large country with a diversity of ecosystem which supports one of the richest avifauna of the world, and is divided into several ecologically and biologically diverse regions (Marini and Garcia, 2005). Several studies have addressed various aspects of avian haemosporidian parasites in different habitats and pointed to a high lineage richness (Fecchio et al., 2011, 2013, 2017; Belo et al., 2012; Lacorte et al., 2013 Ferreira Junior. et al., 2017). However, most of these studies were conducted on regional scales. Here, we present a comparative study based on a broad spatial scale of avian haemosporidian lineages from seven different habitats throughout Brazil.

Our objective was twofold. First, we analysed the richness and distribution of genetically distinct avian haemosporidian lineages on a broad scale across geographically and environmentally distinct habitats. Secondly, we analysed the specificity of lineages to a particular habitat to understand if there is some regional specificity in lineages and/or if lineages restricted to a specific habitat have some preference for specific groups of avian hosts.

2. Materials and Methods

2.1. Database

A total of 1161 parasite lineages sampled from birds captured in seven different habitats (Amazon rainforest, Atlantic rainforest, tropical dry forest, Pantanal, Cerrado, Caatinga and Restinga) across Brazil were selected for this study (Table 1). These samples are part of haemosporidian parasite database from Malaria Lab from published and unpublished studies conducted by our research group. Samples were collected between 2000 to 2015 from different sampling sites across Brazil (Figure 1). Molecular identification of haemosporidian was performed as described in this chapter 1.

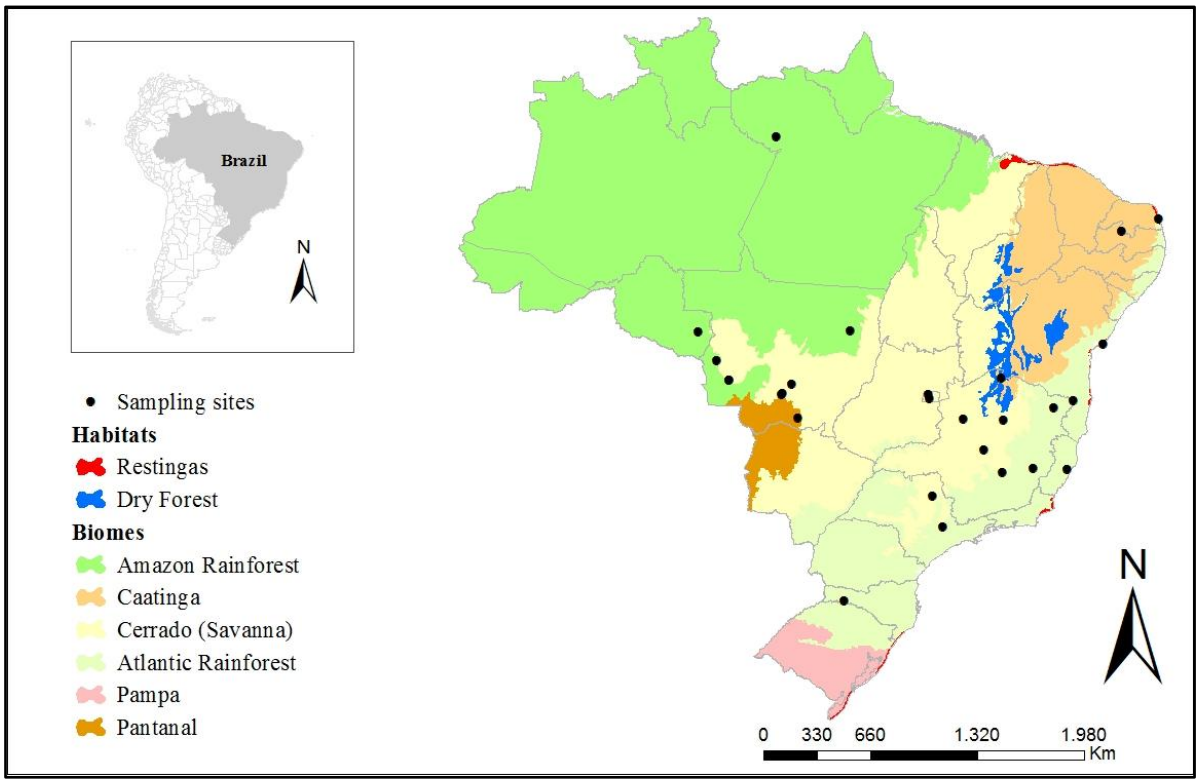

Figure1. Map of Brazil showing the distribution of the seven habitats studied. Sampled localities are marked with dots.

2.4. Statistical Analysis

For the analysis, we created a bipartite graph from an incidence matrix, using the graph incidence function on the igraph package. We used the "heatmap. 2" function of gplots package to generate dendrogram to show the similarity of parasite composition among the habitats (Warnes et al., 2016). We created a bipartite graph from an incidence matrix with the

habitats in one part and the lineages in the other part to visualize the net. For the bipartite graph we used the "graph incidence" function on the igraph package. To generate a dendrogram to show the similarity of parasite composition between the habitats, we used the "heatmap. 2" function on the gplots package. All the statistical analysis was done using R programming (R Core Team, 2017). For more accurate comparative analysis, we used lineages reported four time or more in database. This is important because lineages detected a few time can give a false idea of habitat specialization. The results of all lineages and the exclusive lineages per habitat were plot as box plot.

3. Results

3.1. General pattern of parasite prevalence

A total of 1161 sequences of mitochondrial cytochrome *b* gene from haemosporidian parasites were detected in Brazil from 166 bird species and 36 families. This represented 207 unique parasite lineages of *Plasmodium/Haemoproteus* across the seven different habitats accessed in this study. In total, 33.4% (69/207) unique parasite lineages were obtained a minimum of four times (Table1). *Plasmodium* was the most common genera, accounting for 67% (779/1161) of the sequences, representing 146 unique lineages. The *H.* (*Parahaemoproteus*) subgenus was detected in 20.5% (236/1161) of the samples, representing 39 lineages; while *H.* (*Haemoproteus*) was detected in 12.5% (146/1161) of the sampled birds, representing 22 lineages (Figure 2). The relative prevalence of *Plasmodium* was the highest in all habitats except in Caatinga and Restinga, where *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) were the most common parasite groups, respectively (Table 1).

Table1. Summary information from each sampling habitat, including infection data.

Habitat	Number of localities	Infected bird species	Number of obtained sequences	Lineages[b]			Total number of lineages
				P.	***H.***	***P.H.***	
Amazon rainforest	5	33	90	28(88.9%)	5 (11.1%)	0	33
Atlantic rainforest	7	42	172	52 (94.7%)	0	5 (5.3%)	57
Tropical dry forest	1	52	222	40 (70.3%)	05 (4.9%)	14 (24.3%)	59
Pantanal	2	06	08	06 (100%)	0	0	06
Cerrado	7	93	327	62 (76.7%)	12 (6.1%)	20 (17.1%)	94
Caatinga	1	25	186	17 (38.7%)	05 (50%)	10 (11.3%)	32
Restinga	2	36	156	18 (30.7%)	03 (7.6%)	07 (61.5%)	28
Total	25	166	1161	146(67.1%)	39(20.4%)	22(12.5%)	207

[b] = Represents unique parasite lineages detected in each habitat; values in parenthesis represent relative prevalence from each parasite group in seven Brazilian habitats. *P. = Plasmodium H. =Haemoproteus P.H =Parahaemoproteus*

3.2. Parasite richness among different habitats

We compared the richness of lineages among the seven habitats: Cerrado presented the richest parasite community with a total of 94 lineages (62 *Plasmodium*, 12 *H.* (*Haemoproteus*) and 20 *H.* (*Parahaemoproteus*), followed by tropical dry forests that presented 59 unique parasite lineages (40 *Plasmodium*, five *H.* (*Haemoproteus*) and 14 *H.* (*Parahaemoproteus*) and Atlantic rainforest (52 *Plasmodium* and five *H.* (*Parahaemoproteus*)) (Figures 3A and B). Parasite richness was nearly similar among the Amazon rainforest, (28 *Plasmodium* and five *H.* (*Haemoproteus*) lineages), Caatinga (17

Plasmodium, five *H.* (*Haemoproteus*) and 10 *H.* (*Parahaemoproteus*) and Restinga (18 *Plasmodium*, three *H.* (*Haemoproteus*) and seven *H.* (*Parahaemoproteus*). Pantanal had the lowest richness, with only six *Plasmodium* lineages detected (Figure 3C-F). *Plasmodium* lineage richness was higher in all habitats compared to both *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) (Table S1).

