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# Avian Haemosporidian Parasites From The Brazilian Amazon

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# AVIAN HAEMOSPORIDIAN PARASITES FROM THE BRAZILIAN AMAZON

by

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This dissertation, submitted by Jeffrey Andrew Bell in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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Jeffrey Andrew Bell
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In memory of my father, George Bell

ABSTRACT

Birds possess the most diverse assemblage of haemosporidian parasites, including three genera, *Plasmodium, Haemoproteus,* and *Leucocytozoon.* Currently there are over 200 morphologically identified avian haemosporidian species, although the true diversity is unknown, due to high genetic diversity and insufficient sampling in highly diverse regions, such as the Neotropics. Brazil, specifically the Brazilian Amazon supports the world's highest avian diversity and expected equally diverse yet undescribed community of avian haemosporidians. This study includes the largest sampling of avian haemosporidians in Brazil, and the first large scale survey of the Brazilian Amazon. A total of 4521 blood samples were collected from 447 host species, from 17 host orders and 49 host families. Samples were collected from five distinct Brazilian biomes, Amazonia (3381 samples), Atlantic Forest (39 samples), Caatinga (185 samples), Cerrado (790 samples), and Pantanal (126 samples). I developed a new real-time PCR assay to screen such large numbers of blood samples for the presence of avian haemosporidians. A 182 bp region of the conserved rDNA genes of avian haemosporidians was amplified. The real-time PCR assay proved as reliable as the two most widely used molecular screening methods, but has the additional benefit of screening for all three genera in a single reaction, saving time and expense. From positive samples a portion of the cytochrome *b* gene was amplified using two modified sets of nested PCR primers. One set amplified *Haemoproteus/Plasmodium* together and the

second set amplified *Leucocytozoon*. Sanger sequencing data was used to identify haemosporidian lineages for phylogenetic analysis. Of the 4521 samples screened, 730 were infected (16% prevalence) with *Haemoproteus* or *Plasmodium.* Due to expected low prevalence of *Leucocytozoon*, I attempted nested PCR amplification for only a subset of 1000 samples, and found no *Leucocytozoon* infections. More than three times as many blood samples were infected with *Plasmodium* (574 positive samples) than *Haemoproteus* (178 positive samples). These infections included individuals with coinfections of two lineages of *Haemoproteus*, two lineages of *Plasmodium*, or lineages of both *Haemoproteus* and *Plasmodium*. Haemosporidian prevalence differed between Brazilian biomes and avian host families. Haemosporidian diversity matched host diversity with 365 genetic lineages recovered, 86 *Haemoproteus* and 279 *Plasmodium*. More than 90% of these lineages (331) were novel lineages, never before described. The high number of novel lineages recovered from Brazil increases the known diversity of haemosporidian genetic lineages by 15 percent. An alignment containing these 365 newly discovered Brazilian lineages combined with all quality lineages from the MalAvi database was used for phylogenetic reconstruction. The Bayesian inference phylogeny produced showed a pattern of repeated lineage introduction into Brazil followed by diversification into unique lineages, endemic to Brazil. In the Amazonian biome, samples were collected from six distinct areas of avian endemism; Belém (323 samples), Guiana (353 samples), Imerí (164 samples), Inambari (1437 samples), Rondônia (1004 samples), and Tapajós (100 samples). The areas of endemism in Amazonia directly affected haemosporidian parasite diversity and distribution. Infection prevalence varied significantly between areas of endemism, with higher prevalence south of the Amazon

River. Compositional analysis on avian and parasite communities showed that parasite communities differ between areas of endemism and is attributed to differences in host communities. Areas of endemism with more similar host communities supported more similar parasite communities as well. Individual areas of endemism supported genetically more similar parasite communities, with a significant portion of genetic variation partitioned among areas of endemism. Haemosporidians are known to track host distribution, and analysis of genetic variation analysis showed that individual host families were infected by genetic lineages that were more genetically similar. Although area of endemism did not produce a significant phylogenetic signal in either *Haemoproteus* or *Plasmodium*, S-DIVA analysis did show a phylogeographic structuring in both genera, with the existence of area of endemism specific clades. This was especially true for *Haemoproteus*, where many lineages were concentrated within a Rondônia specific clade. The overall phylogeographic pattern was weaker for *Plasmodium*, but for several lineages area of endemism did appear to have phylogenetic signal. For *Haemoproteus* and *Plasmodium*, dispersal between areas of endemism was the most important event in their evolutionary history, likely due to lineages dispersing between avian hosts. Analysis of the effect of four host life history characteristics (nest height, nest type, foraging height, flocking behavior) on haemosporidian parasitism showed area of endemism as the only predictive variable when all samples were analyzed together. Only when each area of endemism was analyzed separately was host life history variation found to predict infection probability, although differing between areas of endemism. For *Haemoproteus*, nest height (Guiana, Rondônia), foraging height (Tapajós), and flocking (Belém) were found to significantly predict the probability of

infection, whereas for *Plasmodium* nest type (Inambari), foraging height (Guiana, Imerí) and flocking (Belém) were significant predictors. Host phylogenetic constraints on haemosporidian parasitism varied between areas of endemism. The 48 genetic lineages recovered from the Belém area of endemism were used for coevolutionary analysis of haemosporidian parasites and their avian hosts. Cost-event analysis showed that host switching was the most important event in the evolutionary history of haemosporidian parasites from the Belém area of endemism. Global cospeciation analysis showed a significant cospeciation signal between haemosporidian parasites and their avian hosts. The cospeciation signal was mostly due to strong coevolutionary links between *Haemoproteus* parasites and their non-passerine hosts. However, some *Plasmodium* lineages did show strong coevolutionary links with their passerine hosts, which contradicts what is known of the evolutionary history of avian *Plasmodium* parasites. Cospeciation analysis supports the presence of unique coevolutionary relationships between some haemosporidian parasites and their avian hosts. Along with rampant host switching, cospeciation has played a role in the highly diverse community of avian haemosporidians within Amazonian and throughout Brazil. Brazil supports a unique and diverse haemosporidian community, much of it contained within Amazonia, where unique biogeography has shaped the diversification and distribution of these parasites. The role of vectors is this region is unknown, since basic information on vector biology is lacking. Research is needed to determine the role that vectors have played in the distribution, and diversity of haemosporidian parasites within Amazonia and throughout Brazil.

# CHAPTER I

# INTRODUCTION

Parasites can have important impacts on the health, demography, behavior, and evolution of their hosts (Combes 1996, Combes *et al.* 1996, Parker *et al.* 2006, Atkinson *et al.* 2008). These aspects make parasites important elements in the studies of biodiversity and species interactions in ecological communities (Combes 1996, Combes *et al.* 1996, Brooks and Hoberg 2000, 2001, Whiteman and Parker 2005, Parker *et al.* 2006). Areas of high host diversity should support a similarly diverse assemblage of parasites with dynamic ecological and coevolutionary relationships. Parasites therefore can serve as models to make inferences about host ecology, population biology, and evolutionary history (Whiteman and Parker 2005, Nieberding and Morand 2006, Nieberding and Olivieri 2007). For these reasons there is a critical need to study host-parasite interactions in highly diverse regions like the Neotropics, where little is known about the avian parasite fauna and associated host-parasite interactions. The goal of this study is to determine such interactions within one important group of avian parasites, the haemosporidians within the Brazilian Amazon.

## Background on Avian Haemosporidians

Haemosporidians are protozoan parasites that infect vertebrate blood cells and are transmitted by dipteran vectors (Garnham 1966, Coatney *et al.* 1971, Schall 1996, Valkiūnas 2005, Telford 2009). Haemosporidians are one of the most widely studied groups of vertebrate parasites, because members of the genus *Plasmodium* have severe

impacts on human health (Cox 2010, Hay *et al.* 2010) and their evolutionary history is still not fully resolved (Perkins 2014).

Haemosporidians belong to the order Haemosporida in the phylum Apicomplexa (Valkiūnas 2005). Members of this phylum contain a non-photosynthetic plastid, the apicomplast. This organelle is essential for both cell survival and, in parasitic forms, for invading host cells (Roberts and Janovy 2008). Birds possess the highest diversity of haemosporidian parasites encompassing three genera; the sister taxa *Plasmodium* and *Haemoproteus* (families Plasmodiidae and Haemoproteidae) and *Leucocytozoon* (family Leucocytozoidae) (Valkiūnas 2005). *Plasmodium* is widely distributed in vertebrate hosts with both nucleated (birds, reptiles, and amphibians) and anucleated (mammals) red blood cells. In contrast, *Haemoproteus* and *Leucocytozoon* are only found in vertebrates with nucleated red blood cells, with *Leucocytozoon* species only found in birds (Valkiūnas 2005). Although each group infects the host blood stream, the morphology (Figure 1), ecology, and transmission of each genus differs and phylogeny of avian haemosporidians within the order Haemosporida is still not resolved (Perkins and Schall 2002, Martinsen *et al.* 2008, Outlaw and Ricklefs 2011, Perkins 2014, Borner *et al.* 2016) (Figure 2).

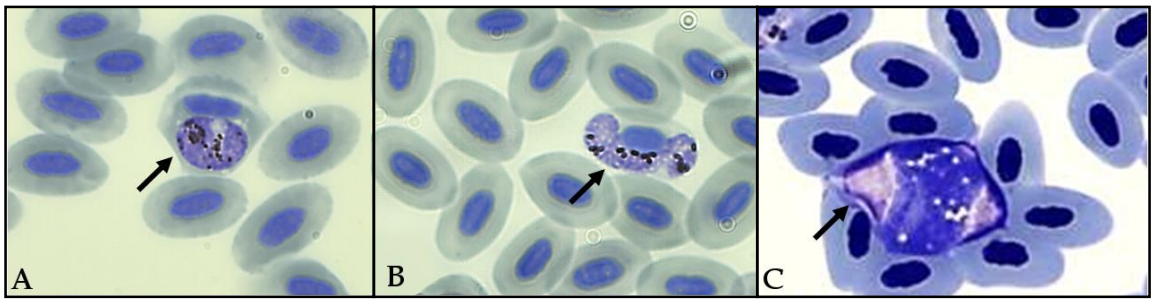

Figure 1. Blood films showing three genera of avian haemosporidians; A) *Plasmodium* B) *Haemoproteus* C) *Leucocytozoon*. All images were taken at 1000X magnification.

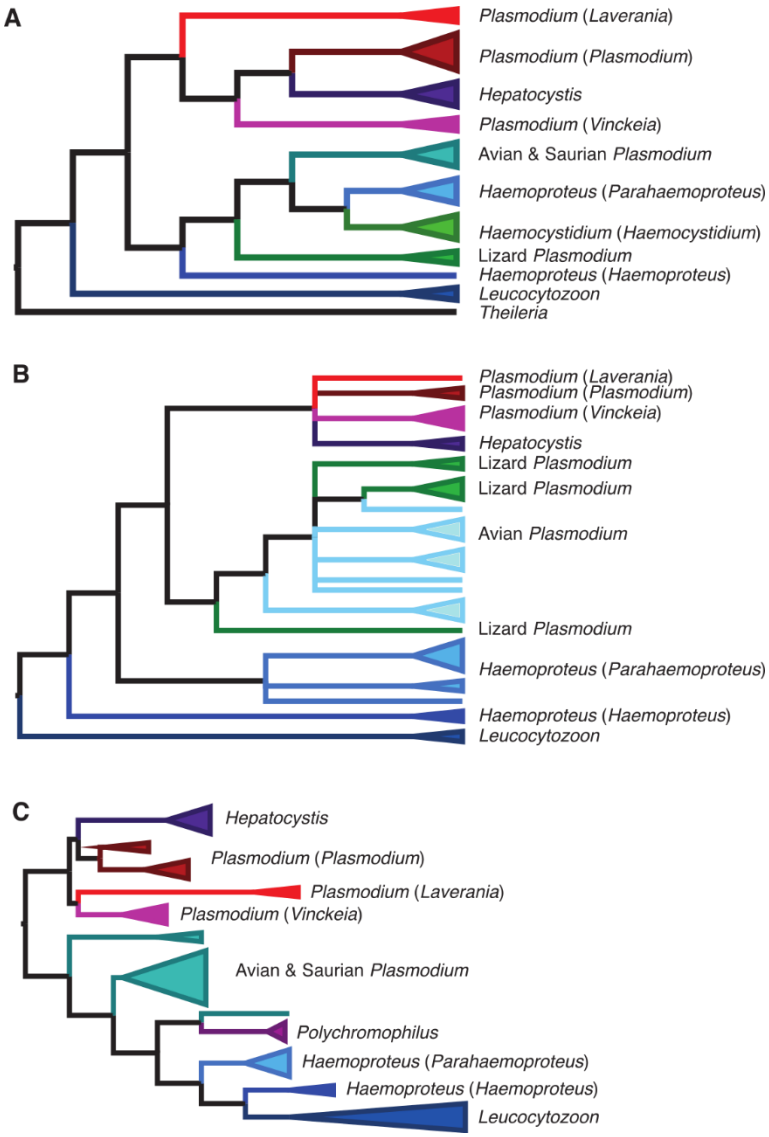


Figure 2. Three phylogenetic hypotheses for the order Haemosporida, (A) Perkins and Schall 2002, (B) Martinsen *et al.* 2008, (C) Perkins 2014. Figure taken from Perkins 2014.

Avian haemosporidians are a widely distributed group both in terms of hosts, infecting almost all known orders of birds, and geographically, being found in all continents except Antarctica (Valkiūnas 2005). There are slightly more than 200 named species of avian haemosporidians, all of which have been characterized and differentiated morphologically by studying blood films (=morphospecies) (Valkiūnas 2005). The use of polymerase chain reaction (PCR) amplification of the cytochrome *b* gene has revealed

many new lineages that are only known from nucleic acid sequence (=genetic lineage). Only a few of these lineages have been matched to known morphospecies (Valkiūnas *et al.* 2008a). At the time of this writing there were 2118 identified genetic lineages of avian haemosporidians in the MalAvi database, the largest database of avian haemosporidian sequences (Bensch *et al.* 2009, http://mbio-serv2.mbioekol.lu.se/MalAvi/). Efforts to link these lineages to known species are continuing (Valkiūnas *et al.* 2008a, 2008b), indicating the potential diversity of this group as well as the need for further studies using both microscopic examination and molecular analysis.

*Plasmodium* is the most widely known haemosporidian genus, due to species causing human malaria. One species of avian *Plasmodium* (*Plasmodium relictum*) was widely used until the 1950s as a model for understanding human malaria. It was eventually replaced by the rodent *Plasmodium* species (Valkiūnas 2005). The studies of avian *Plasmodium* now mostly revolve around understanding parasite-host association in bird populations (Valkiūnas 2005). *Plasmodium* is found in over 70 families of birds, occurring in all orders except Coliiformes, and Trogoniformes (Atkinson 2008a) and is globally distributed except in Antarctica (Valkiūnas 2005). There are 38 named species (Valkiūnas 2005) and 663 genetic lineages of *Plasmodium* (MalAvi database) transmitted by four genera of mosquitoes; *Culex, Aedes, Culiseta*, and *Anopheles* (Valkiūnas 2005).

*Haemoproteus* is the most diverse of the haemosporidian genera with 133 named species (Valkiūnas 2005) and 868 genetic lineages (MalAvi database). *Haemoproteus* has been found in birds from over 70 avian families (Atkinson 2008b). Recently, phylogenetic analysis has split the genus into two subgenera, *Haemoproteus* and *Parahaemoproteus* (Valkiūnas 2005, Figure 2). The subgenus *Parahaemoproteus* is the

most diverse containing over 90% of named species and includes those parasites transmitted by ceratopogonid midges, genus *Culicoides*. The subgenus *Haemoproteus* contains only the few species transmitted by hippoboscid flies and was originally known only from columbiform birds, but recently identified in seabirds, families Frigatidae (Levin *et al.* 2011) and Laridae (Levin *et al.* 2012) and most likely to occur in other families as well. Further studies are needed to understand the distribution of this subgenus.

*Leucocytozoon* is the least studied group of haemosporidians (see Lutz *et al.* 2015 for review); however, it is quite diverse. There are currently 35 named species (Valkiūnas 2005) and 557 genetic lineages (MalAvi database) found in members of 133 bird families (Forrester and Greiner 2008). Much of what is known of *Leucocytozoon* natural history comes from studies of those species that cause the disease leucocytozoonosis in waterfowl and poultry (Forrester and Greiner 2008). *Leucocytozoon* are parasitic exclusively in birds. Black flies (Simuliidae) transmit all species except *Leucocytozoon caulleryi* which is transmitted by *Culicoides* (Valkiūnas 2005).

### Life Cycles of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*

Avian haemosporidians have generally similar life cycles; all are transmitted by dipteran vectors and have similar life stages. Yet, they differ in how and where these stages occur in the host. Life cycle descriptions of haemosporidians below come from Valkiūnas (2005). All haemosporidians are transmitted by infective cells (=sporozoites) released by the vector during blood feeding. The sporozoites will then infect host cells and go through a stage of asexual reproduction (=merogony), which initially forms meronts or shizonts. The site of merogony differs in the different genera, with only

*Plasmodium* showing merogony in blood cells. Meronts will form uninuclear merozoites which will be released to infect host cells. Several stages of merogony usually occur allowing the parasite to both acclimate to its host and rapidly increase in number. The sexual stage occurs when merozoites eventually infect host blood cells and form gametocytes. Gametocytes produce the gametes needed for sexual reproduction in the vector. Two gametocytes are formed in host blood cells, the large macrogametocyte and the smaller microgametocyte. Once gametocytes are formed the parasite is infective to vectors. Once taken in during blood feeding the gametocytes will exit the host blood cells to form gametes (=gametogenesis) within the vector's midgut. The macrogametocyte forms a single macrogamete and the microgamete forms eight threadlike microgametes by exflagellation. Fertilization results in formation of the zygote that develops into a mobile, elongated ookinete. The ookinete penetrates the midgut of the vector and develops into an oocyst. The oocyst then undergoes a stage of asexual division (=sporogony), which produces many elongated sporozoites. Once released the sporozoites enter the haemocoele of the vector and eventually penetrate the salivary glands. The vector is now able to infect new vertebrate hosts, with sporozoites released during feeding.

In haemosporidians the infection in avian hosts includes several periods; 1) prepatent, 2) acute, 3) crisis, 4) chronic, 5) latent, and 6) relapse. These periods correspond with the life cycle of the parasite (Valkiūnas 2005). The prepatent period occurs during merogony outside of the blood stream before merozoites enter blood cells. Once merozoites infect blood cells the acute period begins and parasitemia rises quickly. Parasitemia peaks and symptoms develop during the crisis period. The production of

gametocytes indicates the beginning of the chronic period when the host can infect vectors. The parasitemia will generally decrease and in some cases may be eliminated by the host immune response during the latent period. In most hosts there will be a relapse of high parasitemia levels, occurring during the breeding and/or migratory period of the host. This relapse period, seen in most haemosporidians, facilitates infection of newly hatched birds as the parents serve as parasite source for vectors. It is thought that stress, hormonal changes, and photoperiod may serve as signals for the parasite relapse (Valkiūnas 2005).

The life cycle of *Haemoproteus* (Figure 3) follows the general haemosporidian pattern. Infected *Culicoides* or hippoboscid flies inject sporozoites that travel into the bloodstream and begin merogony. Merogony first begins outside of red blood cells (exoerythrocytic) in endothelial cells and macrophages. The first meronts are most frequently formed in the lungs and less often in other organs, such as the liver, spleen, heart, or skeletal muscle. At least two generations of exoerythrocytic merogony occur, the first producing merozoites that infect capillary endothelial cells, myofibroblasts, and reticular cells of the spleen and the second producing merozoites that infect blood cells. The first generation is responsible for maintaining the chronic infection status of *Haemoproteus*. The second generation produces meronts in the spleen and large meronts (megalomeronts) in skeletal muscle, both of which produce merozoites that penetrate red blood cells (erythrocytes) causing the production of gametocytes. Gametocytes are taken in by blood feeding vectors. The time period between injection of sporozoites in an avian host to production of gametocytes ranges from 11 to 21 days.

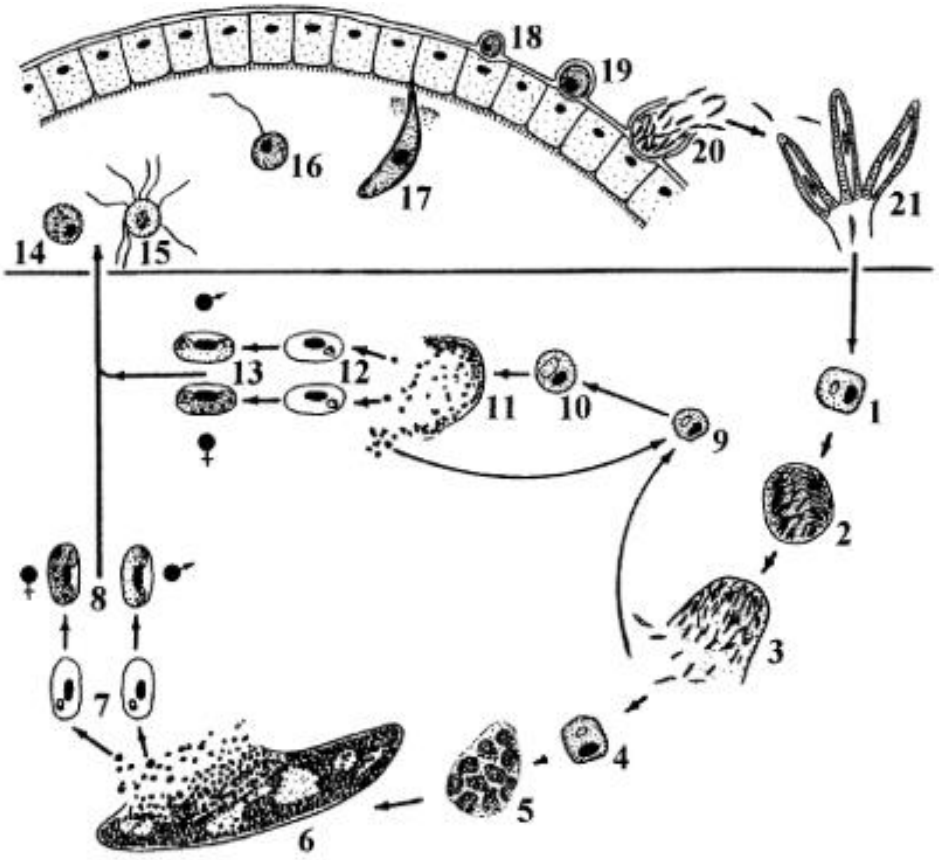


Figure 3. Diagrammatic representation of the life cycle of bird haemoproteids (*Haemoproteus mansoni* as an example): Upper part, in vector, lower part, in bird: 1 - sporozoite in endothelial cell; 2,3 –exoerythrocytic meronts of the first generation with elongated merozoites; 4 – merozoites in endothelial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

The life cycle of avian *Plasmodium* is the most complex of all haemosporidians with merogony occurring both within (erythrocytic) and outside of red blood cells (exoerythrocytic). It is the only haemosporidian genus where merogony occurs in the blood (Figure 4). Exoerythrocytic merogony is divided into primary, which occurs before erythrocytic merogony, and secondary, which occurs after. Infected mosquitoes