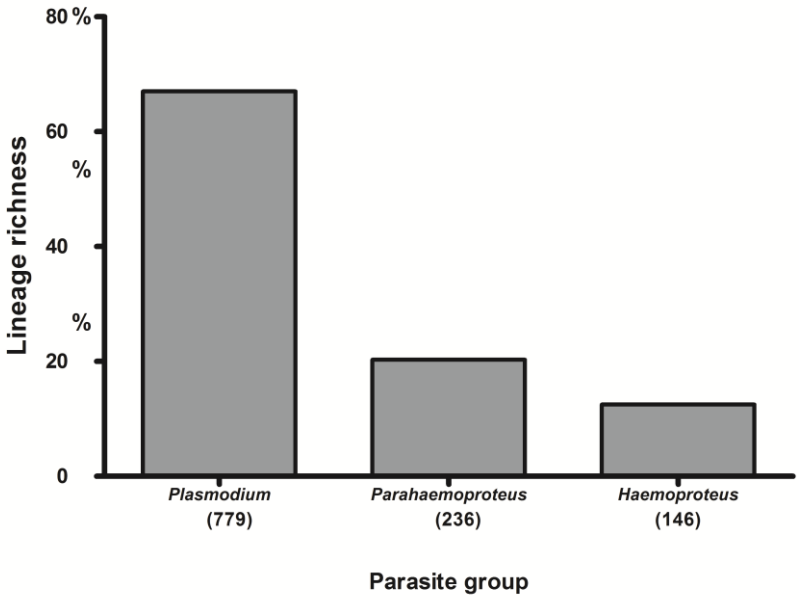

Figure2. Overall lineage richness of the three parasites across the seven different habitats.

3.3. Parasite habitat range

From the 69 lineages that were detected at a minimum of four times, 48 were habitat generalist (i.e. detected at least in two different habitats) and 21 were habitat specialist (exclusively detected in a single habitat). Habitat generalist lineages included 36 *Plasmodium*, five *H.* (*Haemoproteus*) and seven *H.* (*Parahaemoproteus*) lineages. Among all 36 *Plasmodium* lineages, three (DENPET03, PADOM09 and PADOM11) were detected in six habitats; two lineages (BAFLA03 and PADOM17) were detected in five habitats, three lineages (BAFLA04, TUAMA01 and TULEU06) were detected in four habitats. Twelve lineages were obtained from three habitats and 16 were obtained from two habitats (Table S1). Among five *H.* (*Haemoproteus*) lineages, two (COPIC01 and SocH3) were detected in three habitats, while the remaining three (COSQU01, COTAL01, PIRUB01) were detected in two habitats (S1). Similarly *H.* (*Parahaemoproteus*) lineage ELALB01 was detected in five different habitats, and two lineages (MYMAC02 and PAPOL03) were obtained from three

different habitats. The remaining four lineages (CARUF01, COPIL01, PAPOL07 and PISUL01) were detected in two different habitats.

Habitat specialist lineages included 14 *Plasmodium*, one *H.* (*Haemoproteus*) and six *H.* (*Parahaemoproteus*) lineages (Table 2). Among *Plasmodium* lineages, five (COLIN01, COLIN11, THSAY02, TRMEL02 and VIOLIO01) were detected exclusively in the Atlantic rainforest, three (CYCYA01, LENAT02 and WIPOE01) in the Amazon rainforest, three (FULEU01, THAMB01 and THAMB07) in tropical dry forests, two (MYSWA01 and TULEU07) in Cerrado and one (CORPIL01) in Caatinga. Among *Parahaemoproteus* lineages (*Haemoproteus erythrogravidus*, NEOFASE01 and PACPEC02) were specific to Cerrado, PAPOL01 to Tropical dry forest and TARUF02 and UN203 were specific to Restinga habitat. *H.haemoproteus* was the least specific parasite in terms of habitat; only one lineage SocH2 was exclusively detected in Caatinga habitat.

Pantanal and the Amazon rainforest showed the highest lineage similarity across the seven studied habitats. Parasite composition from these two habitats presented close similarity to the Atlantic rainforest. Close relatedness among parasite assemblages was also observed between Cerrado and tropical dry forests. Parasite composition from the Restinga had the highest uniqueness followed by Caatinga (Figure 4). Among the studied habitat Cerrado showed the highest lineage richness followed by tropical dry forest and Atlantic rainforest while was the least rich habitat (Figure 5).

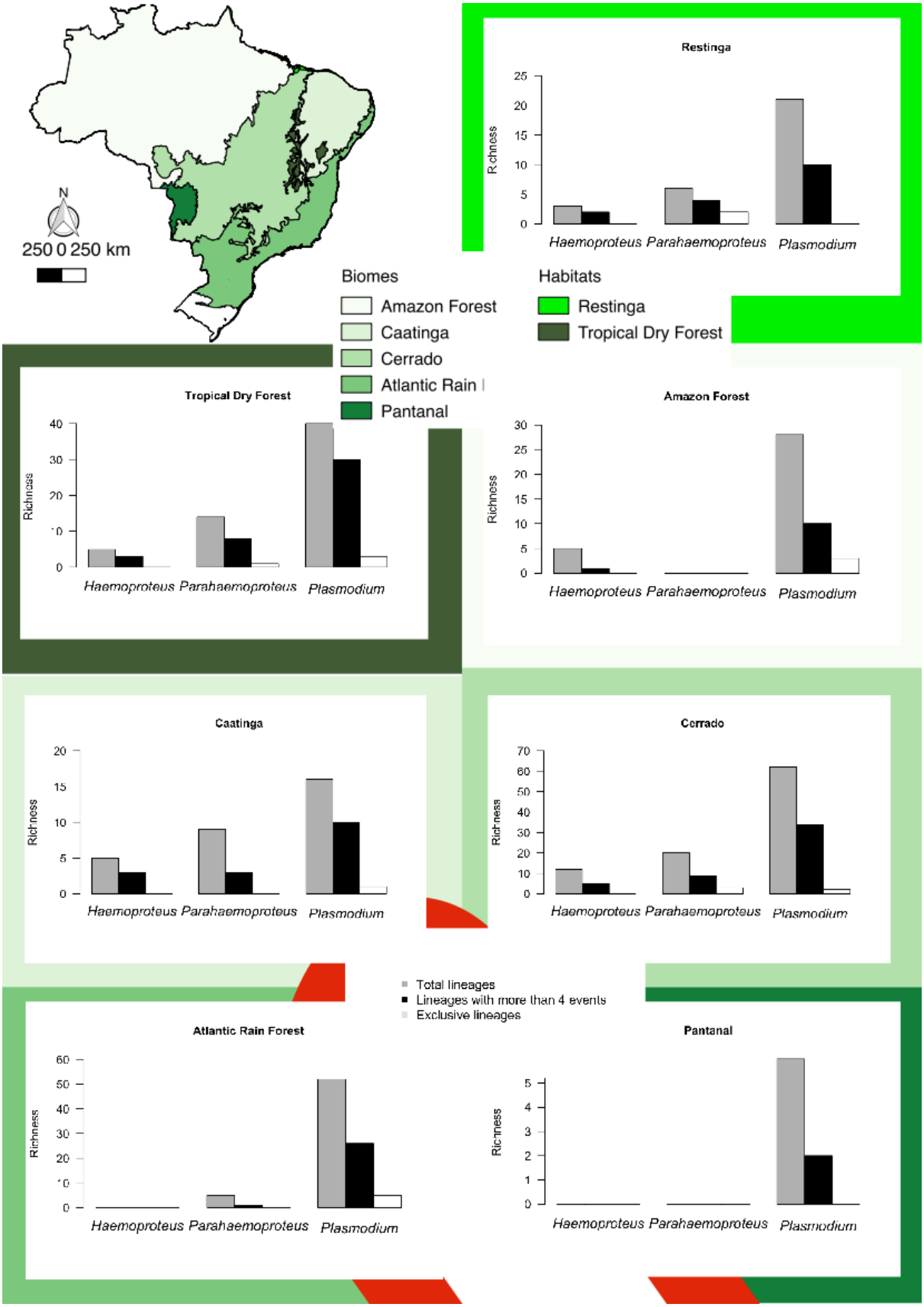

Figure3. Lineage richness of avian haemosporidian parasites among the seven different habitats (five biomes and two phytophysiognomy). Grey in the graph represents overall lineages, Black represent lineages detected at least four time and white represent lineages exclusive to habitat.

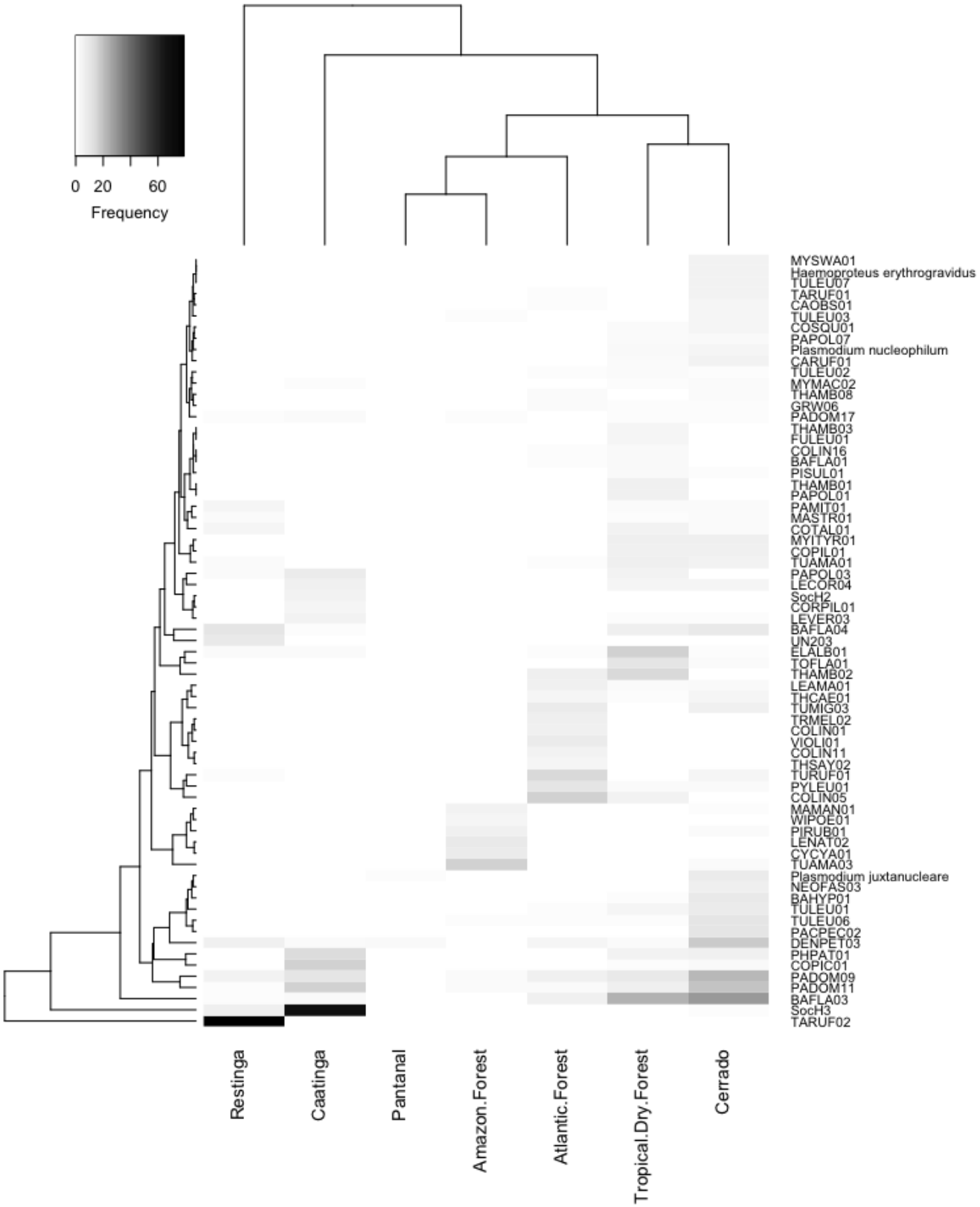

Figure4. Dendrogram showing lineages similarity and differences across the seven habitats

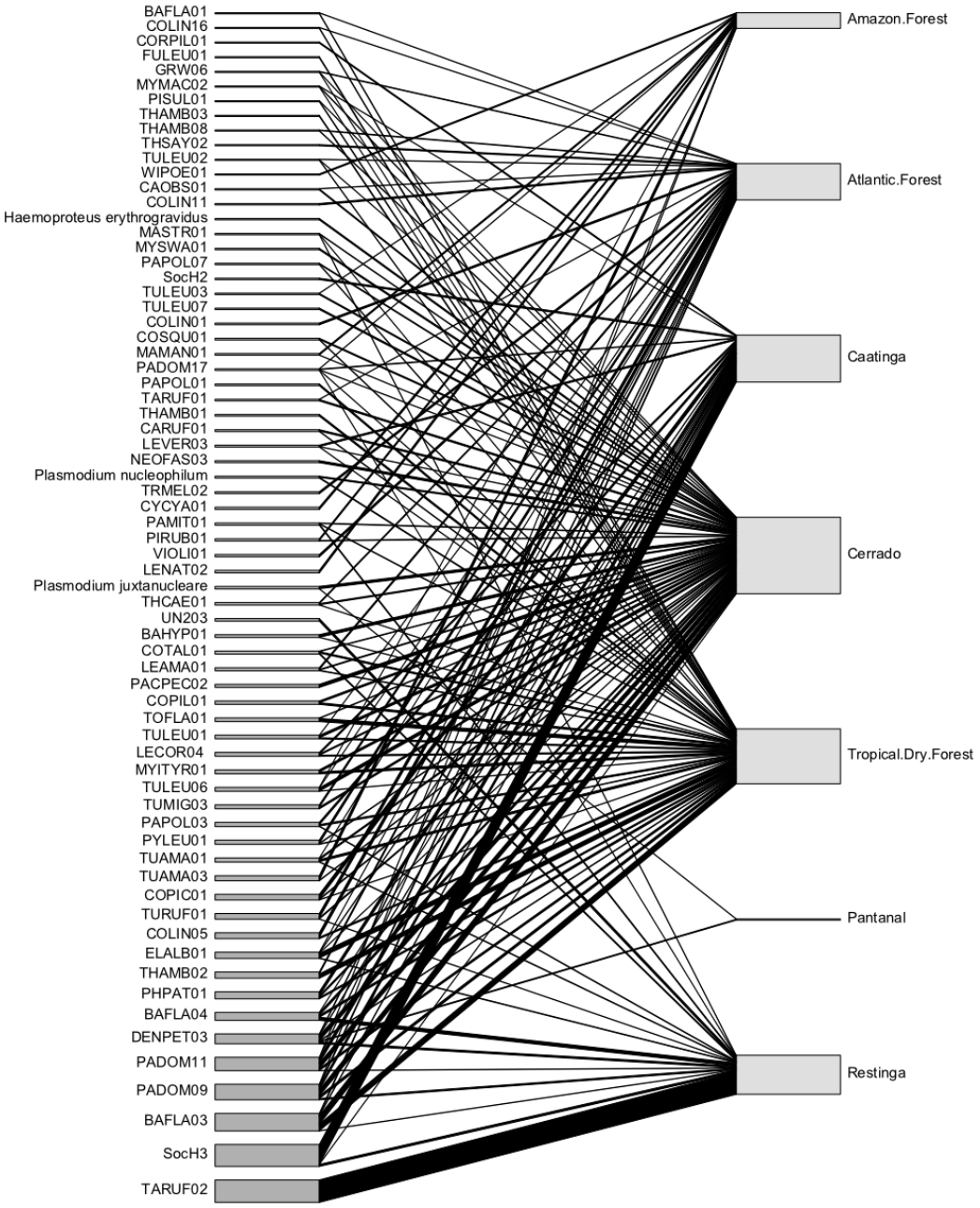

Figure5. Bipartite graphs showing lineages similarity and differences across the seven habitats.

3.4. Parasite host range

Most generalist lineages infected a higher range of host species ranging from two to 29; the highest number of species was infected by *Plasmodium* lineage PADOM09 (29 species), BAFLA03 (21 species), DENPET03 and PADOM11 (20 species each) and BAFLA04 (13 species). Among *H.* (*Parahaemoproteus*) lineages ELALB01 was detected in (12 species), PAPOL03 in (five Species), CORPIL01 in four and PAPOL07 and PISUL01 in three species respectively, while SocH3 and COPIC01 were the *H.* (*Haemoproteus*) lineages infecting eight and four species respectively (Table S1).

In contrast habitat specialist lineages were either restricted to only one specie or infecting few numbers of species (1-5) than generalist lineages (Table 2). Eight *Plasmodium* lineages were detected from single host each, four from two hosts and only two were detected from three different hosts in their respective habitats.

Table2. Summary information of specialist lineages from each sampling habitat, including infection data.

Parasite/Lineage	Species infected	Amazon forest	Atlantic forest	Caatinga	Cerrado	Dry forest	Pantanal	Restinga	Total
Haemoproteus		**0**	**0**	**01**	**-**	**-**	**-**	**-**	**01**
SocH2	02	-	-	05	-	-	-	-	
Parahaemoproteus		**-**	**-**	**-**	**3**	**1**	**-**	**2**	**06**
Haemoproteus erythrogravidus	02	-	-	-	5	-	-	-	05
NEOFAS03	03	-	-	-	7	-	-	-	07
PACPEC02	02	-	-	-	11	-	-	-	11
PAPOL01	04	-	-	-	-	6	-	-	06
TARUF02		-	-	-	-	-	-	79	79
UN203	05	-	-	-	-	-	-	9	9
Plasmodium		**03**	**05**	**01**	**02**	**03**	**0**	**0**	**14**
COLIN01	01	-	6	-	-	-	-	-	06
COLIN11	01	-	5	-	-	-	-	-	05
CORPIL01	01	-	-	4	-	-	-	-	04
CYCYA01	01	8	-	-	-	-	-	-	08
FULEU01	02	-	-	-	-	4	-	-	04
LENAT02	01	9	-	-	-	-	-	-	09
MYSWA01	03	-	-	-	5	-	-	-	05
THAMB01	01	-	-	-	-	6	-	-	06
THAMB03	01	-	-	-	-	4	-	-	04
THSAY02	02	-	4	-	-	-	-	-	04
TRMEL02	03	-	7	-	-	-	-	-	07
TULEU07	02	-	-	-	5	-	-	-	05
VIOLI01	02	-	8	-	-	-	-	-	08
WIPOE01	01	4	-	-	-	-	-	-	04
Grand Total		**22**	**30**	**09**	**33**	**20**	**0**	**88**	**193**

Bold numbers represent the number of lineages obtained of that particular parasite.