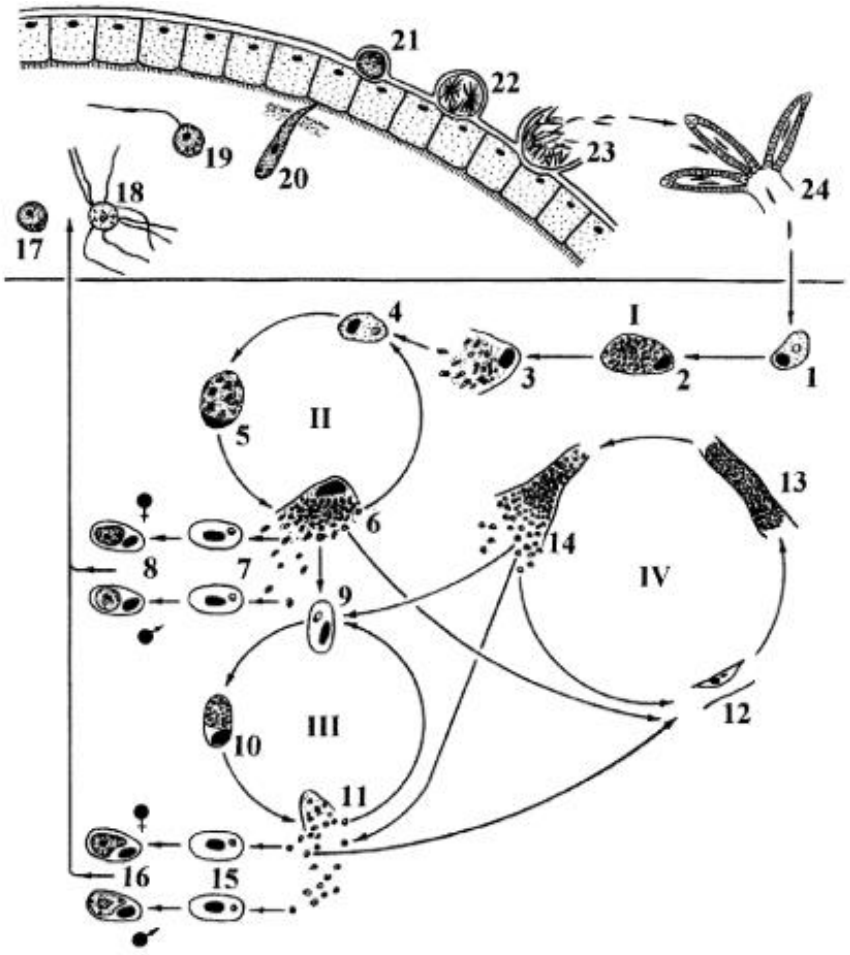


Figure 4. Diagrammatic representation of the life cycle of avian *Plasmodium* (*Plasmodium relictum* as an example): Upper part, in vector, lower part, in bird: I, II – primary exoerythrocytic merogony; III – erythrocytic merogony; IV – secondary exoerythrocytic merogony; 1 - sporozoite in reticuloendothelial cell; 2,3 – cryptozoites; 4 – merozoites in macrophage; 5,6 – metacryptozoites; 7 – merozoites in erythrocytes; 8 – gametocytes; 9 – merozoites in erythrocyte; 10,11 – erythrocytic meronts; 12 – merozoite in endothelial cell of capillaries; 13, 14 – phanerozoites; 15 – merozoites in erythrocytes; 16 – gametocytes; 17 – macrogamete; 17- exflagellation of microgametes; 19 – fertilization of macrogamete; 20 - ookinete penetrating the peritrophic membrane; 21 – young oocyst; 22, 23 – sporogony; 24 – sporozoites in the salivary glands of vector (From Valkiūnas 2005).

inject sporozoites into the bloodstream to begin primary exoerythrocytic merogony that includes two separate generations. The first generation occurs in the reticular cells of various organs and gives rise to meronts called cryptozoites. The cryptozoites produce merozoites that infect macrophages beginning the second generation where meronts

called metacryptozoites are produced. The merozoites of metacryptozoites reinfect macrophages to continue primary exoerythrocytic merogony, infect red blood cells to begin the erthryocytic stages, and infect endothelial cells to begin secondary exoerythrocytic merogony (Figure 4). Merozoite infection of red blood cells causes both the production of gametocytes and also begins erythrocytic merogony. Erythrocytic merogony forms meronts in the blood cells, called trophozoites. The trophozoites along with the blood cells they inhabit will later rupture releasing merozoites that can continue erythrocytic merogony, induce the production of gametocytes in blood cells, infect endothelial cells to begin secondary exoerythrocytic merogony, and produce symptoms in symptomatic hosts. In secondary exoerythrocytic merogony, capillary endothelial cells are infected by merozoites released by either metacryptozoites (primary exoerythrocytic merogony) or trophozoites (erythrocytic merogony). Meronts called phanerozoites are formed in capillary endothelial cells of several organs and release merozoites that infect blood cells, either continuing erythrocytic merogony or forming gametocytes. Secondary exoerythrocytic and erythrocytic merogony maintain parasite levels during chronic infections. Gametocytes formed by either exoerythrocytic or erythrocytic merogony are taken in by blood feeding mosquitoes and form gametes. The time period between injection of sporozoites in an avian host to production of gametocytes occurs more quickly than in *Haemoproteus* and can occur in as short as seven days.

The life cycle of *Leucocytozoon* parasites is unique in haemosporidians in that gametocytes are formed in both red and white blood cells (Figure 5). Infected black flies inject sporozoites into the bloodstream to begin merogony in parenchymal cells of the liver. The sporozoites will gradually penetrate hepatocytes to form meronts. As these

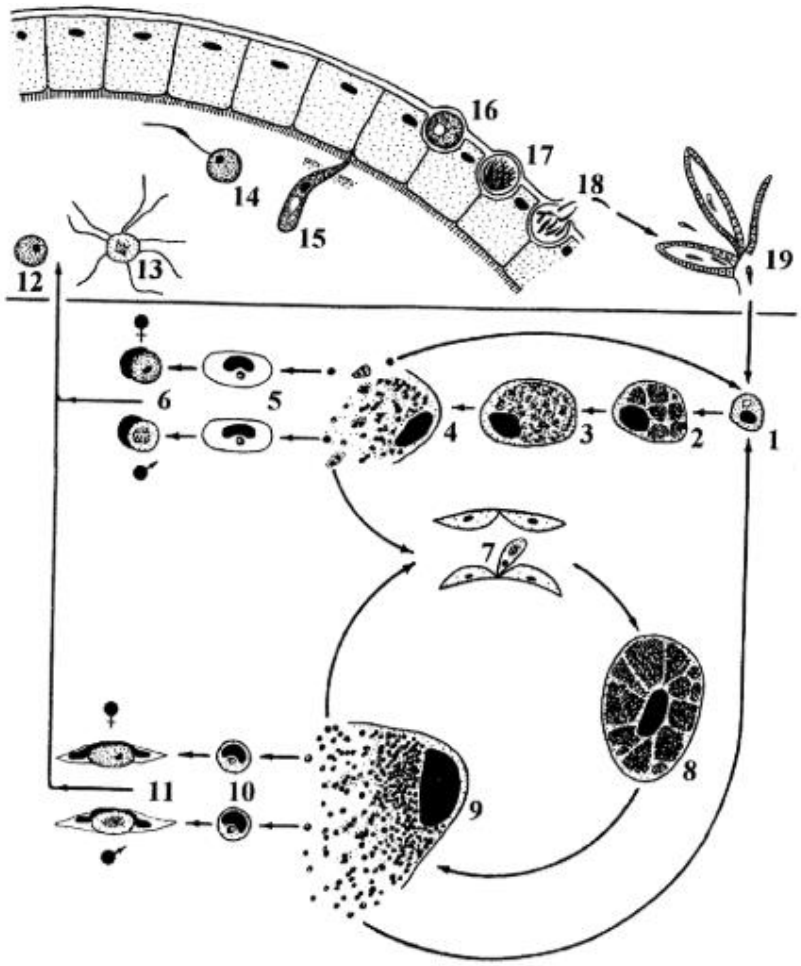


Figure 5. Diagrammatic representation of the life cycle of leucocytozoids (*Leucocytozoon simondi* as an example): Upper part, in vector, lower part, in bird: 1 - sporozoite or merozoites in the parenchymal liver cell (hepatocyte); 2 – 4, – hepatic meronts; 5 – merozoites in erythrocytes; ,6 – gametocytes in roundish host cells; 7 – syncytium (=a fragment of hepatic meront with two or more nuclei) or merozoites in reticuloendothelial cell; 8, 9 – megalomeronts; 10 – merozoites in mononuclear leukocytes; 11 – gametocytes in fusiform host cells; 12 – macrogamete; 13 – exflagellation of microgametes; 14 – fertilization of macrogamete; 15 - ookinete penetrating the peritrophic membrane; 16 – young oocyst; 17, 18 – sporogony; 19 – sporozoites in the salivary glands of vector (From Valkiūnas 2005).

hepatic meronts increase in size they will both release merozoites into the blood stream and eventually break apart into fragments called syncytia. The merozoites will infect red blood cells leading to the formation of gametocytes. The syncytia also enter the blood stream moving to several organs where they are phagocytized by macrophages to give

rise to the second phase of merogony. Large meronts called megalomeronts are formed generally in the spleen and release merozoites that infect lymphocytes and monocytes to form large fusiform gametocytes. Either type of gametocyte is taken in by blood feeding black flies and produces gametes. In general it takes about 8 to 10 days from injection of sporozoites to production of gametocytes.

**Tools Used for the Detection and Characterization of Avian Haemosporidians**

Studies of avian haemosporidians have a long history being first described by Danilewsky (Danilewsky 1884) and later used as a model for human malaria (Atkinson and van Riper 1991, Valkiūnas 2005, Cox 2010). With the discovery of rodent malaria (Vinke and Lips 1948) avian haemosporidians lost their importance as laboratory models. Consequently, they were relegated to the status of a group of limited interest, studied mainly in connection with impacts of these parasites on wild and domestic bird populations (Valkiūnas 2005).

The past two decades have seen a dramatic increase in the study of these parasites as tools to test evolutionary theories of parasite-host interactions (Ricklefs and Fallon 2002, Ricklefs *et al.* 2004, Fallon *et al.* 2005, Martinsen *et al.* 2008, Ricklefs *et al.* 2014, Lutz *et al.* 2015, Olsson-Pons *et al.* 2015) and the cost of parasitism on host populations (Marzal *et al.* 2005, Knowles *et al.* 2010, Martinez-de la Puente *et al.* 2010, Asghar *et al.* 2011, Lachish *et al.* 2011, Asghar *et al.* 2015). The growth in this field is directly tied to the development of a standard nested PCR protocol for amplifying a portion of the haemosporidian cytochrome *b* gene (Bensch *et al.* 2000, Hellgren *et al.* 2004, Waldenström *et al.* 2004) and the subsequent development of the MalAvi database of avian haemosporidian lineages (Bensch *et al.* 2009) (http://mbio-

serv2.mbioekol.lu.se/MalAvi/). Prior to the development of these resources, the main method to identify these parasites was microscopic examination of blood films, which requires expertise in making, staining, and examining such films. Although examination of blood films is an effective way for identifying and quantifying parasites (Valkiūnas *et al.* 2008c), the expertise needed to screen blood films takes time to develop, and chronic infections with low parasitemia can be missed (Jarvi *et al.* 2003, Waldenström *et al.* 2004). Although morphological data remain essential to link genetic lineages with known morphospecies (Valkiūnas *et al.* 2008c), molecular identification requires only minimal training, does not require quality blood films, and is generally accepted to be more sensitive than microscopy (Jarvi *et al.* 2002, 2003, Richard *et al.* 2002, Durrant *et al.* 2006, Fallon and Ricklefs 2008). It is also much faster and allows screening of large numbers of samples in a relatively short time.

The PCR protocols initially developed by Bensch *et al.* (2000), and modified by Hellgren *et al.* (2004), and Waldenström *et al.* (2004) are still widely used today. They rely on using two nested PCR amplifications of a 478 bp fragment of the cytochrome *b* gene, one set of nested PCR for *Haemoproteus/Plasmodium* (Bensch *et al.* 2000, Waldenström *et al.* 2004) and a separate set for *Leucocytozoon* (Hellgren *et al.* 2004). Although effective at both screening and amplifying haemosporidian parasite DNA, the time and amount of reagents necessary for running nested reactions can be limiting when screening large numbers of samples. Fallon *et al.* (2003) worked around this issue by developing an initial standard PCR screening protocol that amplified a 154 bp fragment of the conserved rDNA region of the mitochondrial genome of *Haemoproteus* and *Plasmodium*, although it did not identify *Leucocytozoon*. Only positive samples from

screening were subsequently amplified by regular PCR for cytochrome *b* and sequenced. This increased the speed at which large sets of samples could be screened, but still required the gel electrophoresis of hundreds or thousands of PCR products. Subsequently, researchers who used the Fallon *et al.* (2003) protocol for initial screening moved to various nested PCR protocols, (*e.g.* Fecchio *et al.* 2013, Svensson-Coelho *et al.* 2013), to improve the chances of amplifying haemosporidian DNA from hosts with low intensity of infection.

The use of real-time PCR to screen samples for presence of viral (Lanciotti *et al.* 2000, Wang *et al.* 2014, Yuan *et al.* 2014), bacterial (Ferdin *et al.* 2010, Birdsell *et al.* 2014, Greiman *et al.* 2014), or parasite (Teal *et al.* 2012, Albers *et al.* 2014, Xu *et al.* 2015) DNA has become a useful and common method of determining pathogen prevalence in host populations. Although real-time PCR has been used for avian haemosporidians, it has generally been used to determine level of parasitemia (Bentz *et al.* 2006, Zehtindjiev *et al.* 2008, Knowles *et al.* 2011, van Rooyen *et al.* 2013) or for detecting specific lineages (Asghar *et al.* 2011, Cellier-Holzem *et al.* 2010, Larcombe *et al.* 2013, Biedrzycka *et al.* 2014). The usefulness of real-time PCR as a large scale screening tool for haemosporidian DNA in avian blood samples has been only minimally explored (Friedl *et al.* 2012) and never done for all three genera with a single reaction.

**Ecology and Evolution of Host-Parasite Interactions in Avian Haemosporidians**

Coupled with their broad geographical distribution, their varying host-specificity, and high diversity of host species, avian haemosporidians are excellent models to test different evolutionary theories of parasite–host interactions and the costs of parasitism (Ricklefs and Fallon 2002, Ricklefs *et al.* 2004, Fallon *et al.* 2005, Martinsen *et al.* 2008,

Fecchio *et al.* 2011). Avian haemosporidians can directly decrease host survival (Marzal *et al.* 2008, Martinez-de la Puente *et al.* 2010, Lachish *et al.* 2011) and increase predation rates by raptors (Møller and Nielsen 2007), thus indirectly decreasing survival as well. The introduction of *Plasmodium relicutum* is known to be one of the major causes of extinction of several endemic Hawaiian bird species (Warner 1968, van Riper *et al.* 1986, Atkinson and Samuel 2010). Haemosporidians can have adverse effects on the reproductive performance of their hosts (Hakkarainen *et al.* 1998, Merino *et al.* 2000, Marzal *et al.* 2005, Knowles *et al.* 2010). Infected individuals delay reproduction (Ratti *et al.* 1993), lay fewer eggs (Korpimaki *et al.* 1993, Isaksson *et al.* 2013), and raise fewer chicks to fledging (Sundberg 1995, Marzal *et al.* 2005, Knowles *et al.* 2010, Asghar *et al.* 2011).

Although these are severe costs of parasitism, these affects do not seem to be universal for all haemosporidians, but rather specific to certain host-parasite associations. Incidences where infected hosts do not incur measurable survival and/or reproductive costs have also been documented (Davidar and Morton 1993, Knutie *et al.* 2013, Kulma *et al.* 2014, Zylberberg *et al.* 2015). In rock doves, *Columba livia*, nestlings experimentally infected with *Haemoproteus columbae* showed no decrease in body mass, fledging success, age at fledging, or post fledgling survival when compared to uninfected nestlings (Knutie *et al.* 2013). In purple martins, *Progne subis*, (Davidar and Morton 1993) and white-crowned sparrows, *Zonotrichia leucophrys*, (Zylberberg *et al.* 2015) infection with *Haemoproteus* actually increased host survival and reproductive success. Likewise, in the Hawaii Amakihi, *Hemignathus virens*, chronic *Plasmodium relictum* in breeding adults increased nesting success and offspring survival (Kilpatrick *et al.* 2006).

It has also been shown in collared flycatcher, *Ficedula albicollis*, (Kulma *et al.* 2014) that although infection by *Haemoproteus* delays reproduction, the offspring of infected mothers have no decrease in survival due to larger body size at fledging.

It has been hypothesized that lower investment in immune response is responsible for chronically infected hosts not incurring survival or reproductive costs. In these hosts the cost of infection is actually lower than the cost of mounting an immune response effective enough to successfully combat the infection (Ayres and Schneider 2012, Medzhitov *et al.* 2012, Sorci 2013). Obviously this can only occur in haemosporidian infections that show low virulence to their hosts, and most likely reflect a long coevolutionary history between parasite and host. Not surprisingly, examples of minimal or no infection costs are far more common for *Haemoproteus* (Davidar and Morton 1993, Knutie *et al.* 2013, Kulma *et al.* 2014, Zylberberg *et al.* 2015), which is known to be less virulent and more host specific than *Plasmodium* (Valkiūnas 2005). For example, the same study on white crowned sparrows (Zylberberg *et al.* 2015) has demonstrated that *Haemoproteus* infection increased host survival while *Plasmodium* infection did not. Severe costs of infection are more common for *Plasmodium* (e. g., Atkinson and van Ripper 1991, Merino *et al.* 2000, Valkiūnas 2005, Knowles *et al.* 2010, Lachish *et al.* 2011), which is especially evident when endemic bird populations are exposed to novel *Plasmodium* species (Warner 1968, van Ripper *et al.* 1986, Fix *et al.* 1988, Levin *et al.* 2009, Atkinson and Samuel 2010, Silveira *et al.* 2013). Further studies are warranted to determine the evolutionary context of these drastic differences in the cost of parasitism in avian haemosporidians.

The diverse life history characteristics of birds are useful in modeling the effects

of life history traits on parasite diversity and prevalence (Ricklefs 1992, Young *et al.* 1993, Tella 2002, Fecchio *et al.* 2011, Svensson-Coelho *et al.* 2013, Lutz *et al.* 2015) and can also serve as a model system to study the effects of parasitism on breeding behavior and sexual selection (Hamilton and Zuk 1982, Read 1991, Ricklefs 1992). In haemosporidians, parasite prevalence has been shown to be correlated with breeding season (Young *et al.* 1993), nest type and nest height (Fecchio *et al.* 2011, Lutz *et al.* 2015, Matthews *et al.* 2016), flocking behavior (Fecchio *et al.* 2013, González *et al.* 2014, Lutz *et al.* 2015), social system (Tella 2002), incubation period (Ricklefs 1992), and nesting habitat (Lutz *et al.* 2015). Avian haemosporidians infection has been shown to reduce song complexity and repertoire size (Buchanan *et al.* 1999, Gilmen *et al.* 2007), reduce male body condition (Atkinson and van Ripper 1991, Valkiūnas 2005, Williams 2005), and decrease male display behaviors in lekking species (Bosholn *et al.* 2016).

Birds are highly mobile, with a majority of species migrating between breeding and wintering grounds. Their mobility exposes them to different habitats, different vectors, and different risks of parasitism (Zeller and Murgue 2001, Alerstam *et al.* 2003, Hubálak 2004, Loiseau *et al.* 2012a, Hellgren *et al.* 2013, Oakgrove *et al.* 2014, Gutiérrez-Lopes *et al.* 2015). For example, in Alaska, only one lineage of *Plasmodium* is transmitted where several species of migratory birds breed. However, these same birds are exposed to many *Plasmodium* lineages on wintering grounds (Loiseau *et al.* 2012a, Oakgrove *et al.* 2014). For these species there would be a distinct advantage of flying to parasite free breeding areas due to high susceptibility of nestlings to haemosporidian infection (Edman and Scott 1987, Scott *et al.* 1988, Scott and Edman 1991). It has been speculated that avian migration evolved in part as a means to escape parasitism (Møller

and Szep 2010, Altizer *et al.* 2011), especially in host species that move between fresh and saltwater habitats (Mendes *et al.* 2005) or breed on small islands (Gutiérrez-Lopes *et al.* 2015).

Since most haemosporidians are life-long infections (Valkiūnas 2005, Atkinson *et al.* 2008), these infections travel with the bird during migration to infect new vectors and eventually new hosts. Birds can also introduce haemosporidians into areas where they are not found, shaping the worldwide distribution of these parasites (Altizer *et al.* 2011). Hitchhiking parasites become especially important with the increasing effects of climate change on bird movements (Lukas and Kry 2003, Miller-Rushing *et al.* 2008, Visser *et al.* 2009, Şekercioğlu *et al.* 2012), arthropod distribution (Khasnis and Nettleman 2005, Pascual *et al.* 2009), and consequently the dynamics of haemosporidian transmission (Møller 2010, Garamszegi 2011). Birds, therefore, serve as a model system to study the effect of animal movement on parasite transmission, introduction of parasite lineages into new habitats, climatic change of parasitism rates, and host-parasite interactions and co-evolution (Jenkins *et al.* 2012). The more data gathered on avian haemosporidian dynamics, the better we understand the complex interplay between parasite, vector, and host, which drives many important and emerging diseases. This is especially true in tropical regions that harbor a high diversity of both avian hosts and arthropod vectors with a presumably high diversity of haemosporidians (Clark *et al.* 2014). The Amazon basin of Brazil supports the highest diversity of avian hosts making it the ideal study region for investigating these issues.

## Avian Haemosporidian Studies from South America: Emphasis on the Brazilian Amazon

Avian haemosporidians are among the most studied Neotropical bird parasites. A review by White *et al.* (1978) summarized the prevalence of avian Neotropical haemosporidians, including blood parasite records from 35,555 birds (955 species). However, only 100 samples were from Amazonia (White *et al.* 1978). Other studies reported opportunistic haemosporidian sampling from southern Amazonian Brazil (Lainson *et al.* 1970) and Amazonian Bolivia (Bennett *et al.* 1991). Although there is a continuing interest in South American haemosporidians with large scale surveying conducted in Argentina (Smith and Ramey 2015), Bolivia (Bennett *et al.* 1991), Chile (Forrester *et al.* 1977), Colombia (Bennett and Borrero 1976, Valkiūnas *et al.* 2003, González *et al.* 2015), Ecuador (Svensson-Coelho *et al.* 2013, Harrigan *et al.* 2014, Moens and Pérez-Tris 2016), Peru (Jones *et al.* 2013, Smith and Ramey 2015), and Venezuela (Belo *et al.* 2012, Mijares *et al.* 2012), the only studies on Amazonian haemosporidians come from Bolivia (Bennett *et al.* 1991) and Ecuador (Svensson-Coelho *et al.* 2013, Moens and Pérez-Tris 2016). Studies of Brazilian haemosporidians come from its other biomes (Figure 6), namely the Cerrado (Fecchio *et al.* 2007, 2011, 2013, Belo *et al.* 2011) or the Atlantic forest (Bennett and Lopes 1980, Woodworth-Lynas *et al.* 1989, Ribeiro *et al.* 2004, Sebaio *et al.* 2012, Lacorte *et al.* 2013). The only known publication from the Brazilian Amazon was published recently on the impact of avian malaria on lekking in blue-crowned manakins (Bosholn *et al.* 2016). To date there has not been a large scale survey of avian haemosporidian parasites from the Brazilian Amazon. This highlights the lack of information on avian haemosporidians from Amazonia, especially in Brazil which contains 65% of the Amazon basin (Silva *et al.* 2005). This


Figure 6. Major biomes of Brazil as identified by Oliveira-Ferreira *et al.* 2010.

area contains the highest bird diversity in the world (Mittermeier *et al.* 2003, Marini and Garcia 2005) and an expectedly equally diverse community of avian haemosporidians.

Compared to their avian hosts, little is known about avian haemosporidians from the Brazilian Amazon. Information on diversity, taxonomy, and natural history of avian haemosporidians from the Brazilian Amazonia is minimal to non-existent. However, their diversity is surely high, given the high avian diversity of the Brazilian Amazon (1300

species, 20% endemic) (Mittermeier *et al.* 2003, Marini and Garcia 2005), and that birds are known to harbor a wide range of parasites often with individual hosts carrying multiple genera or species (Poulin and Morand 2000). Therefore it is safe to assume that the diversity of the avian parasites is at least as high as host diversity. Work from the highly diverse area of Malawi, Africa supports this statement with the number of haemosporidians (248 genetic lineages) far exceeding host species number (152) (Lutz *et al.* 2015).

Studying haemosporidians in Amazonia can also give insight into how biogeography can shape avian parasite distribution. The Amazonian biome is not a continuous region but rather comprises eight distinct areas of endemism (Figure 7) formed by major Amazonian rivers (Haffner 1978, 1985, 1987, Cracraft 1985, Silva *et al.* 2002). The Amazon was originally divided into four areas of endemism by Wallace (1852) based on primate distributions. Later, Cracraft (1985) divided Amazonia into seven areas of endemism using bird distributions. They were later expanded to eight areas by Silva *et al.* (2002). Data from other terrestrial vertebrates such as frogs (Ron 2000), lizards (Ávila-Pires 1995), and primates (Silva and Oren 1996) support these distinct areas of endemism. The distribution of butterflies (Brown 1979, Tyler *et al.* 1994, Hall and Harvey 2002) and vascular plants (Prance 1982) within Amazonia also coincide with these eight areas of endemism, thus providing a consistent spatial congruence pattern for different taxonomic groups (Silva *et al.* 2005).

The formation of endemic areas within Amazonia began during the Miocene before the generally accepted origin of most extant Amazonia taxa (Wesselingh *et al.* 2009). This distinct biogeographical pattern and its long geological history have therefore


Figure 7. Amazonian areas of endemism identified by Silvia *et al.* (2005) as modified from Cracraft (1985)

shaped the unique avian communities of Amazonia (Cracraft 1985, Silva *et al.* 2002, 2005, Wesselingh *et al.* 2009) with dispersal between these areas of endemism serving as the major speciation force in Amazonian birds (Smith *et al.* 2014). The dispersal pattern is more complex than simple movement between adjacent areas of endemism. Instead, it rather suggests that exchanges between Amazonia and other biomes in Brazil were more prevalent than movement within Amazonia (Cracraft and Prum 1988, Prum 1988,

Amorim 2001), mostly due to the lack of large river systems on the northern or southern edges of the Amazonian biome.

It is unknown how the unique biogeography of Amazonia has affected haemosporidian diversity, distribution, and phylogeny. Geographic barriers are known to affect host specificity by limiting the movement of specialist species/lineages (Loiseau *et al.* 2012b, Mata *et al.* 2015, Moens and Pérez-Tris 2016). Since geographic barriers (rivers) limit both host and parasite gene flow, areas delineated by such (like Amazonia) are expected to be dominated by generalist haemosporidian lineages as seen at least in the Ecuadorian Amazon (Moens and Pérez-Tris 2016). Geography has been shown to play a major role in the diversification and distribution of avian haemosporidians (Seghal 2015) with host switching between dispersing hosts being the major force of speciation (Ricklefs *et al.* 2004, Martinsen *et al.* 2008, Ricklefs *et al.* 2014). Initially allopatric speciation and host parasite coevolution take place following host range expansions with secondary sympatry resulting in local shifting of lineages across hosts (Ricklefs *et al.* 2014, Lauron *et al.* 2015). However the underlying mechanisms of how speciation occurs following host switching remains unclear, with only a limited amount of information on vectors available (Seghal 2015).

## Study Objectives

The haemosporidian parasites of Amazonian birds were collected and analyzed to determine parasite diversity, define parasite-host interactions, and determine parasite phylogeny. This study is a component of a larger study looking at all symbionts (ectoparasites, endoparasites, blood parasites, bacteria, viruses) of birds in the southern Amazon region of Brazil. The larger project entitled ***Southern Amazonian Birds and***

***their Symbionts*** is being conducted in collaboration with the Field Museum of Natural History in Chicago, Illinois, the Museu Paraense Emílio Goeldi in Belém, Brazil, and Drexel University in Philadelphia, Pennsylvania. This is a project funded by the National Science Foundation as part of its biodiversity, discovery, and analysis program, project numbers DEB-1120734 and DEB-1503804. The primary investigators are Dr. Jason Weckstein (Drexel University), Dr. John Bates (Field Museum), Dr. Vasyl Tkach (University of North Dakota), and Dr. Alexandre Alexio (Museu Paraense Emílio Goeldi). Samples collected for this study were combined with additional samples from both Amazonia and other biomes of Brazil by project collaborator Dr. Alan Fecchio (Drexel University). The specific study objectives are listed below.

1) Develop a real-time PCR protocol that would allow screening blood samples for all three genera of haemosporidians within a single reaction.
2) Identify, describe, and determine the prevalence of haemosporidian parasites in birds collected from five biomes in Brazil with a focus on Amazonia.
3) Describe host-parasite associations across the Amazonian biome.
4) Construct molecular phylogenies of detected species/lineages.
5) Determine the effect of Amazonian areas of endemism on parasite distribution, diversity, and phylogeny.
6) Determine the effect of host life history traits in Amazonia on parasite prevalence.
7) Describe the coevolutionary history between parasites and their avian hosts within the Belém area of endemism.

# CHAPTER II

# MATERIALS AND METHODS

Avian blood samples were obtained from two sources. First, samples (blood or liver fixed in ethanol and blood films) were collected from the Gurupi Biological Reserve (03º42'12.8"S, 46º45'44"W) as part of the larger project entitled Southern Amazonian Birds and their Symbionts. Second, additional samples of ethanol-fixed blood collected in several regions in Brazil were provided by Dr. Alan Fecchio. DNA was extracted from all samples and screened for the presence haemosporidians using polymerase chain reaction (PCR) as described below. Blood films from Gurupi were screened microscopically.

## Study Regions

A total of 4521 birds were collected from five Brazilian biomes; Amazonia, Atlantic Forest, Caatinga, Cerrado, and Pantanal (Figure 6). Of these samples 3381 were collected from Amazonia including 323 from Gurupi during July, 2013 and 3058 provided by Dr. Fecchio. Of these 3058 Amazonian samples, 720 were collected from the Los Amigos Biological Station (CICRA), Peru (12º34'S, 70º 05'W), located within the farthest westward expanse of the Amazonia biome. Of the remaining biomes, 39 samples were collected from the Atlantic Forest, 185 samples from Caatinga, 126 samples from Pantanal, and 790 from Cerrado. The Cerrado samples were previously screened by different methods (Fecchio *et al.* 2013), but positives were re-amplified and sequenced using our nested PCR approach.

The Amazonian biome comprises eight distinct areas of endemism (Figure 7) and sampling was conducted within six of these areas; Belém, Guiana, Imerí, Inambari, Rondônia, and Tapajós. The 323 samples collected from Gurupi were the only samples from the Bélem area of endemism. Dr. Fecchio provided all of the remaining Amazonian samples; Guiana – 353 samples, Imerí – 164 samples, Inambari – 1437 samples, Rondônia – 1004 samples, and Tapajós – 100 samples. Sampling distribution is provided in Table 1.

## Field Collection and Fixation

To maximize sampling effort, birds from Gurupi were collected using two separated techniques, mist netting and firearms. Both American Ornithologists' Union (Fair *et al.* 2010) and UND Institutional Animal Care and Use Committee guidelines (Project # 1402-1) for ethically collecting and euthanizing birds were strictly followed. All birds collected were euthanized for use as museum specimens and for internal parasite collection.

Mist netting used twenty to thirty twelve-meter mist nets set in lines crossing major habitats. The nets were moved every three to six days depending on declining rates of capture success. Netted birds were bled by brachial venipuncture using heparinized capillary tubes. Birds not likely to be captured by mist nets were identified by walking surveys each morning and collected by firearm. Blood and liver samples from all birds were stored in 95% ethanol for later genetic analysis. Between one and three blood films from mist netted birds were prepared, air dried, and fixed with 100% methanol in the field. Preparing blood films from birds collected by firearms was usually not possible.

All blood samples provided by Dr. Fecchio were obtained from birds captured

Table 1. Distribution of the 4521 samples collected from the four biomes and nine areas of endemism of Brazil.

| Biome (n) | Endemic Area (n) | Sample Location (n) | Coordinates |
|---|---|---|---|
| Amazonia (3381) | Belém (323) | Gurupi (323) | 03º42'12.8''S, 46º45'44"W |
| | Guiana (353) | Negro 01 (178) | 0º24'S, 64º48'W |
| | | PTB (175)[a] | 01º21'S, 56º22'W |
| | Imerí (164) | Negro 02 (164) | 0º35'S, 64º55'S |
| | Inambari (1437) | CICRA- Peru (720) | 12º34'S, 70º05'W |
| | | Madeira 01 (7) [a] | 08º48'S, 64º05'W |
| | | Madeira 03 (39) [a] | 09º06'S, 64º28'W |
| | | Madeira 04 (75) [a] | 09º08'S, 64º30'W |
| | | Madeira 05 (26) [a] | 09º08'S, 64º37'W |
| | | Madeira 06 (42) [a] | 09º09'S, 64º38'W |
| | | Madeira 07 (99) [a] | 09º16'S, 64º45'W |
| | | Madeira 08 (10) [a] | 09º19'S, 64º43'W |
| | | Purus 01 (211) | 04º59'S, 62º08'W |
| | | Purus 02 (208) | 05º43'S, 63º12'W |
| | Rondônia (1004) | CHU (117) [a] | 12º13'S, 60º44'W |
| | | COM (136) [a] | 13º48'S, 59º41'W |
| | | Madeira 02 (7) | 09º02'S, 64º14'W |
| | | Madeira 09 (102) | 09º19'S, 64º42'W |
| | | Madeira 10 (67) | 09º27'S, 64º21'W |
| | | Tapajós A (151) | 04º30'24.11"S, 56º17'1.5"W |
| | | Tapajós B (137) | 04º42'46.61"S, 56º26'23.93"W |
| | | Tapajós D (142) | 04º41'36.72"S, 56º38'18.56"W |
| | | Tapajós H (60) | 05º04'25.3"S, 56º51'24.81"W |
| | | Tapajós IL (85) | 04º30'45.3"S, 56º16'39.83"W |
| | Tapajós (100) | Tapajós I (61) | 05º13'37.12"S, 56º55'46.88"W |
| | | Tapajós J (39) | 05º06'46.11"S, 56º26'39.83"W |
| Atlantic Forest (39) | | Natal (39) | 05º55'24"S, 35º10'30"W |
| Caatinga (185) | | Aiuaba (62) | 06º36'06"S, 40º07'28"W |
| | | Serido (123) | 06º34'56"S, 37º16'02"W |
| Cerrado (790) | | CER (790) | 15º32'S, 47º33'W |
| Pantanal (126) | | Corumbá (110) | 19º34'S, 57º01'W |
| | | Cáceres (16) | 16º28'S, 58º08'W |

[a] Only data and DNA sequences provided

by mist net that were then banded, and released. As with samples from Gurupi blood was collected from the brachial vein using heparinized capillary tubes. Blood was purged from capillary tubes into collection vials containing 95% ethanol. Blood films were not produced for any of the blood samples provided by Dr. Fecchio.

### Laboratory Processing: Light Microscopy

Blood films (Gurupi only) were stained in a 1:10 solution of Giemsa stain in phosphate buffer (1g of disodium hydrogen phosphate and 0.7g of potassium dihydrogen phosphate per liter of $dH_2O$) for forty minutes and then air dried (Valkiūnas 2005). Films were viewed under 1000X magnification with 100 fields screened to detect parasites. Detected parasites were identified to the lowest taxonomic level possible using the taxonomic keys of Valkiūnas (2005) and multiple pictures were taken of infected blood cells. Blood films that screened positive for parasite infection by real-time PCR but negative by microscopy were rescreened by viewing 200 fields at 1000X magnification. After initial fixation over a year transpired before the slides could be stained due to permitting issues. Such a long time between fixation and staining produced poor results, making it not only difficult to accurately assess parasitemia but rendering it impossible to identify parasites below the genus level. Therefore the results from microscopic screening blood films are not used for any statistical analysis or discussed further.

### Laboratory Processing: Molecular Methods

Samples collected from CHU, COM, Madeira River, and PTB collection sites (Table 1) were screened and amplified by project collaborators following the protocols of Fallon *et al.* (2003) and Waldenström *et al.* (2004) respectively. Collaborators only supplied host data and DNA sequences for these samples. Samples from Cerrado (Table

1) had previously been screened and amplified (Fecchio *et al.* 2013). Since a different region of the cytochrome *b* gene was amplified previously, positive samples were reamplified with the nested PCR approach detailed below and a subset of samples were rescreened to test the efficacy of the new real-time PCR approach. For all other samples host blood (Gurupi and Dr. Fecchio samples) or liver (Gurupi) were processed, screened, and analyzed using the same molecular methods.

**DNA Extraction**

DNA was extracted using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA), following Qiagen tissue protocol for both blood and liver stored in 95% ethanol. Since blood coagulates in 95% ethanol, sterilized wooden applicators were used to transfer a small portion of the clot representing approximately 2 $mm^3$ into each extraction tube. This sample method of transferring blood was used for liver samples as well. After the transfer, blood and liver samples were dried at 60$^{\circ}$C for one hour in extraction tubes to evaporate residual ethanol in the samples. Prior to extraction the extraction tubes were spun for one minute at 6000 g to bring samples to the bottom. Both liver and coagulated blood samples required overnight incubation at 56$^{\circ}$C for proper digestion.

**DNA Amplification Using Real-time and Standard PCR**

Design of primers that could successfully amplify all three genera in a single real-time reaction required determining a gene region that is more conserved than the standard 478 bp fragment of the cytochrome *b* gene (Hellgren *et al.* 2004, Waldenström *et al.* 2004). The rDNA region of the mitochondrial genome was a good target because it is quite conserved in avian haemosporidians and has been previously used to screen for

*Haemoproteus* and *Plasmodium* infections (Fallon *et al.* 2003). Available avian haemosporidian mitochondrial sequences from GenBank (Table 2) that contained the conserved rDNA region were aligned using BioEdit v7.2.0 (Hall 1999).

Table 2. List of GenBank sequences used to design real-time PCR primers to detect haemosporidian rDNA. Accession numbers and the associated haemosporidian species/lineage are given.

| Accession Number | Haemosporidian species/lineage |
|---|---|
| FJ168562 | *Haemoproteus columbae* |
| AY733087 | *Haemoproteus* sp. jb1. JA27 |
| AB302215 | *Leucocytozoon caulleryi* |
| FJ168564 | *Leucocytozoon fringillinarum* |
| FJ168563 | *Leucocytozoon majoris* |
| NC009336 | *Leucocytozoon sabrezesi* |
| AB250690 | *Plasmodium gallinaceum* |
| AB250415 | *Plasmodium juxtanucleare* |
| KC138226 | *Plasmodium lutzi* |
| NC012426 | *Plasmodium relictum* |

Although the primers described by Fallon *et al.* (2003) did not match *Leucocytozoon* sequences, a region adjacent to these primers proved to be sufficiently conserved for detection of all three genera. The forward primer R330F and reverse primer R480RL were designed, flanking a 182 base pair fragment (Figure 8, Table 3).

All reactions were carried out using iTaq universal SYBR Green Supermix on a CFX96 real-time thermocycler (Bio-Rad, Hercules, CA). The total volume of the reactions was 15 µl, with 7.5 µl of SYBR Green Supermix, 0.6 µl of each primer (10 µM concentration), 3.3 µl of molecular grade water, and 3 µl of DNA template (the volume established empirically, approximately 20 ng/µl). The following cycling conditions were used: 95˚C for 30 seconds, followed by 35 cycles of 95˚C for 30 seconds and 53˚C for 35

Table 3. Primer sequences for real-time and nested PCR protocols, along with sequence of positive control used for real time PCR reactions. Sequencing primers are also listed.

| Protocol/Primer | Primer Sequence |
|---|---|
| Real-Time PCR – *Haemoproteus, Plasmodium, Leucocytozoon* | |
| R330F[a] | 5'- CGTTCTTAACCCAGCTCACG - 3' |
| R480RL[a] | 5'- GCCTGGAGGTWAYGTCC – 3' |
| | |
| *P. relictum* Pos. Control (NC012426) | 5'-GGGAACAAACTGCCTCAAGACGTTCTTAACCC AGCTCACGCATCGCTTCTAACGGTGAACTCTCAT TCCAATGGAACCTTGTTCAAGTTCAAATAGATTG GTAAGGTATAGCGTTTACTATCGAATGAAACAAT GTGTTCCACCGCTAGTGTTTGCTTCTAACATTCCA TTGCTTATAACTGTATGGACGTAACCTCCAGGCA AAGAAATGACCGGTC – 3' |
| Nested PCR – *Haemoproteus* and *Plasmodium* | |
| H332F[a] | 5' - GAGAATTATGGAGYGGATGGTG - 3' |
| HAEMNR2[b] | 5' - AGAGGTGTAGCATATCTATCTAC- 3' |
| | |
| H350F[a] | 5' – GGTGTTTTAGATATATGCATGC - 3' |
| HAEMR2[c] | 5' - GCATTATCTGGATGTGATAATGGT - 3' |
| | |
| Nested PCR – *Leucocytozoon* | |
| HAEMNFI[d] | 5' - CATATATTAAGAGAAITATGGAG - 3' |
| HAEMNR3[d] | 5' - ATAGAAAGATAAGAAATACCATTC - 3' |
| | |
| L350F[e] | 5' - GGTGTTTTAGATACTTA -3' |
| L890R[e] | 5' - TACAATATGTTGAGGTGTTTG - 3' |
| | |
| Sequencing – *Haemoproteus* and *Plasmodium* | |
| FIFI[f] | 5' – GGGTCAAATGAGTTTCTGG - 3' |
| R2[f] | 5' - GCTGTATCATACCCTAAAGG - 3' |
| | |
| Sequencing – *Leucocytozoon* | |
| L545F[e] | 5' - ACAAATGAGTTTCTGGGGA - 3' |
| L825R[e] | 5' - GCAATTCCAAATAAACTTTGAA - 3' |

[a] Designed for this study, [b] Waldenström *et al.* 2004, [c] Bensch *et al.* 2000, [d] Hellgren *et al.* 2004,[e] Lutz *et al.* 2015, [f] Ishtiaq *et al.* 2007

seconds (with a plate read) followed by a final melt curve analysis using instrument default settings. Positive and negative controls were included in all runs. The positive control used was a synthetic double stranded DNA product (G-Block - IDT DNA, Coralville, IA) designed from a 220 bp fragment of the conserved rDNA region of *Plasmodium relictum* (Accession # NC012426) (Table 3). This positive control of

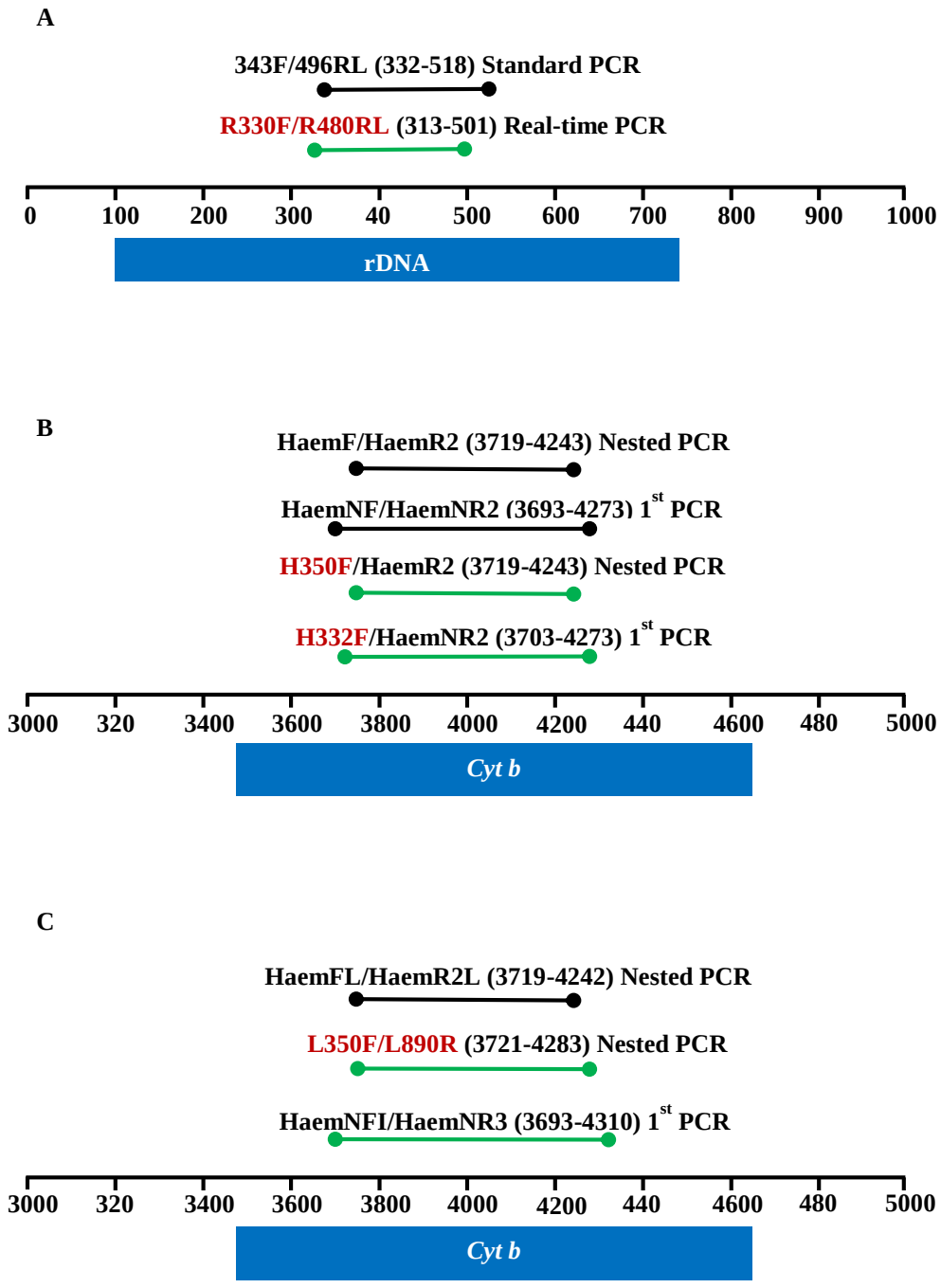


Figure 8. Primer positions of rDNA primers for standard and real-time PCR (A) and cytochrome *b* primers for nested PCR for *Haemoproteus/Plasmodium* (B), and *Leucocytozoon* (C). Blue bars denote location of the target genes on the mitochondrial genome of *Plasmodium relictum* (NC012426). The spans of amplified DNA fragments are indicated in parentheses behind each primer pair. Fragments in green are those that we recommend for use in avian haemosporidian detection (A) and amplification by nested PCR (B, C). Primers in red represent new primers developed for avian haemosporidians either herein or in (Lutz *et al.* 2015).

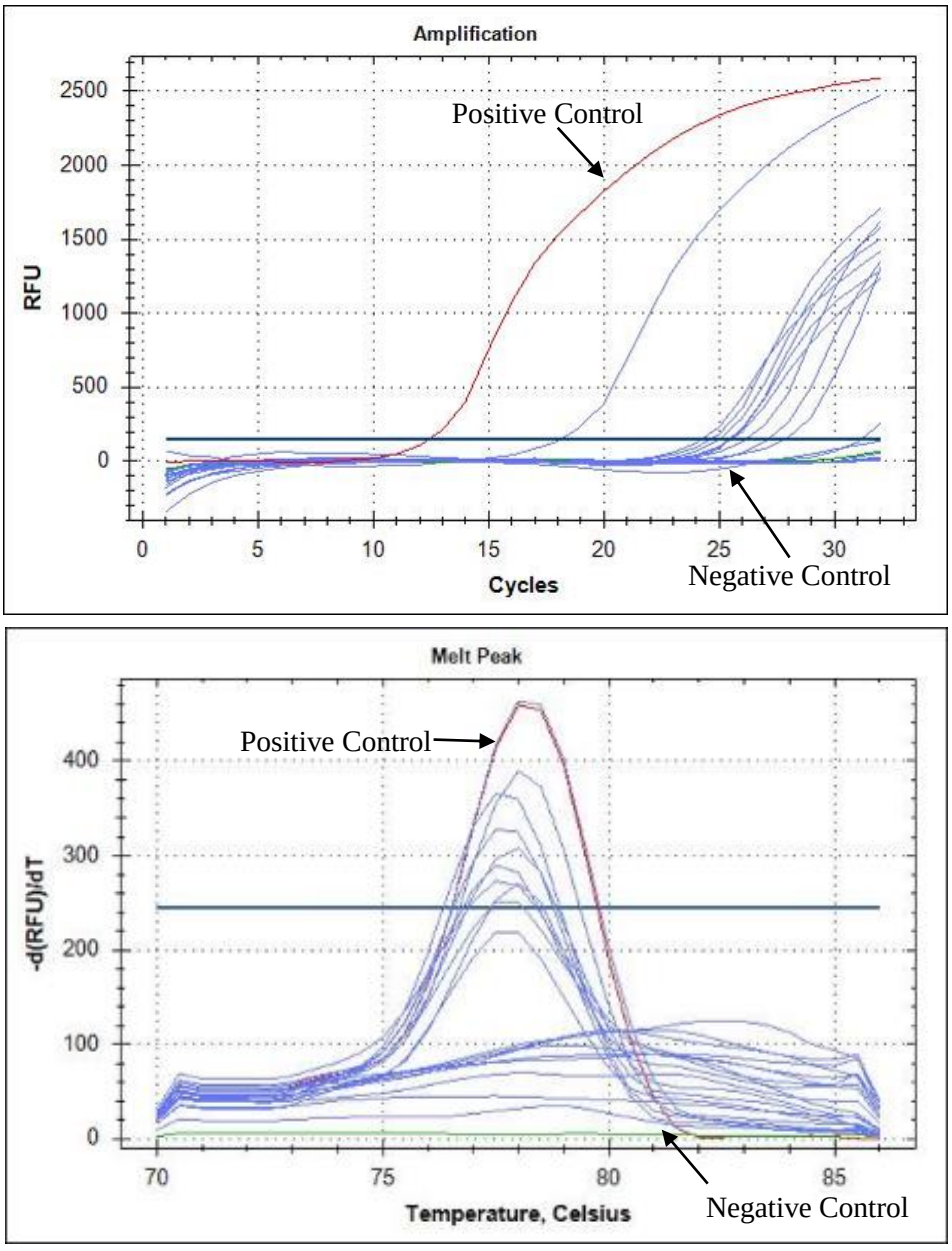


Figure 9. Amplification and melt peak curves from real-time PCR amplification of rDNA from avian blood samples. Positive (*Plasmodium relictum*) control, shown in red, and negative (water) control, shown in green, are indicated in the curves.