4. Discussion

Here we combined a broad sampling from seven ecologically distinct habitats to compare the richness and distribution of avian malaria parasite lineages throughout Brazil. *Plasmodium* was the most common parasite group in Brazil, accounting for 67% of the detected lineages. This pattern was observed in five out of the seven habitats evaluated here; *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) were most common in one habitat each, in Caatinga and in Restinga, respectively. This pattern of high prevalence of *Plasmodium* differs from other haemosporidian communities assessed in China, Philippines, West Africa, Northern Denmark, Venezuela, Colombia and Chile where *Haemoproteus* parasites were the most common (Merino et al., 2008; Møller and Nielsen, 2007; Belo et al., 2012; Silva-Iturriza et al., 2012; González et al., 2014; Zhang et al., 2014). *Haemoproteus* in the above cited studies is basically *H.* (*Parahaemoproteus*). Most of the bird species infected in these studies are non-Columbiformes and subgenus *H.* (*Haemoproteus*) mainly infects Columbiformes and some sea birds (Valkiūnas, 2005). These differences in prevalence among the two genera on a global and regional scale in Caatinga and Restinga might be explained by the fact that environmental conditions differ globally as well as regionally and the avian host distribution varied in space and time. Caatinga is a seasonally dry ecosystem while restinga is distinct type of costal habitat with nutrient poor and acidic soil. Possibly these characteristic may favor the vector propagation; biting midges (Diptera: Ceratopogonidae) for *H.* (*Parahaemoproteus)* and hippoboscid flies (Hippoboscidae) for *H.* (*Haemoproteus*) in these habitats, which could increase prevalence. Alternatively, high prevalence of the two well sampled species; *Columbina minuta* (62%) in Caatinga and *Tachyphonus rufus* (42%) in restinga might be the reason for this different pattern of relative prevalence of the two subgenera compared to other habitats.

We encountered a high haemosporidian lineage richness in Brazil when compared to other regional or local studies (Ricklefs et al., 2005; Belo et al., 2012; Lacorte et al., 2013; González et al., 2014). *Plasmodium* showed higher lineage richness than *Haemoproteus*. Similar pattern is observed in South and North America. However, studies conducted on global scale (Sub-Sahara, India/South East Asia, Australia/Papa New Guinea, Eastern Europe and Western Europe) confirmed a higher global *Haemoproteus* richness rather than *Plasmodium* (Clark et al., 2014). This unique biogeographical pattern of high *Plasmodium* lineage richness in Brazil might be associated with high heterogeneity of avian hosts, vectors

and environmental conditions. Indeed, Brazil is an ecologically diverse region supporting one of the richest avifauna (Marini and Garcia, 2005). We observed differences in lineage richness on habitat level; Cerrado showed the highest lineages richness followed by tropical dry forest and Atlantic rainforest while Pantanal was the least diverse region. Amazon, Pantanal and Atlantic forest showed similarity in lineages. Cerrado and tropical dry forest showed a similar lineage composition. These differences may be associated with differences in bird communities as the abiotic attributes of these habitats are different from one another. The Amazon and Atlantic rainforests receive a constant rainfall. On the other hand, Tropical dry forest Caatinga and Cerrado are seasonally dry ecosystems with variation in temperature and precipitation around the year. Moreover, the uneven sampling in the present study may have some effect on the richness pattern of avian haemosporidian parasites. However for the complete understanding of this unique biogeographical pattern of high *Plasmodium* and low *Haemoproteus* prevalence in Brazil, an extensive and diverse sampling of both host and vector on habitat level is required.

4.1. Host and habitat generalist lineages

Among the 69 well-sampled lineages (lineages detected a minimum of four times), 48 were habitat-generalist (36 *Plasmodium*, seven *H.* (*Parahaemoproteus*), and five *H.* (*Haemoproteus*), appearing in two or more host species or habitats. *Plasmodium* lineages showed a broader host and habitat range when compared to *H.* (*Parahaemoproteus*) and *H.* (*Haemoproteus*). The most habitat generalist *Plasmodium* lineage, PADOM09, was detected in 29 species sampled in six habitats, while the most habitat generalist *H.* (*Parahaemoproteus*); lineage, ELALB01, was detected in 12 species from five different habitats. The *H.* (*Haemoproteus*) lineage SocH3, the most generalist, was detected in eight bird species from three different habitats. Similarly other *Plasmodium* lineages (PADOM11, PADOM17, DENPET03, BAFLA03 and BAFLA04) all have a broader host and habitat range over *H.* (*Parahaemoproteus*) (PAPOL03, CORPIL01, CARUF01, and POPOL07) and *H.haemoproteus* lineages (COPIC01, COSQOU01 and COTAL010). This high degree of generalism of *Plasmodium* lineages might be the reason for its high prevalence, richness and broader host and habitat range as the 'Niche breadth' hypothesis (Brown, 1984) suggests that host generalist parasite lineages will be more prevalent and will efficiently expand their distribution and host range than the host specialist (Drovetski et al., 2014). These generalist *Plasmodium* lineages (PADOM09, PADOM11, PADOM17 and DENPET03 has also been reported from other South and North American regions showing a broad range of generalism

(Bensch et al., 2009). Phylogenetic studies suggested host switching in avian haemosporidians parasites (Ricklefs and Fallon, 2002; Ellis et al., 2015; Fecchio et al., 2018), closely related avian host could facilitate this process of host switching and subsequent diversification (Hayakawa et al., 2008; Fecchio et al., 2018). The high richness of *Plasmodium* in Brazil may be as a result of high richness of avian host and subsequent diversification. Additionally, the presence of generalist lineages in genus *Haemoproteus* suggests that generalism is not only restricted to *Plasmodium*, but genus haemoproteus may contain generalist lineages. However the degree of generalism may differ between the two genera.

4.2. Host and habitat specialist lineages

We analyzed the specificity of parasite lineages to habitats and found 21 lineages exclusively detected in specific habitats including 14 *Plasmodium*, one *H.* (*Haemoproteus*) and six *H.* (*Parahaemoproteus*) lineages. Interestingly we found 14 *Plasmodium* lineages that were host and habitat specialist. Eight out of 14 parasite lineages infected a single host each in one habitat, despite the fact that all these bird species are found in more than one habitat except one specie (*Willisornis poecilinotus*) which has geographic distribution only in Amazon rainforest. While in contrast, habitat specialist *H.* (*Haemoproteus*), and *H.* (*Parahaemoproteus*) were infecting two or more host species. Studies reported high degree of specificity in *Haemoproteus* lineages (Valkiūnas, 2005). However, Pinheiro and co-workers (2016) studying infected bird communities in Brazil, found no correlation between prevalence and specificity and proposed a new hypothesis, "The Integrative Hypothesis of Parasite Specialization", which suggests both trade-off and resource breadth processes drive performance and specificity in a host-parasite system. Specialist parasites with high performance in a host have similar performance in phylogenetically close host while generalist are infecting greater host but with low performance. Thus, it is the phylogenetic distance of the host species that determined host specificity of a parasite rather than the number of host being infected by the parasite. The current scenario indicates the existence of host and habitat specialist lineage in both genera, emphasizing that the degree of specialization may vary across host, habitat and regions. One example is the *Haemoproteus witti* a generalist parasite detected in many species but behave as specialist in humming bird (Moens et al., 2016). It has been reported that the sub genus *H.* (*Haemoproteus*) mainly infects bird of the order Columbiformes (Valkiūnas, 2005). However, we detected *H.* (*Haemoproteus*) lineages SocH2, COPIC01, COTAL01, and SocH3 from a non-

Columbiformes host *Myiarchus tyrannulus*, *Coccyzus melacoryphus*, *Coryphosphangus pileatus* and *Pachyramphus polychopterus*. These findings suggest two scenarios. One possibility is that the subgenus *Haemoproteus* (*Haemoproteus*) may use non-Columbiformes as host, or it may be abortive infections and detection of such infections is rare in avian haemosporidian studies, although both of these possibilities need confirmation through microscopic examination of these parasites. Additionally, TARUF02 a sub genus *Parahaemoproteus* lineage exclusive to Restinga habitat was detected 70/79 times from a single host *Tachyphonus rufus*. The high specificity of this lineage in *Tachyphonus rufus* might be associated with its vector preference for this particular host as recent studies demonstrated the existence of host preference in vector (Martínez-de la Puente et al., 2015; Valkiūnas, 2005). Alternatively, this lineage may be well adapted to the host immune system of *Tachyphonus rufus* and persist better or it may be the case of sampling artifact; other species may even have died before sampled. However, there is a lack of data on vector part of avian haemosporidian parasites in these habitats, and in order to completely understand host and habitat specificity mechanism of these parasites, extensive host vector studies are required throughout Brazil.

4.3. Conclusion

In conclusion, we detected a high richness of avian haemosporidian parasites in Brazil, with higher prevalence and richness of *Plasmodium* when compared to *Haemoproteus*. However, parasite group prevalence varies with respect to habitat, as we found higher prevalence of *H.* (*Haemoproteus*) in Caatinga and higher prevalence of *H.* (*Parahaemoproteus*) in Restinga. Our results suggest the existence of both host and habitat generalist and specialist lineages for both genera (*Plasmodium* and *Haemoproteus*). Moreover, further studies with diverse avian host sampling in different habitats would be valuable to understand the distribution and specificity of avian haemosporidian lineages as a great proportion (67%) of the lineages evaluated here were detected less than four times.