*Plasmodium relictum* produced a melt curve peak at 78.5$^0$C (Figure 9).

This protocol was initially tested on samples positive for *Plasmodium*, *Haemoproteus*, or *Leucocytozoon*, samples with mixed infections, and known negative samples, from a previous study of haemosporidians from Malawi, Africa (Lutz *et al.*

2015). These samples had been previously screened by nested PCR and microscopy (Lutz *et al.* 2015) and were from 16 host species, representing 15 genera, 13 families, and 7 orders.

To further test this protocol 94 samples were selected from the 790 samples collected from the Cerrado biome that had previously screened for haemosporidian parasites (Fecchio *et al.* 2013) using the screening protocol described by Fallon *et al.* (2003). These samples were obtained from four host species, *Myiarchus swainsoni*, *Neothraupis fasciata*, *Nystalus chacuru*, and *Volatinia jacarina*, and were rescreened with the real-time protocol and also amplified using nested PCR protocols (described below) to amplify the cytochrome *b* gene. This not only allowed for testing the effectiveness of the real time protocol, but also enabled comparison between the three different screening methods (single PCR, nested PCR, real-time PCR). Results for these screening methods were analyzed using a 2x3 chi-square contingency table using the package Rcmdr (Fox 2005) within R (version 3.2.2; R Development Core Team 2015).

Two modified nested PCR protocols were used to amplify fragments of the cytochrome *b* gene (Table 3). The protocol for *Haemoproteus/Plasmodium* was based on the standard protocol of Waldenström *et al.* (2004), but with newly designed forward primers, H332F and H350F (Figure 8, Table 3), which match more closely with available GenBank sequences. The protocol produces a 477 bp fragment, which is only one base pair shorter than the fragment produced by the Waldenström *et al.* protocol (2004). The *Leucocytozoon* protocol uses the initial primer sets described by Hellgren *et al.* (2004) but with newly designed nested primers (Lutz *et al.* 2015) (Figure 8, Table 3). This new

protocol produces a 526 bp fragment that encompasses the 478 bp fragment produced by the Hellgren protocol (Lutz *et al.* 2015).

All nested PCRs were run using OneTaq Quick-Load 2X Master Mix with standard buffer (New England Biolabs, Ipswich, MA) in 20 µl reactions. The initial PCR amplifications included 10 µl of OneTaq Master Mix, 1 µl of each primer (10 µM concentration), 3 µl of molecular grade water, and 5 µl of template (the volume established empirically, approximately 20 ng/µl). The nested PCR amplifications differed in using 5 µl of water and 3 µl of PCR product as template. The following protocol was used for all reactions; 95˚C for 3 minutes, then followed by 20 cycles (first amplification)/35 cycles (nested amplification) of 95˚C for 30 seconds, 50˚C for 45 seconds, and 68˚C for one minute, followed by a final elongation at 68˚C for 5 minutes. Negative controls were included in all nested PCR runs. All samples identified as positive by real-time PCR underwent nested PCR amplifications for *Haemoproteus/Plasmodium* using our modified Waldenström protocol. Due to expected low prevalence of *Leucocytozoon* (White *et al.* 1978, Valkiūnas 2005, Forrester and Greiner 2008, Lotta *et al.* 2015) only a subset of 1000 samples from Amazonia were amplified using the modified nested protocol (Lutz *et al.* 2015) for this genus. All PCR products were run on 1.25% agarose gels, stained with ethidium bromide, visualized under UV light, and photographed.

**DNA Sequencing, Sequence Assembly, and Alignment**

Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Bio systems, Foster City, CA). The primers FIFI and R2 (Ishtiaq *et al.* 2007) were used for

sequencing of *Haemoproteus* and *Plasmodium* and the primers L545F and L825R (Lutz *et al.* 2015) were used for *Leucocytozoon* (Table 2). Sequencing reactions were conducted in 96 well plates and reaction products were precipitated with ethanol using the following procedure. Sixty µl of 76% ethanol was added to all wells, the plate was sealed, vortexed, and let to sit for 15 minutes at room temperature. Samples were then spun for 35 minutes at 6000 g (which may correspond to different speeds in different centrifuge rotor sizes). After spinning, ethanol was removed by inverting the plate several times followed by spinning the inverted plate placed on top of paper toweling for 1 minute at 50g. After this step 180 µl of 70% ethanol was added to the plate, it was resealed and spun again for 35 minutes at 6000 g. Ethanol was removed as described above and the unsealed plate was placed on a 60˚C 96 well aluminum heat block for 10 minutes to remove any remaining ethanol. Samples were then re-suspended with 10 µl of dH2O, and run on an ABI 3100 DNA sequencer (Applied Bio systems, Foster City, CA).

Forward and reverse sequences were visualized and assembled using Sequencher v.5.0.1 (Gene Codes Corp., Ann Arbor, MI). Chromatograms that showed the presence of multiple infections were scored as co-infections. Co-infections were separated using the program PHASE 2.1.1 (Stephens *et al.* 2001, Stephens and Donnelly 2003) following the protocol of Harrigan *et al.* (2014). We failed to separate individual sequences from eight samples with co-infections. These samples were removed from all subsequent analyses.

Assembled sequences were aligned using BioEdit v7.2.0 (Hall 1999) and collapsed to unique haplotypes using the FaBox haplotype collapser and converter tool (Villesen 2007). Sequence identities were verified with a local BLAST against the MalAvi database (Bensch *et al.* 2009) using BioEdit v7.2.0 (Hall 1999). New lineages

were named after the host of origin following standard protocol (Bensch *et al.* 2009), using a six letter code produced by using the first three letters of both the host genus and species epithet followed by a number to denote multiple lineages from a single host species. For example lineage WILPOE01 represents the first lineage obtained from *Willisornis poecilinotus*. All sequences were deposited in GenBank (Accession No. KU562119 – KU562842) and the MalAvi database. All sequences detected along with lineage name, sampling location, avian host, and Genbank Accession number are located within Appendix A.

## Evolutionary and Ecological Analysis of Avian Haemosporidians

### Phylogenetic Reconstruction

Assembled sequences of unique lineages were used to construct molecular phylogenies. The GTR+I+G model of nucleotide substitution was implemented for all phylogeny reconstruction as determined by jModelTest (Darriba *et al.* 2012). Lineages were organized into three separate alignments for phylogenetic analysis: 1) lineages from all Brazilian biomes, 2) lineages from the Brazilian Amazon (excluding CICRA), and 3) lineages from the Belém area of endemism, Gurupi collection site (Table 1).

To determine how the newly identified lineages from this study fit within the known phylogeny of *Haemoproteus* and *Plasmodium* all available lineages were downloaded from the MalAvi database (Bensch *et al.* 2009) and aligned with the new Brazilian lineages. Any poor quality or overly short sequences from MalAvi were removed from the alignment. The final alignment contained 1262 sequences. The program Beast v1.82 (Drummond *et al.* 2012) was used for Bayesian inference phylogeny using a strict molecular clock with a 1.2% sequence divergence per million

years (Ricklefs and Outlaw 2010). The coalescent tree prior was implemented with a chain length of 200 million permutations sampled every thousand steps. The resulting log file was analyzed with the program Tracer (Rambault *et al.* 2014) to determine if the chain length was appropriate for the analysis, producing effective sample sizes for all measures above 200 (Drummond *et al.* 2012). The consensus tree was produced using TreeAnnotator (Drummond *et al.* 2012) discarding 10% of trees as burn in and viewed using FigTree (Rambault 2009). Due to the high computer capacity required to run this analysis it was uploaded and run on the CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway v3.3 (Miller *et al.* 2010).

For clarity of visualizing phylogenetic patterns the lineages from Brazilian Amazon were split into two separate alignments based on parasite genus, *Haemoproteus* or *Plasmodium*. For both genera, *Leucocytozoon fringillarum* (FJ168564) served as the outgroup. A Bayesian inference and a maximum likelihood phylogeny was constructed for both genera using the programs Mr. Bayes (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) and RAxML (Stamatakis 2014) respectively. In Mr. Bayes the analysis was run until the standard deviation of split frequencies stabilized below 0.01. Twenty-five percent of resulting trees were discarded as burn in. In RAxML 1000 bootstraps were performed to obtain branch support values. All trees were visualized in FigTree (Rambault 2009).

To determine the effect of area of endemism on the phylogeny of *Haemoproteu*s and *Plasmodium* lineages within Amazonia a phylogeographical ancestral state reconstruction analysis was implemented within the program RASP (Yu *et al.* 2015). Within RASP an S-DIVA (Statistical-Dispersal Vicariance Analysis) analysis was

performed to reconstruct the ancestral distribution in the phylogeny by optimizing a cost matrix, where extinctions and dispersals are more costly than vicariance (Ronquist 1997, 2001, Lamm and Redelings 2009, Yu *et al.* 2010). S-DIVA (Yu *et al.* 2010) differs from traditional DIVA (Ronquist 1997, 2001) analysis by taking into account phylogenetic uncertainties and determining statistical support for ancestral range reconstructions (Nylander *et al.* 2008, Harris and Xiang 2009, Yu *et al.* 2010). The S-DIVA within RASP requires an ultrametric binary tree so Beast v1.82 (Drummond *et al.* 2012) was again used for *Haemoproteus* and *Plasmodium* separately as described above, with *Leucocytozoon fringillarum* (FJ168564) serving as the outgroup. To better visualize the geographic signal within the phylogenies the program GenGIS (Parks *et al.* 2013), which links a phylogenetic tree to a geographic map using latitude and longitude values from the collected DNA sequences, was utilized. For visualization the outgroup was removed.

Lineages from the Belém area of endemism were analyzed in Beast v1.82 (Drummond *et al.* 2012) as described above. Due to the smaller number of lineages (49), lineages from both genera were combined for this analysis and no outgroup was used. The resulting tree was used for coevolutionary analyses as described below.

**Analysis of Molecular Variance**

Analysis of molecular variance (AMOVA) was performed in Arlequin 3.5.1.2 (Excoffier and Lischer 2010) to determine the extent of partitioning within Amazonian haemosporidian lineages due to area of endemism. In this analysis evolutionary distance as measured by sequence divergence was taken into account when partitioning genetic variance among hierarchal levels of population structure (Fitzpatrick 2009). In Arlequin, sequence divergence was calculated by the Tamura-Nei model of nucleotide substitution

(Tamura and Nei 1993) and the frequency of each lineage that occurred in each area of endemism was used to estimate the proportion of total covariance distributed among versus within the areas of endemism. Statistical significance was determined by Monte Carlo permutation test based on 1000 iterations. AMOVAS were run for all lineages combined and also for *Haemoproteus* and *Plasmodium* lineages separately. To determine how genetic covariation was partitioned within and among host families in Amazonia, AMOVAS as described above were also conducted. As with analyses for area of endemism, initially all lineages were combined and then split by parasite genus.

**Coevolutionary Analysis**

To understand the coevolutionary history of haemosporidians and their avian hosts two separate cophylogenetic analyses (CoRe-PA and PACo) were conducted using samples collected from the Belém area of endemism (Table 1).

CoRe-PA (Merkle *et al.* 2010) is an event cost analysis, which tries to determine the most probable coevolutionary history based on specific event costs. This analysis identifies the events that provide the best explanation of the co-phylogenetic patterns. The events include codivergence (cospeciation), sorting (extinction), duplication (within host speciation), and host switching (Merkle *et al.* 2010). A tanglegram produced from host and parasite trees is used as the starting point for all analyses. The parasite tree was produced from only those lineages identified in samples from Gurupi Brazil (Table 1) in Beast software as described previously. For the host tree, avian cytochrome oxidase I (COI) sequences were obtained from Genbank (Table 4), aligned in BioEdit v7.2.0 (Hall 1999), and used to construct a Bayesian tree within Beast. For five host species, *Micrastur mintoni*, *Pheugopedius genibarbis*, *Philydor erythropterum*, *Poecilotriccus*

*fumifrons*, and *Xiphorhynchus spixii*, COI sequences were not available in Genbank so sequences from *Micrastur gilvicollis*, *Philydor erythrocercum*, *Pheugopedius coraya*, *Poecilotriccus sylvia*, and *Xiphorhynchus elegans* were used in their place since they represent the closest related species with available COI sequence.

The host tree was constructed using the same methods as the parasite tree with the following two exceptions. First, a sequence divergence rate of 2.1% per million years (Weir and Schluter 2008) was used for the strict molecular clock and second, the Yule speciation tree prior (Drummond *et al.* 2012) was used. Five separate analyses, each with a different cost matrix for the four events, were conducted (Table 5). The costs for each of the five analyses were determined from previous studies (Bensch *et al.* 2000, Ricklefs and Fallon 2002, Ricklefs *et al.* 2004, Szymanski and Lovette 2005, Križanauskiené *et al.* 2006, Beadell *et al.* 2009, Santiago-Alarcon *et al.* 2014). For each analysis 100 randomizations were conducted to determine if the number of each event differed significantly from random association between the host and parasite trees (Table 5).

PACo (Balbuena *et al.* 2013), unlike CoRe-PA is a global test to determine the congruence between the two phylogenies and identifies the host-parasite associations that contribute significantly to the cophylogenetic structure (Balbuena *et al.* 2013). This analysis implements a procrustean approach using host and parasite genetic distance matrices to produce a residual sum of squares ($m^2_{xy}$) that measures the fit of the parasite phylogeny to the host phylogeny, which is assessed statistically by comparing it to 10,000 random permutations of the host/parasite association.

Within PACo, to test the contribution of each host-parasite link to the global cospeciation signal the squared residual for each link and the associated 95%

Table 4. Host species used in coevolutionary analysis, host code, and accession number.

| Host species | Host Code | Accession Number |
|---|---|---|
| *Aratinga jandaya* | Ajan | KF525368 |
| *Attila cinnamoneus* | Acin | JQ174112 |
| *Campephilus rubricollis* | Crub | JQ174248 |
| *Campylorhynchus turdinus* | Ctur | JQ174275 |
| *Cercomacra cinerascens* | Ccin | JQ174359 |
| *Coereba flaveola* | Cfla | JN801299 |
| *Columbina passerina* | Cpas | JN801583 |
| *Dysithamnus mentalis* | Dmen | JN801648 |
| *Formicivora grisea* | Fgri | JQ174857 |
| *Isleria hauxwelli* | Ihau | JN801853 |
| *Micrastur gilvicollis* | Mgil | JN801798 |
| *Myiophobus fasciatus* | Mfas | JQ175453 |
| *Myrmotherula axillaris* | Maxi | JX487698 |
| *Pachyramphus rufus* | Pruf | JQ175660 |
| *Pheugopedius coraya* | Pcor | JN802043 |
| *Philydor erythrocerum* | Pery | JX487747 |
| *Phlegopsis nigromaculata* | Pnig | JN801914 |
| *Piaya cayana* | Pcay | JN801921 |
| *Piculus flavigula* | Pfla | JQ175823 |
| *Piprites chloris* | Pchl | JN801933 |
| *Poecilotriccus sylvia* | Psyl | JQ175931 |
| *Poliptila guianensis* | Pgui | JN801947 |
| *Psarocolius bifasciatus* | Pbif | JN801699 |
| *Pyriglena leuconota* | Pleu | JN801960 |
| *Pyrrhura lepida* | Plep | JQ176082 |
| *Ramphocelus carbo* | Rcar | JQ176111 |
| *Rhynchocyclus olivaceus* | Roli | JX487850 |
| *Rhytipterna simplex* | Rsim | JN801974 |
| *Sporophila americana* | Same | JQ176248 |
| *Tachyphonus cristatus* | Tcri | JN802009 |
| *Tachyphonus luctuosus* | Tluc | JQ176359 |
| *Tachyphonus rufus* | Truf | KM896605 |
| *Thalurania furcata* | Tfur | JX487917 |
| *Thamnomanes caesius* | Tcae | JX487977 |
| *Thamnophilus aethiops* | Taet | JN802031 |
| *Thamnophilus amazonicus* | Tama | JQ176437 |
| *Thamnophilus doliatus* | Tdol | JN802037 |
| *Thraupis episcopus* | Tepi | JQ176458 |
| *Tolmomyias flaviventris* | Tfla | JQ176518 |
| *Willisornis poecilinotus* | Wpoe | JX487579 |
| *Xenops minutus* | Xmin | JX488034 |
| *Xiphorhynchus elegans* | Xele | JN802104 |

Table 5. Cost-event coevolutionary analysis of the distribution of haemosporidian lineages from Gurupi, Brazil amongst their avian hosts.

| Event Costs[a] | | | | Total Costs | Codivergence | Duplication | Sorting | Switching |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 39 | 0-4[b] | 9[b] | 0 | 35-39[c] |
| 0 | 2 | 1 | 3 | 108 | 13-16[b] | 3[c]-9[b] | 8[c]-17[b] | 27-30[c] |
| 0 | 0 | 1 | 2 | 67 | 8-12[b] | 6[c]-8[b] | 3[c]-7[b] | 30-32[c] |
| 1 | 1 | 1 | 1 | 48 | 0[c]-9[b] | 2[c]-9[b] | 0 | 35[c]-46[c] |
| 1 | 1 | 1 | 0 | 2 | 0 | 2 | 0 | 46 |

[a] Event costs of codivergence, duplication, sorting, and host switching respectively
[b] The number of events significantly exceeds that of randomized trees ($p < 0.05$)
[c] The number of events is significantly less than that of randomized trees ($p < 0.05$)

confidence interval were estimated using a jackknife approach. Links with low squared residuals contribute little to $m^2_{xy}$ and likely represent coevolutionary links (Balbuena *et al.* 2013). All PACo analyses were implemented in R (version 3.2.2; R Development Core Team 2015) using the code provided by Balbuena *et al.* (2013).

**Host Specificity Determination**

To determine host specificity of haemosporidian lineages the host specific index, $S_{TD}$* (Poulin and Mouillet 2005) was calculated for all lineages discovered using the program TAXOBIODIV2 (http://www.otago.ac.nz/parasitegroup/downloads.html). $S_{TD}$* is the average taxonomic distance among host species infected by a parasite, weighted by prevalence in each host. Higher $S_{TD}$* scores indicate increased host generalization. The taxonomic distance between each host pair is calculated as the number of taxonomic classification steps needed to reach a common node that separates the two host species, which is then weighted by the parasite prevalence in each host (Poulin and Mouillet 2005). Any lineages that was recorded only once was omitted since they provide no

information on host range, and lineages recorded multiple times in a single host species were given a default value of 1 (Poulin and Mouillet 2005).

**Composition Analysis**

To determine the effect of area of endemism on host and parasite communities within Amazonia the data were organized into two binary matrices, presence and absence. The first showed the distribution of parasite lineages on the areas of endemism, and the second showed the distribution of bird species. The samples collected from CICRA, Peru were not used for this analysis.

Permutational Multivariate Analyses of Variance (PERMANOVA) was used to determine whether parasite and host assemblages changed between areas of endemism. The Jaccard index was used as a dissimilarity measure and 10,000 permutations for each model were performed. Latitude was also included as an explicative variable to test its effect in the composition of parasite lineages and host species. Analyses were conducted within R (version 3.2.2; R Development Core Team 2015) using the package Vegan (Oksanen *et al.* 2013).

To test for an association between the compositions of host assemblages and parasite assemblages in each locality the Jaccard index was used to measure the pairwise dissimilarities in parasite and in host compositions between localities. A Mantel test was then used to test for a correlation between these two matrices. Mantel statistics were based on Spearman's rank correlation Rho and for each test 5000 permutations were performed.

To test whether parasite assemblage in each area of endemism were composed of lineages that are phylogenetically closer than expected by chance, data for *Plasmodium*

and *Haemoproteus* occurrences were separated by area of endemism to build two binary matrices. The Jaccard index was calculated to measure the pairwise dissimilarities between parasite lineages in the distribution on the areas of endemism, and a matrix for each genus was created with this distance data. Also, matrices of pairwise phylogenetic distance between parasite lineages based on the branch length of the phylogenetic trees were constructed. Then a Mantel test was used to test for a correlation between the matrix of dissimilarities in occurrence and the matrix of phylogenetic distance for each genus. Mantel statistics were based on Spearman's rank correlation Rho and 5000 permutations were performed per test.

The prevalence of *Plasmodium* and *Haemoproteus* in each area of endemism was calculated considering all parasite lineages. After a visual analysis, a chi-square test of independence was used to test if prevalence differs between the endemism areas north and south of the Amazon River.

**Host Life History Analysis**

Host life history traits have been shown to affect the prevalence of haemosporidian parasites (Ricklefs 1992, Young *et al.* 1993, Tella 2002, Fecchio *et al.* 2011, 2013, González *et al.* 2014, Lutz *et al.* 2015, Matthews *et al.* 2016). To understand this relationship in the highly diverse Amazonian bird communities the following four traits were analyzed: 1) nest height, 2) nest type, 3) foraging height, and 4) flocking.

Nest height and nest type can impact haemosporidian prevalence (Fecchio *et al.* 2011, Lutz *et al.* 2015, Matthews *et al.* 2016) and are linked to host encounter rates during nesting, a critical period for infection (Valkiūnas 2005), due to nestlings being more susceptible to dipteran vectors (Blackmore and Dow 1958, Edman and Kale 1971,

Kale *et al.* 1972, Edman and Scott 1987, Scott and Edman 1991). Flocking behavior has also been shown to predict parasitism (Fecchio *et al.* 2011, 2013, González *et al.* 2014, Lutz *et al.* 2015) and since host olfactory cues (kairomones) play a role in attracting dipteran vectors (Withers 1978, Wickler and Marsh 1980, Logan *et al.* 2010) one would expect differences in flocking behavior to affect host-vector encounter rates. Although foraging height did not show previously a correlation with haemosporidian prevalence (González *et al.* 2014, Matthews *et al.* 2016) it was included due to the high levels of foraging stratification in Amazonia (Ridgely and Tudor 1989a, 1989b, Stotz *et al.* 1996).

Nest height was categorized as 1) ground, 2) understory, 3) sub-canopy/canopy, or 4) cliff or bank. Nest type was categorized as 1) open cup, 2) closed cup, or 3) cavity. Foraging height was categorized as 1) ground, 2) understory, 3) sub-canopy/canopy, or 4) understory/sub-canopy/canopy. Flocking behavior is categorized as 1) solitary, 2) single-species flock or family group, or 3) mixed-species flock. These traits were scored for all individuals sampled using *The Birds of South America Volumes I and II* (Ridgely and Tudor 1989a, 1989b), *Neotropical Birds: Ecology and Conservation* (Stotz *et al.* 1996), The Cornell Lab of Ornithology: Neotropical Birds (http://www.neotropical.birds.cornell.edu/portal/home) and WikiAves (http://www.wikiaves.com.br). Analyses were conducted to predict parasitism rates by *Haemoproteus* and *Plasmodium* separately.

Only a subset of samples from Amazonia were analyzed, excluding samples for the collection areas of the CHU, CICRA, COM, Madeira River, and PTB (Table 1). These samples were excluded for two reason. First, the samples from CICRA were collected from the westernmost expanse of the Amazon basin where habitat

characteristics and host communities are much different from other areas sampled within the Inambari area of endemism. Second, the samples from CHU, COM, Madeira River, and PTB were not processed using the same molecular methods as all other samples so they were excluded as well. A total of 1759 samples were used for this analysis, collected from six areas of endemism (Figure 7); Belém (323 samples), Guiana (178 samples), Imerí (164 samples), Inambari (419 samples), Rondônia (575 samples), and Tapajós (100 samples).