5. References

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecol. Lett. 9, 467–484.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22, 711–728.

Atkinson, C.T., Samuel, M.D., 2010. Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on ʻapapane Himatione sanguinea. Journal of Avian Biology. 41, 357–366.

Atkinson, C.T., van Riper III, C., 1991. Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Haemoproteus, and Leucocytozoon, in: Bird-Parasite Interactions: Ecology, Evolution, and Behavior. Oxford University Press, London, pp. 19–48.

Belo, N.O., Passos, L.F., Júnior, L.M.C., Goulart, C.E., Sherlock, T.M., Braga, E.M., 2009. Avian malaria in captive psittacine birds: Detection by microscopy and 18S rRNA gene amplification. Preventive Veterinary Medicine. 88, 220–224.

Belo, N.O., Rodríguez-Ferraro, A., Braga, E.M., Ricklefs, R.E., 2012. Diversity of avian haemosporidians in arid zones of northern Venezuela. Parasitology. 139, 1021–1028.

Bensch, S., Hellgren, O., Pérez-Tris, J., 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome *b* lineages. Molecular Ecology Resources. 9, 1353–1358.

Bensch, S., Pérez-Tris, J., Waldenström, J., Hellgren, O., 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? Evolution .58, 1617–1621.

Bensch Stffan, Stjernman Martin, Hasselquist Dennis, Örjan Östman, Hannson Bengt, Westerdahl Helena, Pinheiro Renato Torres, 2000. Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. Proceedings of the Royal Society of London. Series B: Biological Sciences. 267, 1583–1589.

Bezerra, D.M.M., de Araujo, H.F.P., Alves, R.R.N., 2012. Captura de aves silvestres no semiárido brasileiro: técnicas cinegéticas e implicações para conservação. Tropical Conservation Science. 5, 50–66.

Bertram, M.R., Hamer, S.A., Hartup, B.K., Snowden, K.F., Medeiros, M.C., Outlaw, D.C., Hamer, G.L., 2017. A novel Haemosporida clade at the rank of genus in North American cranes (Aves: Gruiformes). Molecular Phylogenetics and Evolution 109, 73–79.

Borner, J., Pick, C., Thiede, J., Kolawole, O.M., Kingsley, M.T., Schulze, J., Cottontail, V.M., Wellinghausen, N., Schmidt-Chanasit, J., Bruchhaus, I., Burmester, T., 2016. Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. Molecular Phylogenetics and Evolution 94, 221–231.

Braga, É.M., Silveira, P., Belo, N.O., Valkiūnas, G., 2011. Recent advances in the study of avian malaria: an overview with an emphasis on the distribution of Plasmodium spp in Brazil. Memórias do Instituto Oswaldo Cruz. 106, 3–11.

Brown, J.H., 1984. On the Relationship between Abundance and Distribution of Species. The American Naturalist. 124, 255–279.

Clark, N.J., Clegg, S.M., Lima, M.R., 2014. A review of global diversity in avian haemosporidians (Plasmodium and Haemoproteus: Haemosporida): new insights from molecular data. Int. J. Parasitol. 44, 329–338.

Cosgrove, C.L., Wood, M.J., Day, K.P., Sheldon, B.C., 2008. Seasonal variation in Plasmodium prevalence in a population of blue tits Cyanistes caeruleus. Journal of Animal Ecology. 77, 540–548.

Donovan, T.A., Schrenzel, M., Tucker, T.A., Pessier, A.P., Stalis, I.H., 2008. Hepatic Hemorrhage, Hemocoelom, and Sudden Death due to Haemoproteus Infection in Passerine Birds: Eleven Cases. J VET Diagn Invest. 20, 304–313.

Drovetski, S.V., Aghayan, S.A., Mata, V.A., Lopes, R.J., Mode, N.A., Harvey, J.A., Voelker, G., 2014. Does the niche breadth or trade-off hypothesis explain the abundance-occupancy relationship in avian Haemosporidia? Mol. Ecol. 23, 3322–3329.

Duque, J.G., 2004. O Nordeste e as lavouras xerófilas. Banco do Nordeste do Brasil (BNB), Fortaleza.

Ellis, V.A., Collins, M.D., Medeiros, M.C.I., Sari, E.H.R., Coffey, E.D., Dickerson, R.C., Lugarini, C., Stratford, J.A., Henry, D.R., Merrill, L., Matthews, A.E., Hanson, A.A., Roberts, J.R., Joyce, M., Kunkel, M.R., Ricklefs, R.E., 2015. Local host specialization, host-switching, and dispersal shape the regional distributions of avian haemosporidian parasites. PNAS. 112, 11294–11299.

Fallon, S.M., Ricklefs, R.E., Latta, S.C., Bermingham, E., 2004. Temporal stability of insular avian malarial parasite communities. Proc Biol Sci 271, 493–500.

Fallon, S.M., Ricklefs, R.E., Swanson, B.L., Bermingham, E., 2003. Detecting avian malaria: an improved polymerase chain reaction diagnostic. Journal of Parasitology. 89, 1044–1047.

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

Fecchio, A., Ellis, V.A., Bell, J.A., Andretti, C.B., D'Horta, F.M., Silva, A.M., Tkach, V.V., Weckstein, J.D., 2017. Avian malaria, ecological host traits and mosquito abundance in southeastern Amazonia. Parasitology. 144, 1117–1132.

Fecchio, A., Lima, M.R., Silveira, P., Braga, É.M., Marini, M.Â., 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu - Austral Ornithology. 111, 132–138.

Fecchio, A., Lima, M.R., Svensson-Coelho, M., Marini, M.Â., Ricklefs, R.E., 2013. Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. Parasitology. 140, 181–192.

Fecchio, A., Pinheiro, R., Felix, G., Faria, I.P., Pinho, J.B., Lacorte, G.A., Braga, E.M., Farias, I.P., Aleixo, A., Tkach, V.V., Collins, M.D., Bell, J.A., Weckstein, J.D., 2018. Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds. Ecography. 41, 505–515.

Ferreira, F.C., Rodrigues, R.A., Sato, Y., Borges, M.A.Z., Braga, É.M., 2016. Searching for putative avian malaria vectors in a Seasonally Dry Tropical Forest in Brazil. Parasites & Vectors. 9,87.

Ferreira Junior, F.C., Rodrigues, R.A., Ellis, V.A., Leite, L.O., Borges, M.A.Z., Braga, É.M., 2017. Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. PLOS ONE. 12:6, e0178791.

Garamszegi, L.Z., 2011. Climate change increases the risk of malaria in birds. Global Change Biology. 17, 1751–1759.

García-Longoria, L., Garamszegi, L.Z., Møller, A.P., 2014. Host escape behavior and blood parasite infections in birds. Behav Ecol. 25, 890–900.

González, A.D., Matta, N.E., Ellis, V.A., Miller, E.T., Ricklefs, R.E., Gutiérrez, H.R., 2014. Mixed species flock, nest height, and elevation partially explain avian haemoparasite prevalence in Colombia. PLOS ONE. 9:6, e100695.

Gonzalez-Quevedo, C., Davies, R.G., Richardson, D.S., 2014. Predictors of malaria infection in a wild bird population: Landscape-level analyses reveal climatic and anthropogenic factors. Journal of Animal Ecology. 83, 1091–1102.

Green, M.R., Sambrook, J., 2012. Molecular Cloning: A Laboratory Manual (Fourth Edition): Three-volume set, 4th edition. ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Hay, S.I., Myers, M.F., Burke, D.S., Vaughn, D.W., Endy, T., Ananda, N., Shanks, G.D., Snow, R.W., Rogers, D.J., 2000. Etiology of interepidemic periods of mosquito-borne disease. Proceedings of the National Academy of Sciences. 97, 9335–9339.

Hayakawa, T., Culleton, R., Otani, H., Horii, T., Tanabe, K., 2008. Big bang in the evolution of extant malaria parasites. Mol. Biol. Evol. 25, 2233–2239.

Hellgren, O., Pérez-Tris, J., Bensch, S., 2009. A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. Ecology. 90, 2840–2849.

Hellgren, O., Waldenström, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of *Leucocytozoon, Plasmodium,* and *Haemoproteus* from avian blood. Journal of Parasitology. 90, 797–802.

Hess, S.E. Randolph, , C.Chemini, J. Furlanello, P. Arneberg, J.Harwood,, J.Swinton, 2001. Spatial aspects of disease dynamics:The Ecology of Wildlife Diseases, P.J. Hudson, A. Rizzoli, B.T. Grenfell, H.Heesterbeek, A.P. Dobson (Eds.), . ed. Oxford University Press, Oxford.

Hoshen, M.B., Morse, A.P., 2004. A weather-driven model of malaria transmission. Malaria Journal. 3, 32.

Ishtiaq, F., Bowden, C.G.R., Jhala, Y.V., 2017. Seasonal dynamics in mosquito abundance and temperature do not influence avian malaria prevalence in the Himalayan foothills. Ecology and Evolution. 7, 8040–8057.

IUCN, 2016. Rhea americana: BirdLife International: The IUCN Red List of Threatened Species 2016: e.T22678073A92754472. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22678073A92754472.en

Kamiya, T., O'Dwyer, K., Nakagawa, S., Poulin, R., 2014. What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. Biol Rev Camb Philos Soc. 89, 123–134.

Knowles, S.C.L., Palinauskas, V., Sheldon, B.C., 2010. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. J. Evol. Biol. 23, 557–569.

Krasnov, B.R., Poulin, R., Shenbrot, G.I., Mouillot, D., Khokhlova, I.S., 2004. Ectoparasitic "Jacks-of-All-Trades": Relationship between Abundance and Host Specificity in Fleas (Siphonaptera) Parasitic on Small Mammals. 11, 507-516

Lacorte, G.A., Félix, G.M.F., Pinheiro, R.R.B., Chaves, A.V., Almeida-Neto, G., Neves, F.S., Leite, L.O., Santos, F.R., Braga, É.M., 2013a. Exploring the Diversity and Distribution of Neotropical Avian Malaria Parasites – A Molecular Survey from Southeast Brazil. PLOS ONE. 8:3, e57770.

Landesman, W.J., Allan, B.F., Langerhans, R.B., Knight, T.M., Chase, J.M., 2007. Inter-Annual Associations Between Precipitation and Human Incidence of West Nile Virus in the United States. Vector-Borne and Zoonotic Diseases. 7, 337–343.

Lapointe, D.A., Atkinson, C.T., Samuel, M.D., 2012. Ecology and conservation biology of avian malaria. Ann. N. Y. Acad. Sci. 1249, 211–226.

LaPointe, D.A., Goff, M.L., Atkinson, C.T., 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. J. Parasitol. 96, 318–324.

LaPointe, D.A., Goff, M.L., Atkinson, C.T., 2005. Comparative susceptibility of introduced forest-dwelling mosquitoes in hawai'i to avian malaria, *Plasmodium relictum*. Journal of Parasitology. 91, 843–849.

Laurance, S.G.W., Jones, D., Westcott, D., Mckeown, A., Harrington, G., Hilbert, D.W., 2013. Habitat fragmentation and ecological traits influence the prevalence of avian blood parasites in a tropical rainforest landscape. PLOS ONE. 8:10, e76227.

Leal, I.R., da Silva, J., Cardoso, M., Tabarelli, M., Lacher, T.E., 2005. Changing the course of biodiversity conservation in the Caatinga of northeastern Brazil. Conservation Biology 19, 701–706.

Levin, I.I., Parker, P.G., 2014. Infection with *Haemoproteus iwa* affects vector movement in a hippoboscid fly-frigatebird system. Molecular Ecology. 23, 947–953.

Levin, I.I., Valkiūnas, G., Santiago-Alarcon, D., Cruz, L.L., Iezhova, T.A., O'Brien, S.L., Hailer, F., Dearborn, D., Schreiber, E.A., Fleischer, R.C., Ricklefs, R.E., Parker, P.G., 2011. Hippoboscid-transmitted Haemoproteus parasites (Haemosporida) infect Galapagos Pelecaniform birds: Evidence from molecular and morphological studies, with a description of Haemoproteus iwa. International Journal for Parasitology. 41, 1019–1027.

Loiseau, C., Harrigan, R.J., Robert, A., Bowie, R.C.K., Thomassen, H.A., Smith, T.B., Sehgal, R.N.M., 2012. Host and habitat specialization of avian malaria in Africa. Mol Ecol. 21, 431–441.

Loiseau, C., lezhova, T., Valkiunas, G., Chasar, A., Hutchinson, A., Buermann, W., Smith, T.B., Sehgal, R.N.M., 2010. Spatial variation of haemosporidian parasite infection in african rainforest bird species. The Journal of Parasitology. 96, 21–29.

Lutz, H.L., Hochachka, W.M., Engel, J.I., Bell, J.A., Tkach, V.V., Bates, J.M., Hackett, S.J., Weckstein, J.D., 2015. Parasite prevalence corresponds to host life history in a diverse assemblage of afrotropical birds and haemosporidian parasites. PLOS ONE. 10:4, e0121254.

Lugarini, C., Albuquerque, M.C.F. de, Vanstreels, R.E.T., Roos, A.L., Silva, J.C.R., Oliveira, J.B. de, 2018. Endoparasites in birds of guariba state biological reserve, atlantic forest, paraíba state, brazil. Ciência Animal Brasileira 19. e30480

Marini, M.Â., Garcia, F.I., 2005. Bird Conservation in Brazil. Conservation Biology. 19, 665–671.

Martínez-de la Puente, J., Figuerola, J., Soriguer, R., 2015. Fur or feather? Feeding preferences of species of Culicoides biting midges in Europe. Trends Parasitol. 31, 16–22.

Martinsen, E.S., Perkins, S.L., Schall, J.J., 2008. A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. Mol. Phylogenet. Evol. 47, 261–273.

Marzal, A., 2012. Recent advances in studies on avian malaria parasites, in: Malaria Parasites. InTech.

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., De Lope, F., 2008. Effects of malaria double infection in birds: one plus one is not two. Journal of Evolutionary Biology. 21, 979–987.

Marzal, A., de Lope, F., Navarro, C., Møller, A.P., 2005. Malarial parasites decrease reproductive success: an experimental study in a passerine bird. Oecologia. 142, 541–545.

Matthews, A.E., Ellis, V.A., Hanson, A.A., Roberts, J.R., Ricklefs, R.E., Collins, M.D., 2016. Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. J Ornithol. 157, 533–548.

Merino, S., Moreno, J., Vásquez, R.A., Martínez, J., Sánchez-Monsálvez, I., Estades, C.F., Ippi, S., Sabat, P., Rozzi, R., Mcgehee, S., 2008. Haematozoa in forest birds from southern Chile: Latitudinal gradients in prevalence and parasite lineage richness. Austral Ecology. 33, 329–340.

Moens, M.A.J., Valkiūnas, G., Paca, A., Bonaccorso, E., Aguirre, N., Pérez-Tris, J., 2016. Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood parasites of Andean birds. J Anim Ecol. 85, 1234–1245.

Møller, A.P., Erritzøe, J., 1998. Host immune defence and migration in birds. Evolutionary Ecology. 12, 945–953.

Møller, A.P., Nielsen, J.T., 2007. Malaria and risk of predation: a comparative study of birds. Ecology. 88, 871–881.

Motta, R.O.C., Romero Marques, M.V., Ferreira Junior, F.C., Andery, D. de A., Horta, R.S., Peixoto, R.B., Lacorte, G.A., Moreira, P. de A., Paes Leme, F. de O., Melo, M.M., Martins, N.R. da S., Braga, É.M., 2013. Does haemosporidian infection affect hematological and biochemical profiles of the endangered Black-fronted piping-guan (*Aburria jacutinga*)? PeerJ. 1, e45.

Norris, K., 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology. 11, 19–26.

Norte, A.C., Araújo, P.M., Sampaio, H.L., Sousa, J.P., Ramos, J.A., 2009. Haematozoa infections in a Great Tit Parus major population in Central Portugal: relationships with breeding effort and health. Ibis .151, 677–688.

Ortego, J., Cordero, P.J., Aparicio, J.M., Calabuig, G., 2008. Consequences of chronic infections with three different avian malaria lineages on reproductive performance of Lesser Kestrels (*Falco naumanni*). Journal of Ornithology. 149, 337–343.

Outlaw, D.C., Ricklefs, R.E., 2014. Species limits in avian malaria parasites (Haemosporida): how to move forward in the molecular era. Parasitology 141, 1223–1232.

Paaijmans, K.P., Blanford, S., Bell, A.S., Blanford, J.I., Read, A.F., Thomas, M.B., 2010. Influence of climate on malaria transmission depends on daily temperature variation. Proceedings of the National Academy of Sciences. 107, 15135–15139.

Parham, P.E., Michael, E., 2010. Modeling the Effects of Weather and Climate Change on Malaria Transmission. Environ Health Perspect. 118, 620–626.

Patz, J.A., Githeko, A.K., McCarty, J.P., Hussein, S., Confalonieri, U., De Wet, N., others, 2003. Climate change and infectious diseases. Climate change and human health: risks and responses 6, 103–37.

Pérez-Tris, J., Hasselquist, D., Hellgren, O., Krizanauskiene, A., Waldenström, J., Bensch, S., 2005. What are malaria parasites? Trends Parasitol. 21, 209–211.

Perkins, S.L., Schall, J., 2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. Journal of Parasitology. 88, 972–978.

Pigeault, R., Vézilier, J., Cornet, S., Zélé, F., Nicot, A., Perret, P., Gandon, S., Rivero, A., 2015. Avian malaria: a new lease of life for an old experimental model to study the evolutionary ecology of *Plasmodium*. Philos Trans R Soc Lond B Biol Sci 370.

Pinheiro, R.B.P., Félix, G.M.F., Chaves, A.V., Lacorte, G.A., Santos, F.R., Braga, É.M., Mello, M.A.R., 2016a. Trade-offs and resource breadth processes as drivers of performance and specificity in a host–parasite system: a new integrative hypothesis. International Journal for Parasitology. 46, 115–121.