Generalized linear mixed models were used to identify which combination of host life history and ecological factors predicted the probability of an individual bird being parasitized. Independently for each parasite genus, *Haemoproteus* and *Plasmodium*, ability of 15 different logistic regression models (Table 6) to predict the binomial response variable, uninfected versus infected was assessed. Each of the host life history traits (nest height, nest type, foraging height, and flocking behavior) along with area of endemism served as fixed effects and were treated as categorical variables. To account for host phylogenetic constraints on parasitism due to factors not measured, three nested random effects: host family, host genus nested within host family, and host species nested within host genus nested within host family, were included (Table 7). This approach accounted for statistical non-independence in the data due to host phylogenetic constraint, and identified the taxonomic level at which these unexplained effects occurred.

Conclusions are based on the approach to model comparisons and weighted averaging outlined by Burnham and Anderson (2002). Models were ranked by importance based on weights calculated using Akaike's Information Criterion (AIC) (Table 8). The relative importance of each fixed-effect predictor variable was determined

Table 6. Fixed effects in the set of 15 candidate models used for all Amazonia samples and the relative support for each model as calculated by $\Delta AIC_c$. For each parasite genus the model with the $\Delta AIC_c$ value of zero (in bold) is the best-supported model. An "X" indicates that a given trait was used as a fixed effect.

| Model # | Nest Height[a] | Nest Type[b] | Foraging Height[c] | Flocking Behavior[d] | Endemic Area[e] | $\Delta AIC_c$ *Haemoproteus* | $\Delta AIC_c$ *Plasmodium* |
|---|---|---|---|---|---|---|---|
| 1 | X | | | | X | 0.851 | 6.029 |
| 2 | | X | | | X | 1.004 | 1.812 |
| 3 | | | X | | X | **0** | 0.691 |
| 4 | | | | X | X | 0.449 | 2.650 |
| 5 | X | X | | | X | 4.844 | 6.952 |
| 6 | | X | X | | X | 4.468 | 5.530 |
| 7 | X | | | X | X | 3.343 | 8.060 |
| 8 | | X | X | | X | 3.741 | **0** |
| 9 | | X | | X | X | 4.263 | 4.398 |
| 10 | | | X | X | X | 2.894 | 4.183 |
| 11 | X | X | X | | X | 8.518 | 4.848 |
| 12 | X | X | | X | X | 7.373 | 9.597 |
| 13 | X | | X | X | X | 7.081 | 9.168 |
| 14 | | X | X | X | X | 6.775 | 3.950 |
| 15 | X | X | X | X | X | 11.159 | 8.802 |

[a] Nest Height: Ground, Understory, Sub-Canopy/Canopy, Cliff or bank
[b] Nest Type: Open cup, Closed cup, Cavity
[c] Foraging Height: Ground, Understory, Sub-Canopy/Canopy, Understory/Sub-Canopy/Canopy
[d] Flocking Behavior: Solitary/Family group, Single species, Mixed species
[e] Endemic Area: Belém, Guiana, Imerí, Inambari, Rondônia, Tapajós

by calculating the cumulative support for each predictor as the sum of weights of all models containing that predictor. The effect of each predictor and its precision were estimated by calculating weighted average ("model-averaged") regression coefficients and 95% confidence limits (Table 9). To make qualitative comparisons among all categories, graphs illustrating the extent of each effect for which we found significant

Table 7. Tests of statistical significance of host phylogenetic constraints on the probability of parasitism for all Amazonian samples. Phylogenetic effects were examined by including nested random effects of host family, genus (within family), and species (within genus) on the probabilities of parasitism.

| Parasite genus | Host taxonomic level | Chi-squared value | P-value |
|---|---|---|---|
| *Haemoproteus* | | | |
| | Family | 9.56 | < 0.01 |
| | Genus (within Family) | 0.00 | 1 |
| | Species (within Genus) | 3.65 | 0.06 |
| *Plasmodium* | | | |
| | Family | 14.99 | < 0.01 |
| | Genus (within Family) | 0.16 | 0.69 |
| | Species (within Genus) | 13.90 | < 0.01 |

Table 8. AIC-based support for each of the four fixed effects for all Amazonian samples. Values are sums of model weight values for all models in the set, as shown in Table 6. The best supported fixed effect (highest model weight sum) is in bold.

| Fixed Effect | *Haemoproteus* | *Plasmodium* |
|---|---|---|
| Nest Height | 0.27 | 0.09 |
| Nest Type | 0.26 | 0.59 |
| Foraging Height | **0.39** | **0.71** |
| Flocking Behavior | 0.35 | 0.22 |

regression coefficients (coefficients with model-averaged confidence limits not overlapping zero) were produced.

Although main conclusions are based on the multi-model procedures outlined above, two additional results are based on examining output from single models. First, to display variation in the expected probabilities of parasitism for each haemosporidian genus, least-squares mean probabilities of parasitism were calculated from the single model in each set that contained all of the predictor variables identified as important

based on model-averaged coefficients and their confidence limits, those that did not overlap zero (Table 9). Second, the same models, one for each haemosporidian genus, were used to model parasitism rates. The statistical significance of each of the three random effects (host phylogenetic constraints) was determined using a likelihood ratio test that compared the full model (all fixed and random effects present) with a model in which only the focal random effect was removed from the full list.

All models were fit using restricted maximum likelihood implemented with the glmer function from the lme4 package (Bates *et al.* 2016) within R (version 3.2.2; R Development Core Team 2015). Model weights and model-averaged regression coefficients were calculated using the aictab.mer and modavg.mer functions found in the R package AICcmodavg (Mazerolle 2016). The R package lsmeans was used to calculate least-squares means and their confidence intervals. The statistical significance of host phylogeny (random effects) was calculated with Chi-squared likelihood ratio tests using the rand function in the lmerTest package (Kuznetsova *et al.* 2016) within R.

Area of endemism was the strongest predictive factor in the original models as expected due to their importance in both shaping and isolating avian communities in Amazonia (Cracraft 1985, Silva *et al.* 2005, Smith *et al.* 2014). The effect of area of endemism was so significant that it masked any potential impacts due to host life history. The only way to determine the effect of host life history on parasite prevalence was to analyze each area of endemism separately using the same modeling methods. The 15 regression models (Table 10), host phylogenetic constraints (Table 11), AIC support for each life history trait (Table 12) and the ability to predict parasitism (Tables 13, 14) for each of the six areas of endemism were calculated and are given below.

Table 9. Model-averaged regression coefficients and 95% confidence limits used to estimate effects of predictors and precision of effects for all Amazonian samples. For each predictor the regression coefficients are interpreted as describing deviations in parasitism rates from a reference category whose effect is subsumed into the intercept term of the statistical model. Areas in bold represent regression coefficients that are significantly different from zero which were used to predict prevalence of parasitism. All values were multiplied by 100 for clarity.

| | | *Haemoproteus* Model-averaged beta and 95% confidence limits | | | *Plasmodium* Model-averaged beta and 95% confidence limits | | |
|---|---|---|---|---|---|---|---|
| Parameter | Parameter description | Beta | Lower CL | Upper CL | Beta | Lower CL | Upper CL |
| (Intercept) | | **4.87** | **0.22** | **9.52** | **14.19** | **4.55** | **23.83** |
| Nest Height | Understory | -2.34 | -6.73 | 2.05 | 4.06 | -6.19 | 14.31 |
| Nest Height | Sub-Canopy/Canopy | -2.59 | -7.25 | 2.06 | 2.55 | -9.02 | 14.13 |
| Nest Height | Cliff or Bank | 1.21 | -5.95 | 8.36 | 4.26 | -12.68 | 21.20 |
| Nest Type | Close cup | 0.03 | -3.79 | 3.85 | 6.01 | -2.27 | 14.29 |
| Nest Type | Cavity | 0.86 | -2.58 | 4.31 | 6.81 | -0.56 | 14.17 |
| Foraging | Understory | -0.80 | -4.93 | 3.33 | -7.63 | -16.66 | 1.40 |
| Foraging | Sub-Canopy/Canopy | -3.49 | -7.96 | 0.99 | -2.96 | -12.51 | 6.58 |
| Foraging | Under/Sub/Canopy | -1.73 | -5.96 | 2.50 | 0.48 | -8.80 | 9.76 |
| Flocking | Single Species | -0.04 | -3.97 | 3.89 | -0.04 | -3.97 | 3.89 |
| Flocking | Mixed Species | 1.17 | -1.15 | 3.49 | 1.17 | -1.15 | 3.49 |
| Endemism | Guiana | -1.92 | -5.29 | 1.45 | **-10.81** | **-17.79** | **-3.83** |
| Endemism | Imerí | -1.43 | -4.89 | 2.03 | -4.21 | -11.42 | 3.00 |
| Endemism | Inambari | -1.86 | -4.69 | 0.97 | -3.50 | -9.41 | 2.41 |
| Endemism | Rondônia | 2.42 | -0.13 | 4.96 | 1.49 | -3.83 | 6.80 |
| Endemism | Tapajós | 2.97 | -1.05 | 6.99 | 6.13 | -2.12 | 14.39 |

Table 10. Fixed effects in the set of 15 candidate models used for each of the six areas of endemism and the relative support for each model as calculated by $\Delta AIC_c$. For each parasite genus the model with the $\Delta AIC_c$ value of zero (in bold) is the best-supported model. An "X" indicates that a given trait was used as a fixed effect.

| Model # | Nest Height[a] | Nest Type[b] | Foraging Height[c] | Flocking Behavior[d] | $\Delta AIC_c$ for *Haemoproteus/Plasmodium* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Belém | Guiana | Imerí | Inambari | Rondônia | Tapajós |
| 1 | X | | | | 5.02/0.44 | **0**/22.31 | **0**/12.45 | 2.49/5.08 | 1.76/2.10 | 4.81/5.41 |
| 2 | | X | | | 2.84/1.84 | 3.90/21.65 | 1.22/11.80 | 0.80/**0** | 5.85/0.18 | 5.07/3.85 |
| 3 | | | X | | 4.48/**0** | 6.13/**0** | 6.25/**0** | 0.80/5.52 | 6.74/2.65 | **0**/3.08 |
| 4 | | | | X | **0**/0.96 | 4.08/19.47 | 2.36/11.74 | **0**/2.79 | 3.82/**0** | 3.64/**0** |
| 5 | X | X | | | 8.16/4.55 | 3.86/24.89 | 3.66/16.03 | 6.20/5.14 | 4.26/5.74 | 9.45/10.05 |
| 6 | | X | X | | 8.55/4.70 | 6.63/2.95 | 6.42/4.36 | 6.71/10.18 | 7.03/5.90 | 1.51/6.89 |
| 7 | X | | | X | 3.79/3.23 | 3.57/24.10 | 4.01/15.99 | 5.57/7.43 | **0**/5.07 | 6.04/5.38 |
| 8 | | X | X | | 7.64/4.19 | 10.42/3.09 | 7.29/3.15 | 4.56/4.80 | 9.63/5.17 | 3.60/7.87 |
| 9 | | X | | X | 2.50/5.05 | 8.30/22.10 | 4.19/15.04 | 3.76/2.48 | 6.92/3.17 | 7.69/3.99 |
| 10 | | | X | X | 4.46/2.77 | 10.53/2.96 | 8.89/3.51 | 3.71/7.74 | 3.04/5.76 | 2.96/5.34 |
| 11 | X | X | X | | 11.81/8.89 | 10.68/5.76 | 10.02/7.44 | 10.54/8.55 | 9.80/8.76 | 4.94/11.77 |
| 12 | X | X | | X | 6.74/7.40 | 7.26/25.30 | 7.82/19.47 | 9.21/7.45 | 3.60/8.77 | 9.60/10.25 |
| 13 | X | | X | X | 7.50/6.85 | 10.32/7.11 | 10.74/8.40 | 9.71/11.27 | 2.55/9.38 | 6.25/9.90 |
| 14 | | X | X | X | 7.53/7.04 | 14.97/4.85 | 10.77/6.97 | 7.40/6.70 | 7.10/8.32 | 6.49/9.98 |
| 15 | X | X | X | X | 10.80/11.17 | 14.17/8.67 | 14.53/11.74 | 13.48/9.23 | 6.71/12.19 | 9.65/15.10 |

[a] Nest Height: Ground, Understory, Sub-Canopy/Canopy, Cliff or bank
[b] Nest Type: Open cup, Closed cup, Cavity
[c] Foraging Height: Ground, Understory, Sub-Canopy/Canopy, Understory/Sub-Canopy/Canopy
[d] Flocking Behavior: Solitary/Family group, Single species, Mixed species

Table 11. Tests of statistical significance of host phylogenetic constraints on the probability of parasitism for each of the six areas of endemism. Phylogenetic effects were examined by including nested random effects of host family, genus (within family), and species (within genus) on the probabilities of parasitism.

| | | Chi-squared values (* denotes significance at $p < 0.05$) | | | | | |
|---|---|---|---|---|---|---|---|
| Parasite genus | Host taxonomic level | Belém | Guiana | Imerí | Inambari | Rondônia | Tapajós |
| *Haemoproteus* | | | | | | | |
| | Family | 0.00 | 12.18* | 0.00 | 0.00 | 0.00 | 0.26 |
| | Genus (within family) | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Species (within genus) | 27.13* | 0.00 | 0.00 | 0.00 | 0.00 | 0.88 |
| *Plasmodium* | | | | | | | |
| | Family | 13.50* | 16.12* | 0.00 | 10.84* | 6.17* | 2.34 |
| | Genus (within family) | 0.13 | 0.00 | 0.00 | 0.88 | 0.19 | 0.00 |
| | Species (within genus) | 0.14 | 0.00 | 0.00 | 0.00 | 0.45 | 0.00 |

Table 12. AIC-based support for each of the four fixed effects for each of the six areas of endemism. Values are sums of model weight values for all models in the set, as shown in Table 10. The best supported fixed effect (highest model weight sum) is in bold.

| Fixed Effect | Belém (H/P) | Guiana (H/P) | Imerí (H/P) | Inambari (H/P) | Rondônia (H/P) | Tapajós (H/P) |
|---|---|---|---|---|---|---|
| Nest Height | 0.16/0.36 | 0.18/0/02 | **0.56**/0.10 | 0.14/0.12 | **0.80**/0.19 | 0.31/0.11 |
| Nest Type | 0.30/0.21 | 0.20/0.21 | 0.36/0.17 | 0.31/**0.78** | 0.17/0.42 | 0.17/0.18 |
| Foraging Height | 0.15/**0.41** | **0.99/0.41** | 0.06/**0.99** | 0.31/0.12 | 0.25/0.16 | **0.83**/0.19 |
| Flocking Behavior | **0.64**/0.33 | 0.19/0.33 | 0.25/0.14 | **0.44**/0.33 | 0.74/**0.44** | 0.63/**0.73** |

Table 13. Model-averaged regression coefficients and 95% confidence limits used to estimate effects of predictors and precision of effects for *Haemoproteus* in each of the six areas of endemism. For each predictor the regression coefficients are interpreted as describing deviations in parasitism rates from a reference category whose effect is subsumed into the intercept term of the statistical model. Areas in bold represent regression coefficients that are significantly different from zero which were used to predict prevalence of parasitism. All values were multiplied by 100 for clarity.

| | | *Haemoproteus* model averaged beta and 95% confidence limits | | | | | |
|---|---|---|---|---|---|---|---|
| Parameter | Parameter | Belém | Guiana | Imerí | Inambari | Rondônia | Tapajós |
| (Intercept) | | 2.69 (-3.6,8.9) | 1.13 (-5.7,7.9) | -0.13 (-8.5,8.2) | 1.81 (-1.9,5.6) | **12.26 (0.3,24.2)** | 24.47 (-0.6,49.5) |
| Nest Height | Understory | 6.21 (-3.3,15.7) | 0.61 (-6.2,7.4) | -0.03 (-10.9,10.9) | 1.34 (-4.3,6.9) | **-11.26 (-19.2,-3.4)** | 17.0 (-5.1,39.1) |
| Nest Height | SubCanopy/ Canopy | 4.72 (-4.6,13.9) | 0.35 (-6.5,7.2) | 1.16 (-9.7,12.1) | 0.69 (-5.2,6.5) | -11.51 (-19.8,-3.2) | 9.83 (-14.7,34.3) |
| Nest Height | Cliff or Bank | 0.59 (-15.8,16.9) | **16.49 (5.4,27.6)** | 8.79 (-3.7,21.3) | 0.06 (-10.,10.3) | -10.37 (-23.9,3.2) | 20.1 (-28.1,68.3) |
| Nest Type | Close cup | -2.24 (-9.2,4.8) | 0.85 (-3.8,5.5) | 0.11 (-10.8,11.1) | -1.13 (-4.9,2.6) | 1.66 (-5.1,8.4) | -10.7 (-33.2,11.8) |
| Nest Type | Cavity | -3.32 (-8.9,2.3) | 0.18 (-3.8,4.1) | 2.83 (-0.9,6.5) | -0.1 (-2.4,2.1) | 1.9 (-3.3,7.1) | -2.1 (-17.4,13.2) |
| Foraging | Understory | -4.17 (-13.5,5.2) | 0.48 (-5.0,5.9) | 1.21 (-7.8,10.2) | -2.28 (-6.0,1.5) | 5.24 (-2.8,13.3) | **-31.3 (-50.9,-11.7)** |
| Foraging | SubCanopy/ Canopy | -3.94 (-12.2,4.3) | -0.3 (-6.9,6.4) | -0.04 (-9.9,9.8) | -3.38 (-8.3,1.5) | 2.07 (-7.0,11.2) | **-32.8 (-54.9,-10.7)** |
| Foraging | Under/Sub/ Canopy | -0.79 (-9.8,8.3) | 0.25 (-5.4,5.9) | 1.62 (-7.3,10.6) | -2.80 (-6.6,0.9) | -0.24 (-8.1,7.6) | **-27.95 (-46.6,-9.3)** |
| Flocking | Single Species | **8.48 (0.4,16.6)** | -0.68 (-5.0,3.7) | -0.16 (-8.2,7.9) | -1.47 (-5.1,2.2) | -4.66 (-13.4,4.0) | -5.29 (-26.0,15.4) |
| Flocking | Mixed Species | -0.09 (-5.3,5.2) | -0.78 (-3.4,1.8) | 2.06 (-1.6,5.7) | -0.89 (-2.9,1.0) | 4.13 (-0.4,8.7) | 7.69 (-5.4,20.8) |

Table 14. Model-averaged regression coefficients and 95% confidence limits used to estimate effects of predictors and precision of effects for *Plasmodium* in each of the six areas of endemism. For each predictor the regression coefficients are interpreted as describing deviations in parasitism rates from a reference category whose effect is subsumed into the intercept term of the statistical model. Areas in bold represent regression coefficients that are significantly different from zero which were used to predict prevalence of parasitism. All values were multiplied by 100 for clarity.

| | | *Plasmodium* model averaged beta and 95% confidence limits | | | | | |
|---|---|---|---|---|---|---|---|
| Parameter | Parameter | Belém | Guiana | Imerí | Inambari | Rondônia | Tapajós |
| (Intercept) | | 3.76 (-11.5,18.9) | **54.83 (32.1,77.6)** | **58.52 (30.6,86.4)** | 13.81 (-4.4,32.0) | **15.54 (2.9,28.2)** | 17.87 (-6.0,41.7) |
| Nest Height | Understory | 9.48 (-6.6,25.6) | 6.26 (-16.5,29.1) | -3.16 (-36.6,30.3) | 6.43 (-15.7,28.5) | -2.51 (-21.1,16.1) | -18.25 (-49.1,12.5) |
| Nest Height | SubCanopy/ Canopy | 12.5 (-3.5,28.5) | -3.59 (-27.6,20.4) | -6.05 (-42.3,30.2) | 1.84 (-22.6,26.3) | -8.2 (-28.0,11.6) | -21.29 (-59.4,16.8) |
| Nest Height | Cliff or Bank | -1.87 (-29.3,25.6) | 0.36 (-37.7,38.4) | 12.94 (-25.2,51.1) | 17.96 (-24.4,60.3) | -2.18 (-31.7,27.4) | -30.37 (-74.8,14.1) |
| Nest Type | Close cup | 2.28 (-10.4,14.9) | 0.58 (-14.1,15.3) | -18.65 (-52.6,15.3) | 9.81 (-9.2,28.8) | 7.49 (-7.2,22.2) | 14.18 (-24.1,52.5) |
| Nest Type | Cavity | 1.08 (-9.9,12.0) | 8.95 (-3.8,21.7) | 1.43 (-9.2,12.1) | **15.92 (1.7,30.2)** | 1.89 (-10.5,14.2) | -0.48 (-23.2,22.3) |
| Foraging | Understory | 10.95 (-4.1,25.9) | **-49.9 (-67.9,-31.8)** | **-45.4 (-71.9,-18.8)** | -7.33 (-23.7,9.1) | -4.2 (-20.8,12.5) | 8.41 (-22.2,39.0) |
| Foraging | SubCanopy/ Canopy | 14.0 (-0.2,28.2) | **-42.2 (-64.2,-20.1)** | **-57.9 (-87.3,-28.6)** | -8.42 (-30.2,12.4) | -0.09 (-19.7,19.5) | 9.65 (-29.8,49.1) |
| Foraging | Under/Sub/ Canopy | 12.84 (-2.1,27.8) | **-46.4 (-65.2,-27.6)** | **-46.1 (-72.8,-19.4)** | -2.43 (-20.6,15.7) | 1.79 (-15.8,19.4) | 25.58 (-8.0, 59.2) |
| Flocking | Single Species | **8.48 (0.4,16.6)** | -0.67 (-5.0,3.7) | -0.16 (-8.2,7.8) | -1.47 (-5.1,2.2) | -4.66 (-13.4,4.0) | -5.29 (-26.0,15.4) |
| Flocking | Mixed Species | -0.09 (-5.3,5.2) | -0.79 (-3.4,1.8) | 2.06 (-1.6,5.7) | -0.89 (-2.9,1.1) | 4.13 (-0.4,8.6) | 7.69 (-5.4,20.8) |

# CHAPTER III

# A NEW REAL-TIME PCR PROTOCOL TO DETECT AVIAN HAEMOSPORIDIANS

## Results

The real-time PCR protocol successfully identified all single infections of *Plasmodium*, *Haemoproteus,* and *Leucocytozoon* previously detected by standard nested PCR protocol and microscopy (Lutz *et al.* 2015) from samples collected in Malawi, Africa. For all three genera the melt peaks generally occurred between 78 to 79 degrees Celsius, but variability existed, with some lineages producing peaks slightly above or below this range. The assay also detected all samples from the same collection with mixed infections of *Plasmodium/Haemoproteus*, *Plasmodium/Leucocytozoon*, *Haemoproteus/Leucocytozoon,* and *Plasmodium/Haemoproteus/Leucocytozoon*, but due to the use of a single primer set it was generally not possible to discern mixed infections with the real-time PCR assay. The intensity of infection as determined by blood films had no effect on detection by real-time PCR. It successfully detected the presence of haemosporidians in samples with only one infected red blood cell per 100 fields at 1000x magnification.

There was no significant difference between the three different screening protocols used for the 94 samples from Cerrado ($\chi^2 = 0.3429$, df = 2, p = 0.842) (Table 15). The Fallon protocol identified 49 positive samples, the real-time protocol identified 53 positive samples, and our nested PCR protocol for *Haemoproteus/Plasmodium*

Table 15. Results of single, nested, and real-time PCR tests on 94 samples from Cerrado biome of Brazil. Only samples that were positive by at least one screening method are shown, thirty-six samples were negative by all three methods. Forty-two samples were positive by all three screening methods (bold text), samples with divergent results are shown individually.

| Sample ID | Single PCR | Nested PCR | Real-time PCR |
|---|---|---|---|
| **Various (n=42)** | **Positive** | **Positive** | **Positive** |
| CE0049 | Positive | | Positive |
| CE0051 | | Positive | Positive |
| CE0053 | | Positive | Positive |
| CE0058 | | | Positive |
| CE0060 | | Positive | Positive |
| CE0068 | Positive | Positive | |
| CE0071 | | | Positive |
| CE0074 | | | Positive |
| CE0076 | | | Positive |
| CE0578 | Positive | | Positive |
| CE0581 | | Positive | Positive |
| CE0592 | Positive | Positive | |
| CE0594 | | Positive | Positive |
| CE0595 | Positive | Positive | |
| CE0597 | Positive | Positive | |
| CE0598 | Positive | | |
| TOTAL | 49 | 51 | 53 |

identified 51 positive samples (Table 15). The samples were also run using the *Leucocytozoon* nested PCR protocol (Lutz *et al.* 2015) identified by the Fallon protocol and 48 out of 51 samples identified by our nested PCR protocol. Two samples determined to be positive by both the Fallon *et al.* (2003) protocol and the real-time protocol were negative by our nested PCR protocol and three samples were only found positive by the real-time protocol. Both the Fallon protocol and the real-time protocol

failed to identify three samples screened as positives by our nested PCR protocol (Table 15).