Pinheiro, R.B.P., Félix, G.M.F., Chaves, A.V., Lacorte, G.A., Santos, F.R., Braga, É.M., Mello, M.A.R., 2016b. Trade-offs and resource breadth processes as drivers of performance and specificity in a host–parasite system: a new integrative hypothesis. International Journal for Parasitology. 46, 115–121.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics. 14, 817–818.

Poulin, R., 1998. Evolutionary ecology of parasites: from individuals to communities. Evolutionary ecology of parasites: from individuals to communities. Journal of wildlife diseases. 37, 212-214

Poulin, R., 1997. Species Richness of Parasite Assemblages: Evolution and Patterns. Annual Review of Ecology and Systematics. 28, 341–358.

Poulin, R., Mouillot, D., 2004. The relationship between specialization and local abundance: the case of helminth parasites of birds. Oecologia. 140, 372–378.

Prado, D.E., 2003. As caatingas da América do Sul. Ecologia e conservação da Caatinga 2, 3–74.

R core team, 2017. R: a language and environment for statistical computing [WWW Document]. URL https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing.

R. Toby Pennington,, James A. Ratter, 2006. Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography, and Conservation - CRC Press Book

Ribeiro, S.F., Sebaio, F., Branquinho, F.C.S., Marini, M. â., Vago, A.R., Braga, é. M., 2005. Avian malaria in Brazilian passerine birds: parasitism detected by nested PCR using DNA from stained blood smears. Parasitology. 130, 261–267.

Ricklefs, R.E., 1992. Embryonic development period and the prevalence of avian blood parasites. Proceedings of the National Academy of Sciences. 89, 4722–4725.

Ricklefs, R.E., Fallon, S.M., 2002. Diversification and host switching in avian malaria parasites. Proc Biol Sci 269, 885–892.

Ricklefs, R.E., Fallon, S.M., Bermingham, E., Johnson, K., 2004. Evolutionary Relationships, Cospeciation, and Host Switching in Avian Malaria Parasites. Systematic Biology. 53, 111–119.

Ricklefs, R.E., Swanson, B.L., Fallon, S.M., MartÍnez-AbraÍn, A., Scheuerlein, A., Gray, J., Latta, S.C., 2005. Community relationships of avian malaria parasites in southern missouri. Ecological Monographs. 75, 543–559.

Rogers, D.J., Randolph, S.E., 1988. Tsetse flies in Africa: bane or boon? Conservation biology. 2, 57–65.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19, 1572–1574.

Santiago-Alarcon, D., Palinauskas, V., Schaefer, H.M., 2012. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. Biological Reviews. 87, 928–964.

Schoener, E., Banda, M., Howe, L., Castro, I., Alley, M., 2014. Avian malaria in New Zealand. New Zealand Veterinary Journal. 62, 189–198.

Sebaio, F., Braga, E.M., Branquinho, F., Manica, L.T., Marini, M. Ngelo, 2010. Blood parasites in Brazilian Atlantic Forest birds: effects of fragment size and habitat dependency. Bird Conservation International. 20, 432–439.

Sehgal, Ravinder N.M., 2015. Manifold habitat effects on the prevalence and diversity of avian blood parasites. International Journal for Parasitology: Parasites and Wildlife. 4, 421–430.

Shyamsunder Singh, U., Praharaj, M., Sharma, C., Das, A., 2016. Paradigm Shift in Transmission of Vector Borne Diseases. Journal of Emerging Infectious Diseases. 01, 16

Silva JMC, T., Leal, last, 2003. Ecologia e conservac¸a˜o da Caatinga. Editora Universita´ria, Recife, in: Ecologia e Conservacao Da Caatinga. 239-274

Silva-Iturriza, A., Ketmaier, V., Tiedemann, R., 2012. Prevalence of avian haemosporidian parasites and their host fidelity in the central Philippine islands. Parasitol. Int. 61, 650–657.

Silveira, P., Belo, N.O., Lacorte, G.A., Kolesnikovas, C.K., Vanstreels, R.E., Steindel, M., Catão-Dias, J.L., Valkiūnas, G., Braga, É.M., 2013. Parasitological and new

molecular-phylogenetic characterization of the malaria parasite Plasmodium tejerai in South American penguins. Parasitology international. 62, 165–171.

Slowinski, S.P., Fudickar, A.M., Hughes, A.M., Mettler, R.D., Gorbatenko, O.V., Spellman, G.M., Ketterson, E.D., Atwell, J.W., 2018. Sedentary songbirds maintain higher prevalence of haemosporidian parasite infections than migratory conspecifics during seasonal sympatry. PLOS ONE. 13:8, e0201563.

Straube, F.C., Bianconi, G.V., 2002. Sobre a grandeza e a unidade utilizada para estimar esforço de captura com utilização de redes-de-neblina. Chiroptera Neotropical. 8, 150–152.

Valkiūnas, G., 2005. Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton.

Valkiūnas, G., Iezhova, T.A., Brooks, D.R., Hanelt, B., Brant, S.V., Sutherlin, M.E., Causey, D., 2004. Additional Observations on Blood Parasites of Birds in Costa Rica. Journal of Wildlife Diseases. 40, 555–561.

Valkiūnas, G., Iezhova, T.A., Loiseau, C., Sehgal, R.N.M., 2009. Nested cytochrome B polymerase chain reaction diagnostics detect sporozoites of hemosporidian parasites in peripheral blood of naturally infected birds. J. Parasitol. 95, 1512–1515.

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

Vanstreels, R.E.T., da Silva-Filho, R.P., Kolesnikovas, C.K.M., Bhering, R.C.C., Ruoppolo, V., Epiphanio, S., Amaku, M., Junior, F.C.F., Braga, É.M., Catão-Dias, J.L., 2015. Epidemiology and pathology of avian malaria in penguins undergoing rehabilitation in Brazil. Vet Res. 46, 30.

Vanzolini, P.E, A.M.M. Ramos-Costa, L.J. Vitt., 1980. Répteis das Caatingas. Academia Brasileira de Ciências, Rio de Janeiro. pp. 153-154

Varela-Freire, A.A., 2004. Atividade de vôo de esfingídeos (lepidoptera: bombycoidea phingidae) em área protegida de caatinga, Estação Ecológica do Seridó, Serra Negra do Norte/RN.

Velloso, A.L, Everado, V.S.B., Pareyn, G.C.F, 2002. ECORREGIÕES Propostas para o Bioma Caatinga, in: ECORREGIÕES Propostas Para o Bioma Caatinga. The Nature Conservancy do Brasil, Recife. pp. 7-33

Waldenström, J., Bensch, S., Hasselquist, D., Ostman, O., 2004. A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. J. Parasitol. 90, 191–194.

Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D., Ottosson, U., 2002. Cross-species infection of blood parasites between resident and migratory song birds in Africa. Mol. Ecol. 11, 1545–1554.

Walker, J.G., Hurford, A., Cable, J., Ellison, A.R., Price, S.J., Cressler, C.E., 2017. Host allometry influences the evolution of parasite host-generalism: theory and meta-analysis. Philos Trans R Soc Lond B Biol Sci. 372. 20160089

Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber Andy Liaw, W., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., Venables, B., 2016. gplots: Various R Programming Tools for Plotting Data version 3.0.1.1 from CRAN [WWW Document]. URL https://rdrr.io/cran/gplots/

WHO, 2014. WHO | A global brief on vector-borne diseases

WHO, 2010. WHO | World Malaria Report 2010

Wilkinson, L.C., Handel, C.M., Van Hemert, C., Loiseau, C., Sehgal, R.N.M., 2016. Avian malaria in a boreal resident species: long-term temporal variability, and increased prevalence in birds with avian keratin disorder. International Journal for Parasitology. 46, 281–290.

Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C.L., Day, K.P., Sheldon, B.C., 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, Cyanistes caeruleus. Mol. Ecol. 16, 3263–3273.

Zhang, Y., Wu, Y., Zhang, Q., Su, D., Zou, F., 2014. Prevalence patterns of avian plasmodium and haemoproteus parasites and the influence of host relative abundance in southern china. PLOS ONE. 9:9. e107826

6. Supplementary Materials

Chapter 1

Table S1. Birds community of Seridó ecological station sampled during the four different sampling months.