After all the new and amended protocols were tested, the real-time protocol was used to screen 2829 samples collected from three Brazilian biomes; Amazonia, Caatinga, and Pantanal and representing 378 host species. Of these 2829 samples, 740 were identified as positive by real-time PCR. Of those 740 infected, the cytochrome *b* region fragment was successfully amplified in 586 samples (79%) and identification confirmed by sequencing. These infected individuals included single infections of *Plasmodium* and *Haemoproteus* as well as coinfections of two different haemosporidian taxa, including *Haemoproteus/Haemoproteus*, *Haemoproteus/Plasmodium*, and *Plasmodium/Plasmodium*. No *Leucocytozoon* infections were detected in the 1000 samples tested, which is in agreement with previous reports from the region (White *et al.* 1978, Valkiūnas 2005, Forrester and Greiner 2008, Lotta *et al.* 2015).

## Discussion

The real-time protocol presented herein is highly effective at determining the presence of haemosporidian parasites in avian blood and liver samples. It reliably identified all known positive samples from a recently published study of haemosporidians from birds sampled in Malawi (Lutz *et al.* 2015) and matched the results of two other standard molecular screening methods. The real-time protocol also successfully detected parasites in more than 2,800 samples from Brazil. The results of these three screening methods (single PCR, nested PCR, real-time PCR) were not significantly different when used to screen the same blood samples, showing that similar results were obtained

regardless of the screening method employed. This is important for the comparability of results from studies where these different screening methods have been used.

Limitations exist for any screening method for haemosporidians, whether using microscopy or molecular techniques. Birds with low parasitemia during the chronic phase of infection are always difficult to detect with microscopy creating the potential for misidentification of these birds as uninfected (Jarvi *et al.* 2003, Waldenström *et al.* 2004). Increasing the area of the blood film screened reduces the probability of false negative results (Valkiūnas *et al.* 2008c), but adds considerable time to the screening process, twenty to twenty-five minutes per slide (Jarvi *et al.* 2003) Even after adding additional screening time some infections will be missed. For example, a blood film from an individual with low parasitemia rarely contains all stages of haemosporidian development that are necessary for identification and/or adequate characterization of morphological species.

With molecular techniques, including nested PCR, low intensity infections can also be missed (Valkiūnas *et al.* 2008c). Molecular screening techniques based on PCR and Sanger sequencing also have lower ability to distinguish and identify mixed infections (Valkiūnas *et al.* 2006). This is compounded by the fact that the host DNA is much more concentrated in samples than parasite DNA which somewhat affects the ability to detect haemosporidian DNA (Freed and Cann 2006) or to PCR amplify larger fragments of parasite DNA, a necessity for the nested PCR protocol. This is evident in the results from this study, where only 77% of the 740 samples identified as positive by real-time PCR were also identified as positive by nested PCR.

The goal of any new screening method is to provide an accurate estimate of

parasite prevalence and to provide advantages over already established methods. The real-time PCR protocol proved as effective as the two most widely used molecular screening methods for haemosporidian parasites in birds (Waldenström *et al.* 2004, Fallon and Ricklefs 2008). Although all three methods likely leave a small proportion of samples undetected, there are distinct advantages of the real-time protocol. The main advantage of this protocol is its ability to reliably and quickly detect haemosporidian infections. Since real-time PCR eliminates gel electrophoresis, the result for a full 96 or 384-well PCR plate are available in one hour (or sooner if fast running protocol and corresponding PCR mix is used). With the Fallon *et al.* (2003) or Waldenström *et al.* (2004) protocols not only is cycling time between 2.5 to 3.5 times longer respectively, there is also the added time of gel electrophoresis before results can be determined. Thus, the real-time protocol dramatically increases throughput of sample screening.

Of the three methods, only this real-time protocol uses a single reaction to screen for *Leucocytozoon* in addition to *Plasmodium* and *Haemoproteus* infections. The Fallon *et al.* (2003) protocol was not designed to target *Leucocytozoon*. To amplify *Leucocytozoon* DNA with nested PCR a separate set of nested PCR amplifications are needed, the most widely used is the protocol of Hellgren *et al.* (2004). Inability to screen for all three genera in one nested PCR protocol increases the time and expense of screening for *Leucocytozoon* infections. This has led to a strong bias towards screening for *Haemoproteus* and *Plasmodium* only and ignoring *Leucocytozoon*, which explains why it is understudied. This is particularly true in areas of high host diversity, where the increased cost of PCR amplifications can make screening for *Leucocytozoon* prohibitive. Recent studies have shown that the *Leucocytozoon* diversity may be high in regions with

high avian diversity (Lutz *et al.* 2015) and in specific host populations (Reeves *et al.* 2015). Availability of a screening method that can amplify all three genera can aid in understanding the true diversity and ecology of all three genera of avian haemosporidian parasites. Until now, the only screening methods that could detect all three genera in a single procedure were microscopy and the restriction digestion protocol of Beadell and Fleischer (2004), but both take significantly more time than the real-time PCR protocol and still require the use of nested PCR to amplify DNA for sequencing.

Although real-time PCR reagents are somewhat more expensive than those for standard PCR, it is more cost effective to use real-time PCR compared to the cost of running two to three rounds of regular/nested PCRs and associated gels for all samples. The cost advantage is even more evident when time and workforce cost are taken into consideration. This is especially beneficial when screening very large sets of samples.

The real-time PCR assay proved as effective as two currently used molecular screening techniques, a single PCR screening assay (Fallon and Ricklefs 2008) and nested PCR screening assays (Hellgren *et al.* 2004, Waldenström *et al.* 2004). However, the real-time protocol has the distinct advantage of detecting all three genera in a single reaction in at least half the time of these current methods. Therefore, throughput is significantly increased by greatly decreasing screening time and cost without loss of sensitivity. The ability to quickly and reliably screen avian blood samples is crucial for trying to understand the species richness and ecology of haemosporidian parasites, especially from highly diverse areas. The real-time protocol developed serves these purposes and provides a very useful tool in the expanding field of avian haemosporidian research.

## CHAPTER IV

## AMAZONIAN AREAS OF ENDEMISM DETERMINE THE DISTRIBUTION, DIVERSITY, AND PHYLOGENY OF *HAEMOPROTEUS* AND *PLASMODIUM*

### Results

#### Haemosporidians from Five Brazilian Biomes

Of the 4521 samples analyzed, 730 were infected by *Haemoproteus* or *Plasmodium* (16.1% prevalence) (Table 16). No *Leucocytozoon* positive samples were found in the 1000 samples screened for this genus. *Plasmodium* infections were significantly more frequent than *Haemoproteus*, both in terms of overall prevalence ($\chi^2$ = 227.45, df = 1, $p < 0.001$) and number of unique genetic lineages recovered ($\chi^2$ = 204.10 df = 1, $p < 0.001$) (Table 16). Of the 730 infected individuals, 574 (78.6%) were infected with *Plasmodium* with an overall infection prevalence of 12.7%, whereas *Haemoproteus* only accounted for 178 infections (21.4%) with an overall infection prevalence of 3.9% (Table 16).

Ninety three individuals were infected by two different haemosporidian lineages. These coinfections were successfully resolved in 85 samples, with only eight samples

Table 16. Haemosporidian parasitism in 4521 avian blood samples collected from Brazil. Some of the totals include coinfections.

| | *Haemoproteus* | *Plasmodium* | Total |
|---|---|---|---|
| Individuals infected | 178 | 574 | 730 |
| Infection prevalence | 3.9% | 12.7% | 16.1% |
| Novel cytochrome *b* lineages | 77 (89.5%) | 254 (91.0%) | 331 (90.7%) |
| Described lineages (MalAvi) | 9 (10.5%) | 25 (9.0%) | 34 (9.3%) |
| Total lineages identified | 86 (23.6%) | 279 (76.4%) | 365 |

remaining undetermined. Infection by two different lineages of *Plasmodium* was the most common type of coinfection, with 48 instances, although individuals were also infected by two different lineages of *Haemoproteus*, and by *Haemoproteus* and *Plasmodium* together (Table 17).

Table 17. Haemosporidian infection distribution, including single *Haemoproteus* lineage infection (H), single *Plasmodium* lineage infection (P), or coinfections by two different haemosporidian lineages.

| | H | H*H | H*P | P | P*P | Total |
|---|---|---|---|---|---|---|
| Individuals infected | 141 | 15 | 22 | 504 | 48 | 730 |
| Infection prevalence | 3.1% | 0.3% | 0.5% | 11.2% | 1.1% | 16.1% |

A total of 365 unique genetic lineages were recovered, with 279 (76.4%) *Plasmodium* lineages and 86 (23.6%) *Haemoproteus* lineages (Table 16). Three hundred and thirty one lineages (90.7%) were discovered for the first time, with both haemosporidian genera having a similarly high percentage of newly identified lineages, 89.5% in *Haemoproteus* and 91.0% in *Plasmodium* ($\chi^2 = 0.18$, df = 1, p = 0.67) (Table 16). The majority of lineages in both genera were recorded only once, 67 in *Haemoproteus* and 211 in *Plasmodium*. For the remaining lineages recovered from multiple hosts, host specificity indices, $S_{TD}$*, could be calculated. The mean $S_{TD}$* for *Haemoproteus* was 1.93 ± 0.78 (95% CI), whereas for *Plasmodium* it was 2.18 ± 0.26 (95% CI). There was not a significant difference between mean $S_{TD}$* for the two genera (T = 1.01, df = 85, p = 0.314). $S_{TD}$* values for each lineage are listed in Appendix A.

Haemosporidian infection varied between collection areas, with overall infection prevalence varying from 0 to 33.3% (Table 18). Prevalence significantly varied between the five biomes sampled for overall prevalence ($\chi^2 = 12.63$, df = 4, p = 0.013), *Haemoproteus* prevalence alone ($\chi^2 = 144.34$, df = 4, p = < 0.001), and *Plasmodium*

Table 18. Sampling distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Brazil.

| Biome (n) | Area Endemism (n) | Location (n) | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|
| Amazonia (3381) | Belém (323) | Gurupi (323) | 11 (3.4) | 48 (14.9) | 58 (18.0)[a] |
| | Guiana (353) | Negro 01 (178) | 1 (0.6) | 12 (6.7) | 13 (7.3) |
| | | PTB (175) | 2 (1.1) | 27 (15.4) | 29 (16.6) |
| | Imerí (164) | Negro 02 (164) | 2 (1.2) | 22 (13.4) | 24 (14.6) |
| | Inambari (1437) | CICRA- Peru (720) | 6 (0.8) | 59 (8.2) | 64 (8.9)[b] |
| | | Madeira 01 (7) | 0 (0) | 1 (14.3) | 1 (14.3) |
| | | Madeira 03 (39) | 0 (0) | 5 (12.8) | 5 (12.8) |
| | | Madeira 04 (75) | 0 (0) | 6 (8.0) | 6 (8.0) |
| | | Madeira 05 (26) | 0 (0) | 4 (15.4) | 4 (15.4) |
| | | Madeira 06 (42) | 1 (2.4) | 0 (0) | 1 (2.4) |
| | | Madeira 07 (99) | 0 (0) | 17 (17.2) | 17 (17.2) |
| | | Madeira 08 (10) | 0 (0) | 0 (0) | 0 (0) |
| | | Purus 01 (211) | 3 (1.4) | 22 (10.4) | 25 (11.8) |
| | | Purus 02 (208) | 1 (0.5) | 35 (16.8) | 36 (17.3) |
| | Rondônia (1004) | CHU (117) | 0 (0) | 17 (14.5) | 17 (14.5) |
| | | COM (136) | 6 (4.4) | 32 (23.5) | 38 (27.9) |
| | | Madeira 02 (7) | 0 (0) | 1 (14.3) | 1 (14.3) |
| | | Madeira 09 (102) | 0 (0) | 11 (10.8) | 11 (10.8) |
| | | Madeira 10 (67) | 0 (0) | 6 (9.0) | 6 (9.0) |
| | | Tapajós A (151) | 11 (7.3) | 42 (27.8) | 47 (31.1)[c] |
| | | Tapajós B (137) | 5 (3.6) | 17 (12.4) | 22 (16.1) |
| | | Tapajós D (142) | 5 (3.5) | 31 (21.8) | 35 (24.6)[d] |
| | | Tapajós H (60) | 5 (8.3) | 10 (16.7) | 15 (25.0) |
| | | Tapajós IL (85) | 6 (7.1) | 16 (17.7) | 21 (24.7)[e] |
| | Tapajós (100) | Tapajós I (61) | 5 (8.2) | 13 (21.3) | 18 (29.5) |
| | | Tapajós J (39) | 1 (2.6) | 12 (30.8) | 13 (33.3) |
| Atlantic Forest (39) | | Natal (39) | 4 (10.3) | 5 (12.8) | 9 (23.1) |
| Caatinga (185) | | Aiuaba (62) | 5 (8.1) | 10 (16.1) | 15 (24.2) |
| | | Serido (123) | 8 (6.5) | 23 (18.7) | 27 (22.0)[f] |
| Cerrado (790) | | CER (790) | 87 (11.0) | 44 (5.6) | 123 (15.6)[g] |
| Pantanal (126) | | Corumbá (110) | 3 (2.7) | 21 (19.1) | 24 (21.8) |
| | | Cáceres (16) | 0 (0) | 5 (31.3) | 5 (31.3) |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 1, [b] 1, [c] 6, [d] 1, [e] 1, [f] 4, [g] 8

Table 19. Geographic distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Brazil.

| Biome | Area of Endemism | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|
| Amazonia | Belém | 323 | 11 (3.4) | 48 (14.9) | 58 (18.0)[a] |
| | Guiana | 353 | 3 (0.9) | 39 (11.1) | 42 (11.9) |
| | Imerí | 164 | 2 (1.2) | 22 (13.4) | 24 (14.6) |
| | Inambari | 1437 | 11 (0.8) | 149 (10.4) | 159 (11.1) [b] |
| | Rondônia | 1004 | 38 (3.8) | 183 (18.2) | 213 (21.2)[c] |
| | Tapajós | 100 | 6 (6.0) | 25 (25.0) | 31 (31.0) |
| Total | | 3381 | 71 (2.1) | 466 (13.8) | 527 (15.6)[d] |
| Atlantic Forest | | 39 | 4 (10.3) | 5 (12.8) | 9 (23.1) |
| Caatinga | | 185 | 13 (7.0) | 33 (17.8) | 42 (22.7)[e] |
| Cerrado | | 790 | 87 (11.0) | 44 (5.6) | 123 (15.6)[f] |
| Pantanal | | 126 | 3 (2.4) | 26 (20.6) | 29 (23.0) |
| Grand Total | | 4521 | 178 (3.9) | 574 (12.7) | 730 (16.1)[g] |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 1, [b] 1, [c] 8, [d] 10, [e] 4, [f] 8, [g] 22

prevalence alone ($\chi^2 = 51.38$, df = 4, p = < 0.001) (Figure 10, Table 19). The highest overall prevalence was seen in the Atlantic Forest, Caatinga, and Pantanal biomes, (Figure 10, Table 19). *Plasmodium* was most prevalent in Caatinga and Pantanal, and least prevalent in Cerrado, where *Haemoproteus* showed its highest prevalence (Figure 10, Table 19). *Plasmodium* lineages restricted to one biome had a significantly lower mean host specificity index value, $S_{TD}$*, than lineages found in multiple biomes (T = -2.57, df = 64, p = 0.013). Mean $S_{TD}$* for *Haemoproteu*s lineages found in only one biome versus those found in multiple biomes did not differ (T = 0.25, df = 12, p = 0.803).

Samples were obtained from 17 avian orders, 49 families, and 447 host species (Tables 20, 21). Infection prevalence varied between host families and orders, with no infections found in eight orders, although these orders were poorly sampled with only 40 samples collected from these orders combined (Table 21). Passeriformes were the most sampled order with 4151 (91.8%) samples collected, although higher haemosporidian

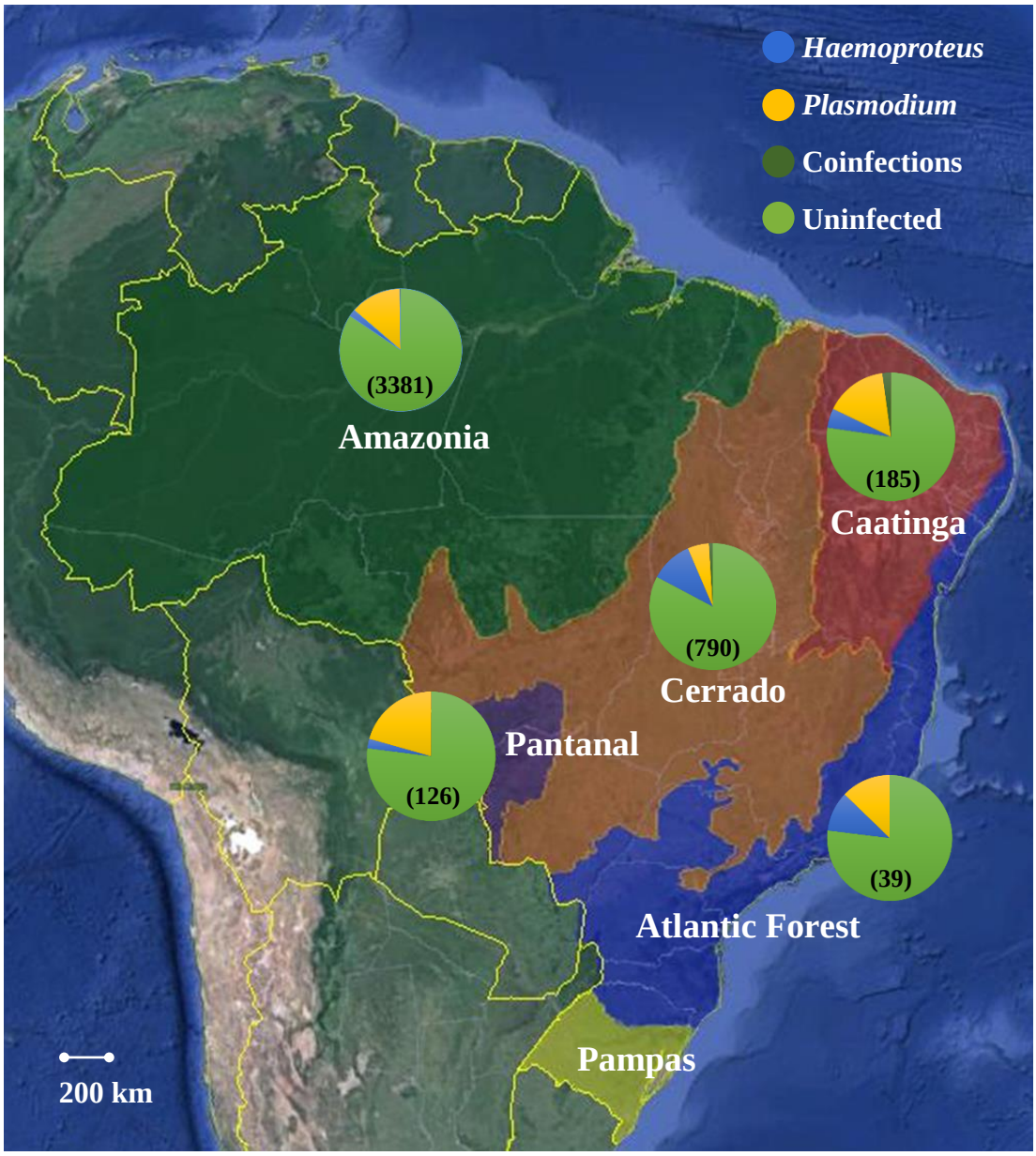


Figure 10. Map of Brazilian biomes. Pie charts indicate the proportion of infected bird samples at each biome, with number of samples in parentheses. The Pampas biome was not sampled. Coinfections indicate samples infected by both *Haemoproteus* and *Plasmodium*

prevalence was seen in the Columbiformes, Falconiformes, Gruiformes, Piciformes, and Psittaciformes (Table 20). *Plasmodium* prevalence was higher than *Haemoproteus* prevalence in all orders, except Columbiformes and Piciformes where *Haemoproteus* prevalence was higher (Table 21).

Within Passeriformes, 25 host families were sampled, and six of these families

Table 20. Host taxonomic distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Brazil.

| Order | Family | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Accipitriformes | Accipitridae | 3 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Anseriformes | Anhimidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Apodiformes | Apodidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Trochilidae | 20 | 68 | 1 (1.5) | 2 (2.9) | 3 (4.4) |
| Caprimulgiformes | Caprimulgidae | 5 | 11 | 0 (0) | 0 (0) | 0 (0) |
| | Nyctibiidae | 2 | 3 | 0 (0) | 0 (0) | 0 (0) |
| Ciconiiformes | Jacanidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Columbiformes | Columbidae | 9 | 49 | 13 (26.5) | 3 (6.1) | 14 (28.6)[a] |
| Coraciiformes | Cerylidae | 3 | 10 | 1 (10.0) | 1 (10.0) | 2 (20.0) |
| | Momotidae | 2 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Cuculiformes | Cuculidae | 3 | 6 | 0 (0) | 1 (16.7) | 1 (16.7) |
| Falconiformes | Falconidae | 4 | 8 | 1 (12.5) | 2 (25.0) | 3 (37.5) |
| Galliformes | Cracidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Gruiformes | Psophiidae | 1 | 3 | 0 (0) | 2 (66.7) | 2 (66.7) |
| | Rallidae | 3 | 3 | 0 (0) | 0 (0) | 0 (0) |
| Passeriformes | Cardinalidae | 5 | 41 | 1 (2.4) | 4 (9.8) | 5 (12.2) |
| | Conopophagidae | 3 | 22 | 0 (0) | 1 (4.5) | 1 (4.5) |
| | Corvidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Cotingidae | 3 | 7 | 0 (0) | 0 (0) | 0 (0) |
| | Dendrocolaptidae | 29 | 547 | 10 (1.8) | 33 (6.0) | 41 (7.5)[b] |
| | Donacobiidae | 1 | 6 | 0 (0) | 2 (33.3) | 2 (33.3) |
| | Emberizidae | 13 | 159 | 5 (3.1) | 17 (10.7) | 22 (13.8) |
| | Formicariidae | 2 | 36 | 0 (0) | 14 (38.9) | 14 (38.9) |
| | Fringillidae | 4 | 6 | 0 (0) | 1 (16.7) | 1 (16.7) |
| | Furnariidae | 26 | 241 | 6 (2.5) | 18 (7.5) | 23 (9.5)[c] |
| | Hirundinidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| | Icteridae | 2 | 5 | 1 (20.0) | 2 (40.0) | 3 (60.0) |
| | Melanopareiidae | 1 | 4 | 0 (0) | 0 (0) | 0 (0) |
| | Mimidae | 1 | 15 | 0 (0) | 3 (20.0) | 3 (20.0) |
| | Parulidae | 2 | 2 | 0 (0) | 0 (0) | 0 (0) |
| | Pipridae | 19 | 486 | 2 (0.4) | 70 (14.4) | 72 (14.8) |
| | Polioptilidae | 4 | 10 | 0 (0) | 2 (20.0) | 2 (20.0) |

Table 20. cont.

| Order | Family | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Passeriformes | Scleruridae | 2 | 9 | 0 (0) | 0 (0) | 0 (0) |
| | Thamnophilidae | 76 | 1168 | 21 (1.8) | 226 (19.3) | 242 (20.7)[d] |
| | Thraupidae | 29 | 350 | 56 (16.0) | 81 (23.1) | 126 (36.0)[e] |
| | Tityridae | 11 | 89 | 3 (3.4) | 4 (4.5) | 7 (7.9) |
| | Troglodytidae | 8 | 86 | 2 (2.3) | 11 (12.8) | 13 (15.1) |
| | Turdidae | 8 | 85 | 0 (0) | 17 (20.0) | 17 (20.0) |
| | Tyrannidae | 73 | 733 | 27 (3.7) | 37 (5.1) | 64 (8.7) |
| | Vireonidae | 6 | 41 | 3 (7.3) | 1 (2.4) | 4 (9.8) |
| Piciformes | Bucconidae | 11 | 62 | 20 (32.3) | 3 (4.8) | 22 (35.5)[f] |
| | Capitonidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Galbulidae | 6 | 38 | 2 (5.3) | 9 (23.7) | 11 (28.9) |
| | Picidae | 13 | 49 | 1 (2.0) | 3 (6.1) | 4 (8.2) |
| | Ramphastidae | 6 | 8 | 0 (0) | 1 (12.5) | 1 (12.5) |
| Psittaciformes | Psittacidae | 9 | 20 | 2 (10.0) | 3 (15.0) | 5 (25.0) |
| Strigiformes | Strigidae | 6 | 9 | 0 (0) | 0 (0) | 0 (0) |
| Tinamiformes | Tinamidae | 2 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Trogoniformes | Trogonidae | 4 | 6 | 0 (0) | 0 (0) | 0 (0) |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 2, [b] 2, [c] 1, [d] 5, [e] 11, [f] 1

were not infected (Table 20). The mostly highly sampled families were Thamnophilidae (1168 samples), Tyrannidae (733 samples), Dendrocolaptidae (549 samples), Pipridae (486 samples), and Thraupidae (350 samples) (Table 20).