Family	Species	PCR Positive Samples				Total	Prevalence
		End Rainy	Mid Rainy	Beginning dry	End Dry		
Bucconidae	*Nystalus maculates*	2/3	1/2	2/2	0/2	5/9	55.55%
Caprimulgidae	*Chordeiles pusillus*	0/1	0	0	0	0/1	0
	Hydropsalis parvulus	0/5	0/2	0/1	0	0/8	0
	Hydropsalis torquata	0	0	0/1	0	0/1	0
Columbidae	*Columbina minuta*	33/70	39/57	154/238	22/35	249/401	62.09%
	Columbina talpacoti	1/1	0	0	0	1/1	100%
	Columbina passerina	0	0	0	0/1	0/1	0
	Columbina picui	2/4	0/2	16/27	02/3	20/36	54.05%
	Leptotila verreauxi	1/2	1/4	1/2	1/2	4/10	40%
	Zenaida auriculata	1/1	0	1/1	5/8	7/10	70%
Corvidae	*Cyanocorax cyanopogon*	0/1	1/4	0	1/1	2/6	33.33%
Cuculidae	*Coccyzus melacoryphus*	0/9	0/1	0	0	0/10	0
Dendrocolaptidae	*Lepidocolaptes angustirostris*	0	0/1	0	0/4	0/5	0
	Sittasomus griseicapillus	1/1	0	0	1/4	2/5	40%
Furnariidae	*Synallaxis frontalis*	0/1	0	0	0	0/1	0
Icteridae	*Molothrus bonariensis*	0	1/1	0	0	1/1	100%
	Icterus jamacaii	0	1/1	0	2/2	3/3	100%

Passerellidae	*Ammodramus humeralis*	06/10	01/4	0	0/3	7/17	41,17%
	Zonotrichia capensis	1/1	0	1/1	0	2/2	100%
Picidae	*Colaptes melanochloros*	0	0	0	0/1	0/1	0
	Picumnus limae	1/1	0	0	0	1/1	100%
	Picumnus fulvescens	0	0	0	1/2	1/2	50%
	Veniliornis passerines	0/2	2/5	2/3	4/6	8/16	50%
Polioptilidae	*Polioptila plumbea*	1/1	1/2	2/3	2/4	6/10	60%
Rhyncocyclidae	*Tolmomyias flaviventris*	0	0/2	0/3	0/2	0/7	0
	Hemitriccus margaritaceiventer	1/1	0/1	0/7	1/8	2/17	11.76%
Strigidae	*Glaucidium brasilianum*	1/2	1/1	2/2	0	4/5	80%
Thamnophilidae	*Formicivora melanogaster*	0	0	0	1/5	1/5	20%
Thraupidae	*Coryphospingus pileatus*	7/11	16/24	11/17	12/26	46/78	58.97%
	Paroaria dominicana	1/1	1/2	0	0	2/3	66.66%
	Sicalis flaveola	1/1	0	0/1	0	1/2	50%
	Sicalis luteola	3/4	0	0	0	3/4	75%
	Sporophila albogularis	1/2	1/1	0	0	2/3	66.66%
	Volatinia jacarina	3/3	3/6	2/2	6/9	14/20	70%
Tityridae	*Xenopsaris albinucha*	0/3	0	0/1	0	0/4	0
	Pachyramphus polychopterus	5/7	4/7	0	0	9/14	64.28%
Troglodytidae	*Cantorchilus longirostris*	0	0/1	0	0	0/1	0
Turdidae	*Turdus amaurochalinus*	1/2	1/4	0	0/2	2/8	25%
	Turdus rufiventris	0	3/3	0	1/1	4/4	100%
Tyrannidae	*Camptostoma obsoletum*	0	0/2	0	0/3	0/5	0
	Casiornis fuscus	2/4	0/8	0	1/2	03/14	21.42%
	Cnemotriccus fuscatus	0/1	0	0	0	0/1	0
	Empidonomus varius	2/6	1/7	0	0	3/13	23.07%
	Elaenia chilensis	8/14	0	0	0	8/14	57.14%
	Myiarchus swainsoni	4/9	0/3	0/2	0	4/14	28.57%

	Myiarchus tyrannulus	1/5	4/18	1/4	6/12	12/39	30.76%
	Myiodynastes maculatus	3/15	2/7	1/1	2/3	8/26	30.76%
	Myiopagis viridicata	0/2	0/1	0	0	0/3	0
	Myiophobus fasciatus	0/1	0	0/1	0	0/2	0
	Phaeomyias murina	10/20	9/20	1/1	0	20/41	48.78%
	Pitangus sulphuratus	1/1)	1/1	0	0	2/2	100%
	Sublegatus modestus	0	1/2	0	1/1	2/3	66.66%
	Tyrannus melancholicus	3/6	1/4	0/1	0	4/11	36.36%
Vireonidae	*Cyclarhis gujanensis*	0/1	0	0	3/5	3/6	50%
	Vireo olivaceus	0/1	2/2	0	0	2/3	66.66%

Table.S2. Co-infection detected in birds of Seridó ecological station.

Mix Infection	Parasite Species	Host Species	Host Family	Sampling Periods				Total
				End Rainy	Mid Rainy	Beginning Dry	End Dry	
Haem/Haem	*H.*(*Haemoproteus*)	*Columbina minuta* :01	Columbidae	01	0	0	0	01
		Zenaida auriculata :02	Columbidae	0	0	0	02	02
		Leptotila verreauxi :01	Columbidae	0	01	0	0	01
		Pitangus sulphuratus :01	Tyrannidae	0	01	0	0	01
SocH2/Haem	*H.*(*Haemoproteus*)	*Zenaida auriculata* :01	Columbidae	0	0	0	01	01
SocH2/ZENAUR01	*H.*(*Haemoproteus*)	*Myiarchus tyrannulus*:01	Tyrannidae	0	0	0	01	01
Plas/Plas	*Plasmodium*	*Coryphosphangus pileatus*:01	Thraupidae	0	0	01	0	01
Plas/PADOM09	*Plasmodium*	*Casiornis fuscus*:01	Tyrannidae	01	0	0	0	01
Plas/PADOM11	*Plasmodium*	*Coryphosphangus pileatus*:01	Thraupidae	0	0	01	0	01
Plas/PHPAT01	*Plasmodium*	*Ammodramus humeralis*:01	Passerellidae	01	0	0	0	01
PADOM17/POLPLU01	*Plasmodium*	*Polioptila plumbea* :01	Polioptilidae	0	0	01	0	01
NYSMAC03/NYSMAC04	*H.*(*Parahaemoproteus*)	*Nystalus maculatus* :02	Bucconidae	0	0	0	02	02
Total	**03**	**12**	**06**	**03**	**02**	**03**	**06**	**14**

In red are the new lineages recovered in this study.

Chapter II

Table S1. Summary information of habitat generalist lineages from each sampling habitat, including infection data. Bold numbers represent total number of sequences obtained for each parasite.

Parasite/Lineage	Amazon forest	Atlantic forest	Caatinga	Cerrado	Dry Forest	Pantanal	Restinga	Total
Haemoproteus	**10**		**93**	**20**	**11**	**-**	**12**	**146**
COPIC01	-		16	3	1	-	-	20
COSQU01	-		-	4	2	-	-	6
COTAL01	-		-	2	5	-	4	11
PIRUB01	6		-	2	-	-	-	8
SocH3	-		69	1	-	-	7	77
Parahaemoproteus	**-**	**9**	**21**	**56**	**54**	**-**	**96**	**236**
ELALB01	-	1	2	1	16	-	2	22
MYMAC02	-	-	1	2	1	-	-	4
PAPOL03	-	-	8	-	5	-	2	15
PAPOL07	-	-	-	3	2	-	-	5
CARUF01	-	-	-	5	2	-	-	7
COPIL01	-	-	-	6	6	-	-	12
Plasmodium	**80**	**163**	**72**	**251**	**157**	**8**	**48**	**779**
DENPET03	-	4	2	18	2	3	6	35
PADOM09	2	6	11	22	9	-	5	55
PADOM11	2	2	16	19	7	-	1	47
BAFLA03	-	6	3	29	23	-	1	62
PADOM17	1	-	2	1	1	-	1	6
BAFLA04	-	-	1	9	7	-	11	28
TUAMA01	-	1	-	5	7	-	2	15
TULEU06	1	1	-	11	1	-	-	14
GRW06	-	2	-	1	1	-	-	4
LEAMA01	-	6	-	3	2	-	-	11
LECOR04	-		6	4	4	-	-	14
LEVER03	-		5	1	1	-	-	7
TURUF01	-	15	-	4		-	1	20
PYLEU01	-	11	-	2	2	-	-	15
THCAE01	-	4	-	4	1	-	-	9
TULEU01	-	1	-	8	4	-	-	13

TULEU02	-	1	-	1	2	-	-	4
PHPAT01	-	-	14	6	5	-	-	25
BAHYP01	-	-	-	9	1	-	-	10
CAOBS01	-	1	-	4	-	-	-	5
COLIN05	-	16	-	-	5	-	-	21
COLIN16	-	1	-	-	3	-	-	4
BAFLA01	-	1	-	-	3	-	-	4
MAMAN01	5	-	-	1		-	-	6
MASTR01	-	-	-	2	1	-	2	5
MYITYR01	-	-	-	7	7	-	-	14
TUAMA03	16	-	-	2		-	-	18
Plasmodium juxtanucleare	-	-	-	8	-	1	-	9
Plasmodium nucleophilum	-	-	-	4	3	-	-	7
TARUF01	-	1	-	5	-	-	-	6
THAMB02	-	7	-	-	15	-	-	22
THAMB02	-	2	-	2		-	-	4
TOFLA01	-	-	-	2	11	-	-	13
PAMIT01	-	-	-	2	2	-	4	8
TUAMA03	16	-	-	2	-	-	-	18
TULEU03	1	-	-	4	-	-	-	5
TUMIG03	-	8	-	6	-	-	-	14
Grand Total	**90**	**172**	**186**	**327**	**222**	**8**	**156**	**1161**