Haemosporidian prevalence varied widely among families, from 0 to 60%. In families with at least twenty samples the highest prevalence was seen in Formicariidae (38.9%), Thraupidae (36.0%), and Thamnophilidae (20.7%) (Table 20). The family Thraupidae showed the highest *Haemoproteu*s prevalence (16%) in Passeriformes, and third highest for any avian family, with *Haemoproteus* prevalence higher in only Bucconidae (32.3%) and Columbidae (26.5%) (Table 20). These last two families were the only families that showed higher *Haemoproteus* prevalence than *Plasmodium*, and

differ from the general pattern of higher *Plasmodium* prevalence (Table 20). The complete list of host species along with infection status can be found in Appendix B.

Table 21. Host order distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Brazil.

| Order | Families | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Accipitriformes | 1 | 3 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Anseriformes | 1 | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Apodiformes | 2 | 21 | 69 | 1 (1.4) | 2 (2.9) | 3 (4.3) |
| Caprimulgiformes | 2 | 7 | 14 | 0 (0) | 0 (0) | 0 (0) |
| Ciconiiformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Columbiformes | 1 | 9 | 49 | 13 (26.5) | 3 (6.1) | 14 (28.6)[a] |
| Coraciiformes | 2 | 5 | 14 | 1 (7.1) | 1 (7.1) | 2 (14.2) |
| Cuculiformes | 1 | 3 | 6 | 0 (0) | 1 (16.7) | 1 (16.7) |
| Falconiformes | 1 | 4 | 8 | 1 (12.5) | 2 (25.0) | 3 (37.5) |
| Galliformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Gruiformes | 2 | 4 | 6 | 0 (0) | 2 (33.3) | 2 (33.3) |
| Passeriformes | 25 | 330 | 4151 | 137 (3.3) | 544 (13.1) | 662 (15.9)[b] |
| Piciformes | 5 | 37 | 158 | 23 (14.6) | 16 (10.1) | 38 (24.1)[c] |
| Psittaciformes | 1 | 9 | 20 | 2 (10.0) | 3 (15.0) | 5 (25.0) |
| Strigiformes | 1 | 6 | 9 | 0 (0) | 0 (0) | 0 (0) |
| Tinamiformes | 1 | 2 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Trogoniformes | 1 | 4 | 6 | 0 (0) | 0 (0) | 0 (0) |
| Total (17 Orders) | 49 | 447 | 4521 | 178 (3.9) | 574 (12.7) | 730 (16.1)[d] |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 2, [b] 19, [c] 1, [d] 22

**Phylogenetic Reconstruction of Brazilian Haemosporidian Lineages**

The final alignment used in the phylogenetic analyses combined sequences of a total of 365 newly obtained lineages of *Haemoproteus* and *Plasmodium* from Brazil with all previously published quality sequences of matching length available in the MalAvi database. The Bayesian analysis resulted in a tree containing several distinct clades (Figure 11). The Brazilian lineages more frequently associated together than with non-Brazilian lineages, with several larger clades solely composed of Brazilian lineages,

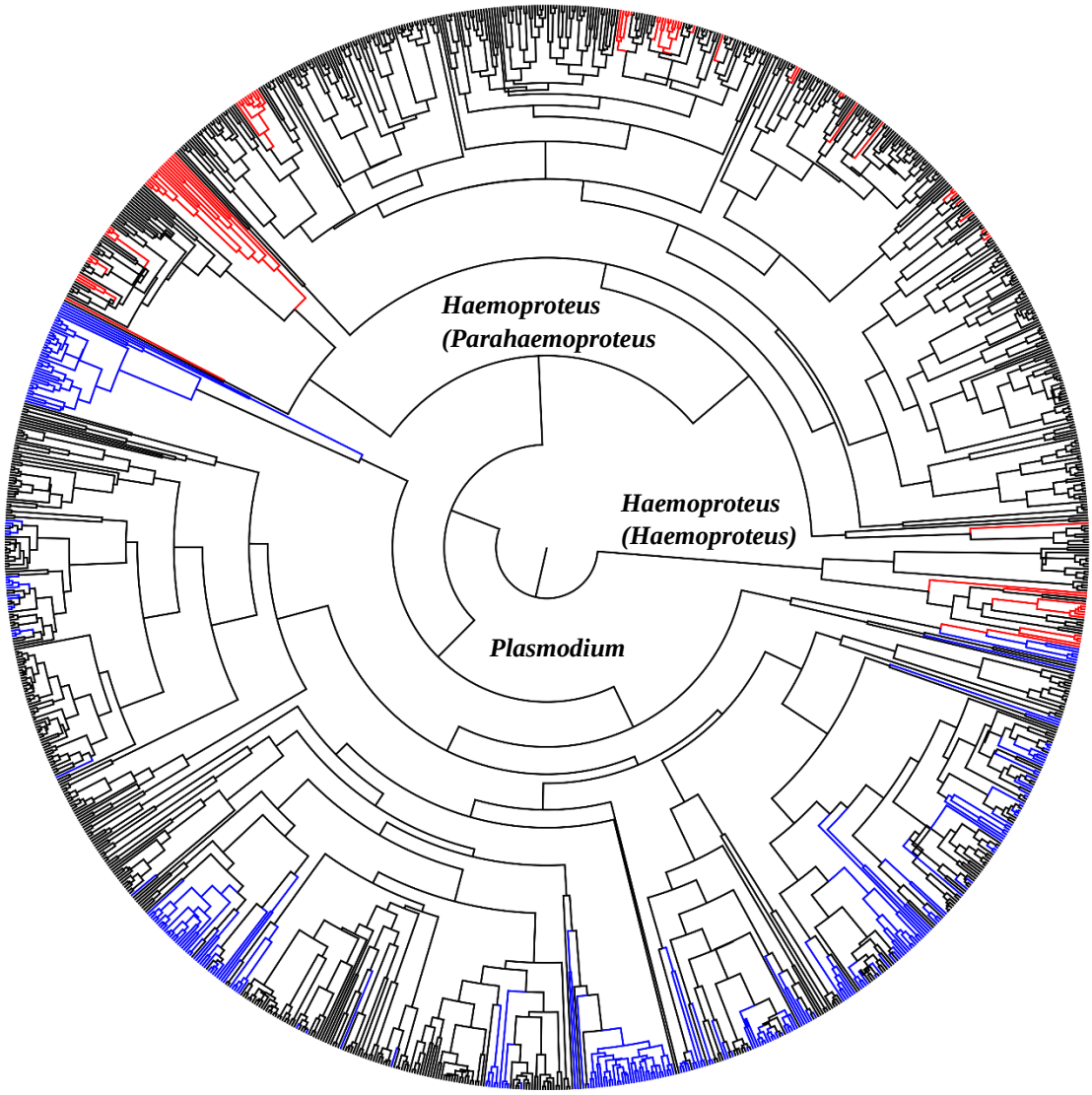


Figure 11. Bayesian inference phylogeny of Brazilian haemosporidian lineages amongst identified lineages from the MalAvi database. *Plasmodium* lineages from Brazil are in blue, and *Haemoproteus* lineages from Brazil are in red.

especially for *Plasmodium* (Figure 11). Brazilian lineages for both genera span the phylogeny and are not restricted to any one specific location with the phylogenetic tree (Figure 11).

**Haemosporidians from Amazonia**

The Amazonia biome was the most widely sampled biome, with 3381 collected samples (Table 19). Five hundred and twenty seven samples from Amazonia (15.6%

prevalence) were infected, with *Plasmodium* accounting for 466 (88.4%) of all infections (Table 19). As with all Brazilian samples, *Plasmodium* prevalence (13.8%) was significantly higher than *Haemoproteus* prevalence (2.1%) ($\chi^2 = 315.61$, df = 1, $p < 0.001$) in Amazonia (Table 22). Of the total 85 resolved coinfections from Brazil, 60 came from Amazonia, with dual *Plasmodium* infection being most common (Table 23). Three hundred and three lineages were recovered from Amazonian birds, 91.4% of them being novel lineages (Table 22). *Plasmodium* lineages significantly out numbered *Haemoproteus* lineages ($\chi^2 = 235.78$, df =1, $p < 0.001$), although the percentages of newly identified lineages did not differ between the two genera ($\chi^2 = 0.44$, df = 1, $p = 0.507$) (Table 22). Most lineages in both genera are presented by only a single sample, but for those recovered more than once the mean host specificity index, $S_{TD}$*, was 2.34 ± 0.73 (95% CI) for *Haemoproteus* and 2.28 ± 0.22 (95% CI) for *Plasmodium*. The host specificity indices did not differ significantly between the two genera (T = 0.19, df = 69, p = 0.847).

The Amazonia biome consists of eight areas of endemism, six of which were sampled during this study (Figure 12, Table 19). Infection prevalence significantly differed between the six areas of endemism: overall prevalence ($\chi^2 = 69.70$, df = 5, $p < 0.001$), *Haemoproteus* prevalence ($\chi^2 = 39.69$, df = 5, $p < 0.001$), and *Plasmodium*

Table 22. Haemosporidian parasitism in 3381 avian blood samples collected from Amazonia. Some of the totals include coinfections.

| | *Haemoproteus* | *Plasmodium* | Total |
|---|---|---|---|
| Individuals infected | 71 | 466 | 527 |
| Infection prevalence | 2.1% | 13.8% | 15.6% |
| Novel cytochrome *b* lineages | 49 (86.0%) | 226 (91.9%) | 277 (91.4%) |
| Described lineages (MalAvi) | 6 (14.0%) | 20 (8.1%) | 26 (8.6%) |
| Total lineages identified | 57 (18.8%) | 246 (81.2%) | 303 |

Table 23. Haemosporidian infection distribution in Amazonia, including single *Haemoproteus* lineage infection (H), single *Plasmodium* lineage infection (P), or coinfections by two different haemosporidian lineages.

| | H | H*H | H*P | P | P*P | Total |
|---|---|---|---|---|---|---|
| Individuals infected | 51 | 10 | 10 | 416 | 40 | 527 |
| Infection prevalence | 1.5% | 0.3% | 0.3% | 12.3% | 1.2% | 15.6% |

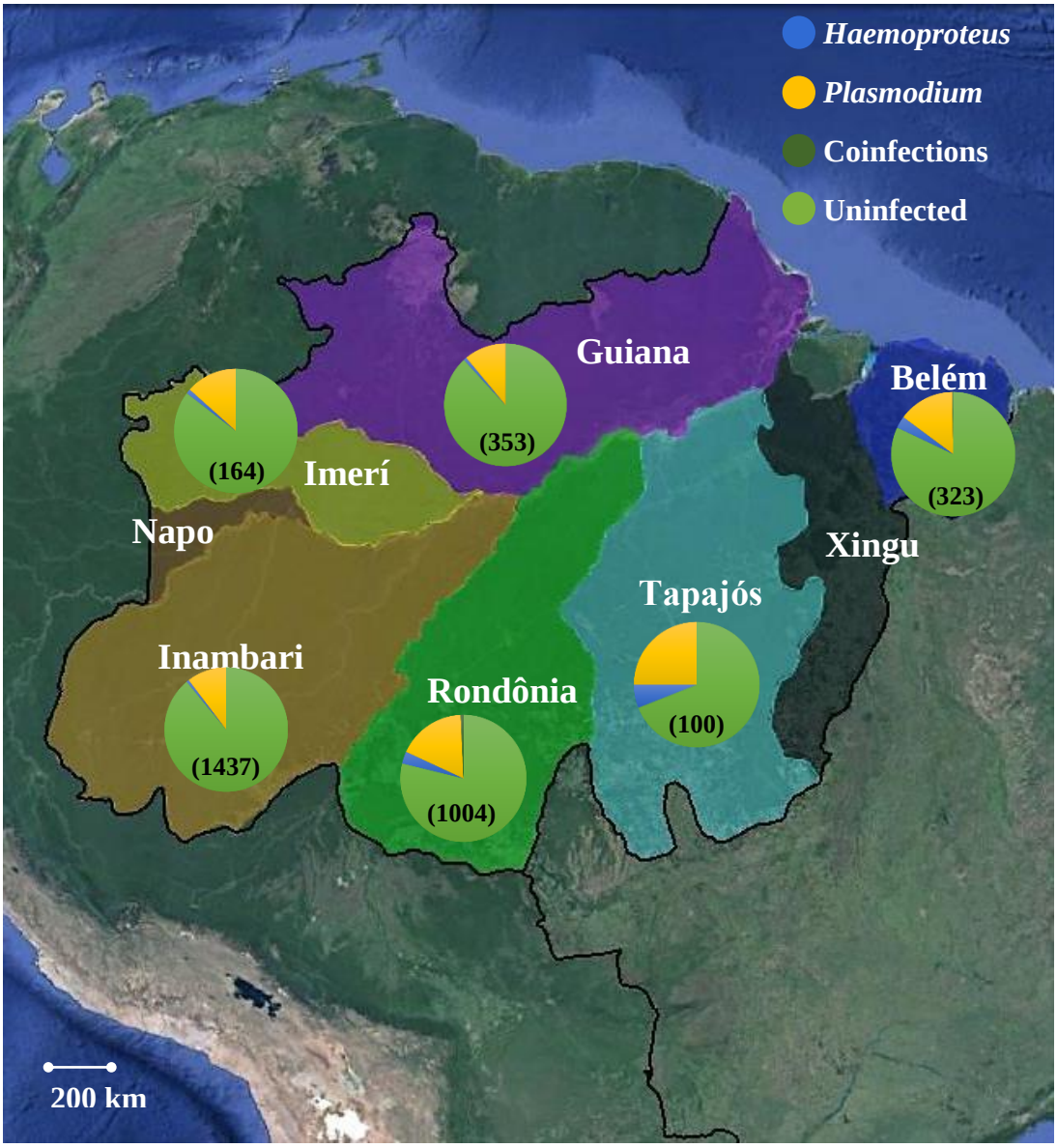


Figure 12. Map of Amazonian areas of endemism. Pie charts indicate the proportion of infected bird samples at area of endemism, with number of samples in parentheses. The Napo and Xingu areas of endemism were not sampled. Coinfections indicate samples infected by both *Haemoproteus* and *Plasmodium.*

prevalence ($\chi^2$ = 43.93, df = 5, p < 0.001) (Figure 12, Table 19). Parasitism differed between the six areas of endemism in overall prevalence ($\chi^2$ = 69.70, df = 5, p < 0.001), *Haemoproteus* prevalence alone ($\chi^2$ = 39.69, df = 5, p < 0.001), and *Plasmodium* prevalence alone ($\chi^2$ = 43.93, df = 5, p < 0.001) (Figure 12, Table 19). Overall infection prevalence ranged from 11.1% in Inambari to 31.0% in Tapajós (Figure 12, Table 19). The areas of Belém, Rondônia, and Tapajós showed significantly higher prevalence for *Haemoproteus*, *Plasmodium*, and both genera combined (Figure 12, Table 19). For both *Haemoproteus* (T = 0.79, df = 6, p = 0.459) and *Plasmodium* (T = -1.69, df = 59, p = 0.097) mean host specificity index values, $S_{TD}$*, did not differ significantly between lineages restricted to one area of endemism from those found in multiple areas of endemism.

The prevalence of Haemosporidia in different avian groups in Amazonia showed the same patterns as the larger Brazilian data set (Tables 24, 25), likely due to the fact that Amazonian samples constituted the bulk of the whole Brazilian data set (Table 19). Amazonian samples included 372 host species from all 17 host orders and including 46 families (Table 25). Only the order Columbiformes showed higher *Haemoproteus* prevalence than *Plasmodium* prevalence in Amazonia (Table 25). Again, Passeriformes were the most frequently sampled order (3107 samples), with most other orders sparsely sampled in Amazonia, and only Piciformes having more than 100 individuals samples (Table 25).

Among Passeriformes, samples were collected from 22 host families and 275 species (Table 25). Thamnophilidae (1142 samples), Dendrocolaptidae (496 samples), Pipridae (486 samples), and Tyrannidae (307 samples) were again the most frequently

sampled families. Infection prevalence varied between families, with Formicariidae (38.9%), Thraupidae (28.6%), and Thamnophilidae (20.7%) and showing the highest prevalence. All infected passerine families had higher prevalence of *Plasmodium* than *Haemoproteus*, even Thraupidae that showed the opposite relationship for all Brazilian samples (Table 24).

**Effect of Amazonian Areas of Endemism on Parasite and Host Communities**

Composition analysis demonstrated that both parasite and host communities differed significantly between areas of endemism and also as a function of latitude (Table 26). Areas of endemism that are more similar in their avian communities are significantly more similar in their parasite communities as well (Mantel statistic r = 0.33, Quartile 0.95 of permutations =0.22, p = 0.005). Haemosporidian prevalence varied widely between areas of endemism (Figure 12, Table 19), with areas of endemism north of the Amazon River (Guiana and Imerí) having significantly lower haemosporidian prevalence than areas of endemism south of the Amazon River (Belém, Tapajós, Inambari and Rondônia) ($\chi^2 = 34.37$, $p < 0.001$). No correlation was found between the phylogenetic distance of parasite lineages and their occurrence in the areas of endemism, for neither *Haemoproteus* lineages (Mantel statistic r = 0.08, Quartile 0.95 of permutations = 0.12, p = 0.11), nor *Plasmodium* lineages (Mantel statistic r = -0.05, Quartile 0.95 of permutations = 0.04, p = 0.99).

**Geographic and Host Taxonomic Structuring of Amazonian Haemosporidian Lineages**

For all Amazonian lineages, and for *Haemoproteus* and *Plasmodium* considered separately, a statistically significant proportion of genetic variation was contained within lineages coming from more than one area of endemism (Table 27). Although

Table 24. Host taxonomic distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Amazonia.

| Order | Family | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Accipitriformes | Accipitridae | 3 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Anseriformes | Anhimidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Apodiformes | Apodidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Trochilidae | 16 | 60 | 1 (1.7) | 2 (3.3) | 3 (5.0) |
| Caprimulgiformes | Caprimulgidae | 3 | 5 | 0 (0) | 0 (0) | 0 (0) |
| | Nyctibiidae | 2 | 3 | 0 (0) | 0 (0) | 0 (0) |
| Ciconiiformes | Jacanidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Columbiformes | Columbidae | 7 | 40 | 9 (22.5) | 2 (5.0) | 10 (25.0)[a] |
| Coraciiformes | Cerylidae | 3 | 10 | 1 (10.0) | 1 (10.0) | 2 (20.0) |
| | Momotidae | 2 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Cuculiformes | Cuculidae | 3 | 5 | 0 (0) | 1 (20.0) | 1 (20.0) |
| Falconiformes | Falconidae | 4 | 8 | 1 (12.5) | 2 (25.0) | 3 (37.5) |
| Galliformes | Cracidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Gruiformes | Psophiidae | 1 | 3 | 0 (0) | 2 (66.7) | 2 (66.7) |
| | Rallidae | 3 | 3 | 0 (0) | 0 (0) | 0 (0) |
| Passeriformes | Cardinalidae | 5 | 41 | 1 (2.4) | 4 (9.8) | 5 (12.2) |
| | Conopophagidae | 3 | 22 | 0 (0) | 1 (4.5) | 1 (4.5) |
| | Cotingidae | 3 | 7 | 0 (0) | 0 (0) | 0 (0) |
| | Dendrocolaptidae | 27 | 496 | 9 (1.8) | 32 (6.7) | 39 (7.9)[b] |
| | Donacobiidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Emberizidae | 6 | 50 | 0 (0) | 10 (20.0) | 10 (20.0) |
| | Formicariidae | 2 | 36 | 0 (0) | 14 (38.9) | 14 (38.9) |
| | Fringillidae | 3 | 5 | 0 (0) | 1 (20.0) | 1 (20.0) |
| | Furnariidae | 22 | 162 | 6 (3.7) | 17 (10.5) | 22 (13.6)[c] |
| | Hirundinidae | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| | Icteridae | 1 | 2 | 1 (50.0) | 1 (50.0) | 2 (100.0) |
| | Parulidae | 2 | 2 | 0 (0) | 0 (0) | 0 (0) |
| | Pipridae | 19 | 486 | 2 (0.4) | 70 (14.4) | 72 (14.8) |
| | Polioptilidae | 3 | 6 | 0 (0) | 2 (20.0) | 2 (20.0) |
| | Scleruridae | 2 | 9 | 0 (0) | 0 (0) | 0 (0) |
| | Thamnophilidae | 72 | 1142 | 21 (1.8) | 219 (19.2) | 235 (20.6)[d] |
| | Thraupidae | 20 | 84 | 6 (7.1) | 19 (22.6) | 24 (28.6)[e] |
| | Tityridae | 8 | 80 | 1 (1.3) | 3 (3.8) | 4 (5.0) |

Table 24. cont.

| Order | Family | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Passeriformes | Troglodytidae | 7 | 80 | 2 (2.5) | 11 (13.8) | 13 (16.3) |
| | Turdidae | 5 | 68 | 0 (0) | 15 (22.1) | 15 (22.1) |
| | Tyrannidae | 59 | 307 | 6 (2.0) | 20 (6.5) | 26 (8.5) |
| | Vireonidae | 4 | 19 | 0 (0) | 1 (5.3) | 1 (5.3) |
| Piciformes | Bucconidae | 9 | 36 | 1 (2.8) | 2 (5.6) | 3 (8.3) |
| | Capitonidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| | Galbulidae | 6 | 36 | 2 (5.6) | 9 (25.0) | 11 (30.1) |
| | Picidae | 10 | 22 | 1 (4.5) | 1 (4.5) | 2 (9.1) |
| | Ramphastidae | 6 | 8 | 0 (0) | 1 (12.5) | 1 (12.5) |
| Psittaciformes | Psittacidae | 4 | 7 | 0 (0) | 3 (42.9) | 3 (42.9) |
| Strigiformes | Strigidae | 5 | 6 | 0 (0) | 0 (0) | 0 (0) |
| Tinamiformes | Tinamidae | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Trogoniformes | Trogonidae | 4 | 6 | 0 (0) | 0 (0) | 0 (0) |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 1, [b] 2, [c] 1, [d] 5, [e] 1,

*Plasmodium* showed significant structuring of genetic variation among areas of endemism, *Haemoproteus* showed a much higher proportion of genetic diversity among areas of endemism (Table 27).

Similar results were also found for the effect of host family on lineage structure in Amazonian. For all lineages, and for each genus separately, a statistically significant proportion of genetic variation was distributed among host families (Table 28). Again, *Haemoproteus* showed a much higher proportion of genetic diversity among host families than *Plasmodium* (Table 28).

**Phylogenetic Reconstruction of Amazonian Haemosporidian Lineages**

*Haemoproteus* and *Plasmodium* lineages from 2661 Amazonian samples, excluding CICRA-Peru, (Table 18) were used for phylogenetic reconstruction, with each genus analyzed separately. A total of 51 *Haemoproteus* lineages (Figure 13) and 214 *Plasmodium* lineages (Figure 14) were included in each respective phylogenetic tree.

Table 25. Host order distribution of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts from Amazonia.

| Order | Families | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Accipitriformes | 1 | 3 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Anseriformes | 1 | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Apodiformes | 2 | 17 | 61 | 1 (1.6) | 2 (3.3) | 3 (4.9) |
| Caprimulgiformes | 2 | 5 | 8 | 0 (0) | 0 (0) | 0 (0) |
| Ciconiiformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Columbiformes | 1 | 7 | 40 | 9 (22.5) | 2 (5.0) | 10 (25.0)[a] |
| Coraciiformes | 2 | 5 | 14 | 1 (7.1) | 1 (7.1) | 2 (14.2) |
| Cuculiformes | 1 | 3 | 5 | 0 (0) | 1 (20.0) | 1 (20.0) |
| Falconiformes | 1 | 4 | 8 | 1 (12.5) | 2 (25.0) | 3 (37.5) |
| Galliformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Gruiformes | 2 | 4 | 6 | 0 (0) | 2 (33.3) | 2 (33.3) |
| Passeriformes | 22 | 275 | 3107 | 55 (1.8) | 440 (14.2) | 486 (15.6)[b] |
| Piciformes | 5 | 32 | 103 | 4 (3.9) | 13 (12.6) | 17 (16.5) |
| Psittaciformes | 1 | 4 | 7 | 0 (0) | 3 (42.9) | 3 (42.9) |
| Strigiformes | 1 | 5 | 6 | 0 (0) | 0 (0) | 0 (0) |
| Tinamiformes | 1 | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Trogoniformes | 1 | 4 | 6 | 0 (0) | 0 (0) | 0 (0) |
| Total (17 Orders) | 46 | 372 | 3381 | 71 (2.1) | 466 (13.8) | 527 (15.6)[c] |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 1, [b] 9, [c] 10

Although nodal support was general high for both genera (Figures 13, 14), both included several large polytomies, especially for *Plasmodium* (Figure 14). Lineages are shaded in both phylogenies to indicate the five host families with the most lineages recovered (Figures 13, 14). In both genera, host families were generally spread throughout the phylogeny, without an overall host family pattern (Figures 13, 14). However, a clade of *Haemoproteus* (*Haemoproteus*) from Columbidae were sister to all other *Haemoproteus* (*Parahaemoproteus*) (Figure 13) and a clade of *Plasmodium* lineages parasitizing Tyrannidae clustered together (Figure 14). The host family pattern within both phylogenies is partly biased by the fact that Thamnophilidae is by far the most sampled

Amazonian family comprising a third of all samples (Tables 24, 25).

Table 26. PERMANOVA results. Model 1 tests for changes in parasite assemblage composition between areas of endemism and in different latitudes, while Model 2 tests for changes in host assemblage composition. df = degrees of freedom; ss = sums of squares; ms= mean squares.

| Model 1: Composition of parasite assemblage | | | | | | |
|---|---|---|---|---|---|---|
| Explanatory Variables | df | ss | ms | F | $R^2$ | p-value |
| Areas of Endemism | 5 | 2.6021 | 0.52042 | 1.2548 | 0.39916 | <0.001 |
| Latitude | 1 | 0.5988 | 0.59878 | 1.4437 | 0.09185 | <0.001 |
| Residuals | 8 | 3.318 | 0.41475 | | 0.50898 | |
| Total | 14 | 6.5188 | | | 1 | |
| Model 2: Composition of host assemblage | | | | | | |
| Explanatory Variables | df | ss | ms | F | $R^2$ | p-value |
| Areas of Endemism | 5 | 2.6021 | 0.52042 | 1.2548 | 0.39916 | <0.001 |
| Latitude | 1 | 0.5988 | 0.59878 | 1.4437 | 0.09185 | <0.001 |
| Residuals | 8 | 3.318 | 0.41475 | | 0.50898 | |
| Total | 14 | 6.5188 | | | 1 | |

Table 27. AMOVA results of genetic structure among areas of endemism in Amazonia. df = Degrees of Freedom; ss = Sums of Squares; % var. = percentage variation. $\Phi_{ST}$ summarizes the proportion of nucleotide diversity among areas of endemism relative to the total. P values were calculated from 1000 randomization.

| | df | ss | % var. | $\Phi_{ST}$ | p-value |
|---|---|---|---|---|---|
| All lineages | | | | | |
| Among areas of endemism | 5 | 292.90 | 2.45 | 0.03 | < 0.001 |
| Within areas of endemism | 532 | 10616.16 | 97.55 | | |
| *Haemoproteus* lineages | | | | | |
| Among areas of endemism | 5 | 240.30 | 13.36 | 0.13 | < 0.001 |
| Within areas of endemism | 67 | 1378.53 | 86.64 | | |
| *Plasmodium* lineages | | | | | |
| Among areas of endemism | 5 | 217.29 | 2.48 | 0.02 | < 0.001 |
| Within areas of endemism | 428 | 7165.46 | 97.52 | | |

**Phylogeographic Signal within Amazonian Haemosporidian Lineages**

S-DIVA analysis of the 51 *Haemoproteus* lineages showed a noticeable impact of area of endemism on parasite relatedness, with several clades formed entirely of lineages

Table 28. AMOVA results of genetic structure among host families in Amazonia. df = Degrees of Freedom; ss = Sums of Squares; % var. = percentage variation. $\Phi_{ST}$ summarizes the proportion of nucleotide diversity among host families relative to the total. P values were calculated from 1000 randomization.

| | df | SS | % Var. | $\Phi_{ST}$ | p-value |
|---|---|---|---|---|---|
| All lineages | | | | | |
| Among host family | 27 | 1306.54 | 10.58 | 0.11 | < 0.001 |
| Within host family | 475 | 8894.20 | 89.42 | | |
| *Haemoproteus* lineages | | | | | |
| Among host family | 15 | 631.40 | 26.77 | 0.27 | < 0.001 |
| Within host family | 56 | 954.06 | 73.23 | | |
| *Plasmodium* lineages | | | | | |
| Among host family | 27 | 936.69 | 9.72 | 0.10 | < 0.001 |
| Within host family | 406 | 6444.27 | 90.28 | | |

recovered from a single area of endemism (Figure 15). At the same time the Mantel test did not find areas of endemism to have significant phylogenetic signal for *Haemoproteus* (Mantel statistic r = 0.08, Quartile 0.95 of permutations = 0.12, p = 0.11). The Rondônia area of endemism is especially well represented in our data set, which allowed more definite conclusion regarding the impact of area of endemism on parasite relatedness. A number of *Haemoproteus* linages from Rondônia tend to cluster together in the phylogenetic tree (Figure 16). Both dispersal (20 instances) and vicariance (14 instances) have played an important role in phylogeographical patterns observed in *Haemoproteus* (Figure 14).

For the 214 *Plasmodium* lineages, the phylogeographical signal is weaker, with many larger clades composed of lineages recovered from several areas of endemism (Figure 17). This is supported by the Mantel test results for phylogeographical signal (Mantel statistic r = -0.05, Quartile 0.95 of permutations = 0.04, p = 0.99). Mapping *Plasmodium* lineages to their geographical location also demonstrates the lack of strong

phylogeographical pattern (Figure 18). Unlike *Haemoproteus*, dispersal (142 events) occurred more frequently than vicariance (80 events). Extinction events were also evident in the *Plasmodium* phylogeny (5 extinction events), which was not seen in *Haemoproteus* (Figure 17).

## Discussion

Haemosporidians and their avian hosts exhibit similar diversity and distribution patterns (Ellis *et al.* 2015), with tropical regions supporting the highest diversity of haemosporidian parasites (see Clark *et al.* 2014 for review). Since haemosporidian diversity is a function of host diversity, regions with hyper-diverse avian fauna such as Brazil (Mittermeier *et al.* 2003, Marini and Garcia 2005, Grenyer *et al.* 2006), should support highly diverse community of haemosporidian parasites. The results of this study, one of the largest sampling efforts within Brazil, support this theory. Haemosporidian diversity from Brazil matched host diversity, with 365 genetic lineages recovered from 447 host species. *Plasmodium* was especially diverse including 78% of all infections found and 76% of all identified genetic lineages. The lack of *Leucocytozoon* infections is in agreement with other studies from the region (White *et al.* 1978, Valkiūnas 2005, Forrester and Greiner 2008, Lotta *et al.* 2015). *Leucocytozoon* records in South America are restricted to the higher altitudes surrounding the Andes and are seemingly absent from all other areas (Matta *et al.* 2014, González *et al.* 2014, Lotta *et al.* 2015).

High haemosporidian diversity composed mostly by *Plasmodium* matches both previous work from both Brazil (Ribeiro *et al.* 2005, Sebaio *et al.* 2012, Lacorte *et al.* 2013) and Ecuador (Svennsson-Coelho *et al.* 2013) and the expectations of Clark *et al.* (2014). The high diversity of *Plasmodium* is likely a function of multiple factors such as

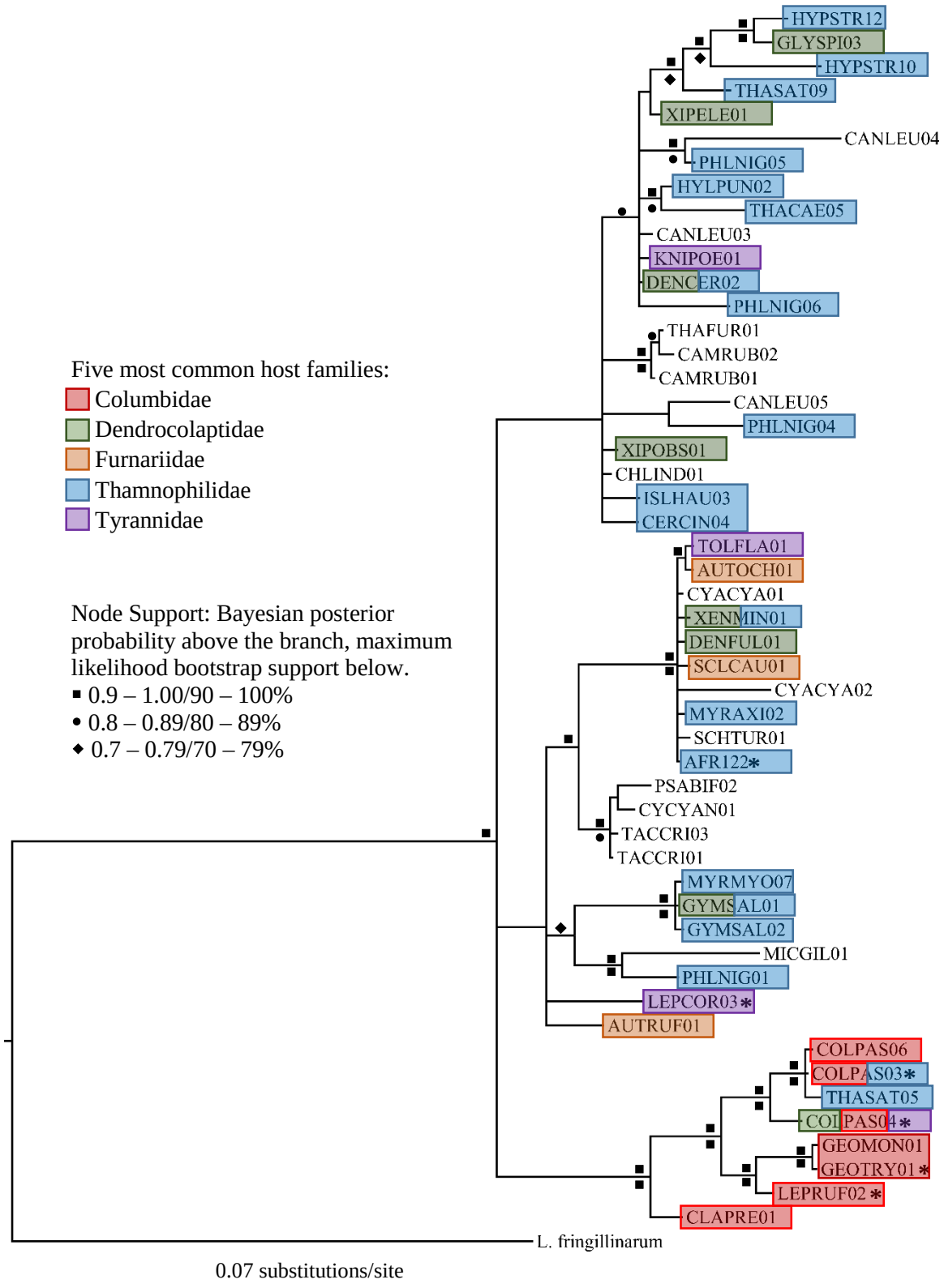


Figure 13. Phylogenetic reconstruction of Amazonian *Haemoproteus* lineages. Lineages previously described (MalAvi) are indicated with an asterisk. The five most common host families are also indicated by colored blocks.

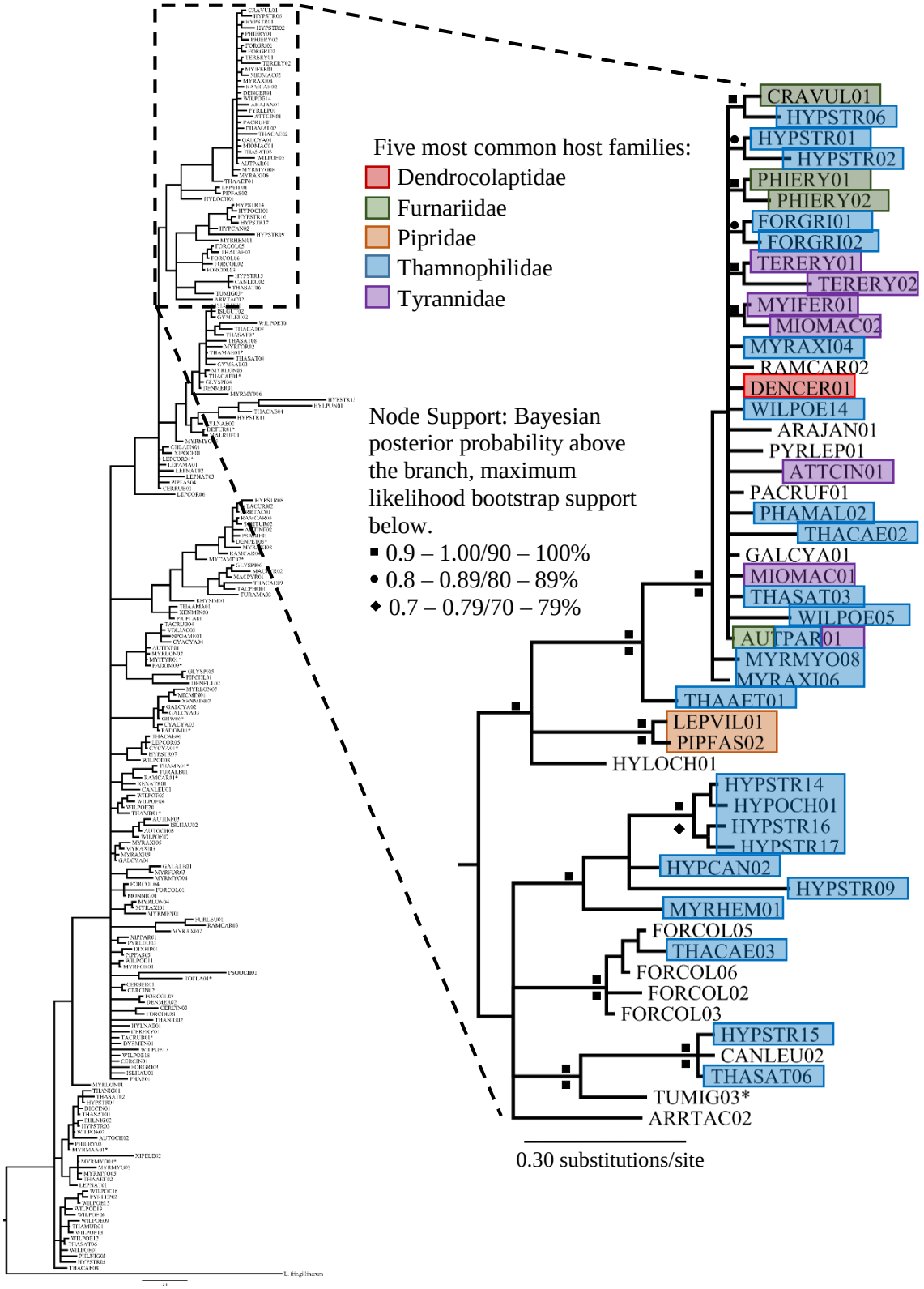


Figure 14. Phylogenetic reconstruction of Amazonian *Plasmodium* lineages. Enclosed subtree shown in detail. Lineages previously described (MalAvi) are indicated with an asterisk. The five most common host families are also indicated by colored blocks.

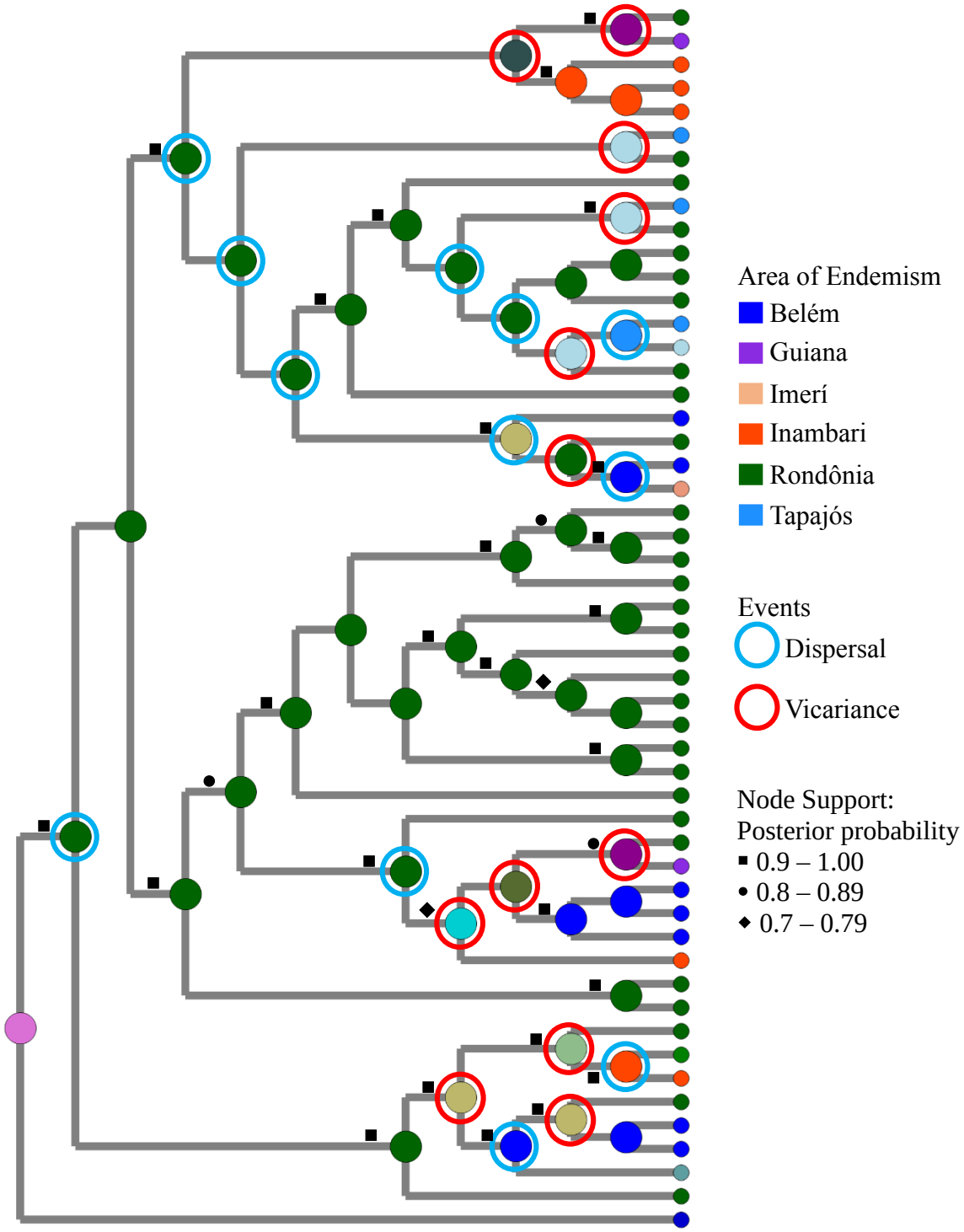


Figure 15. S-DIVA analysis on the impact of area of endemism on *Haemoproteus* phylogeny within Amazonia. Colors represent sampling location, with parent nodes shaded to represent the most likely ancestral area. The speciation events (dispersal, vicariance) responsible for lineage divergence are shown.

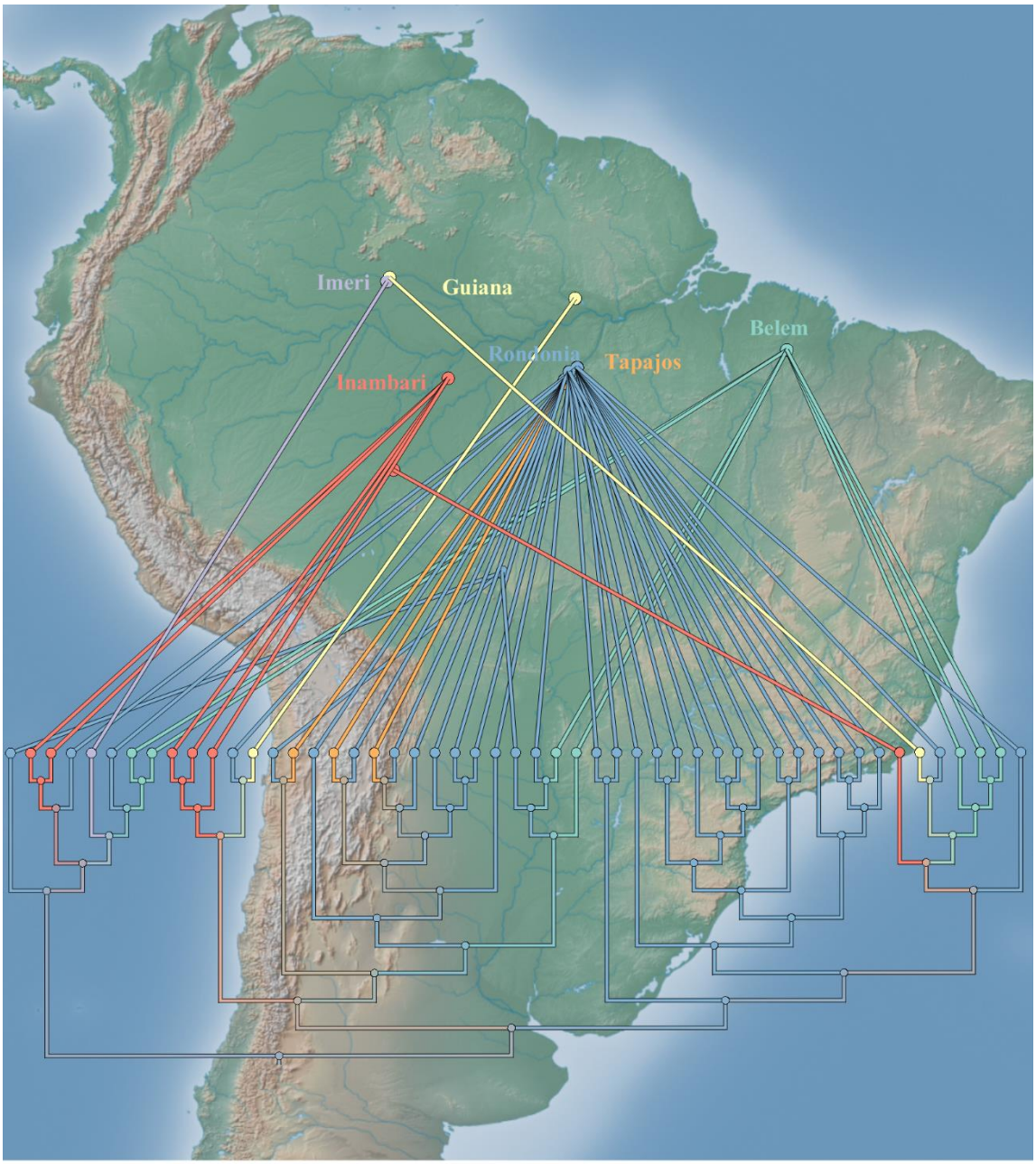


Figure 16. Visualization of the impact of geographic distribution (area of endemism) within the phylogeny of *Haemoproteus* from Amazonia. Same Bayesian phylogenetic tree used for S-DIVA analysis (Figure 15).

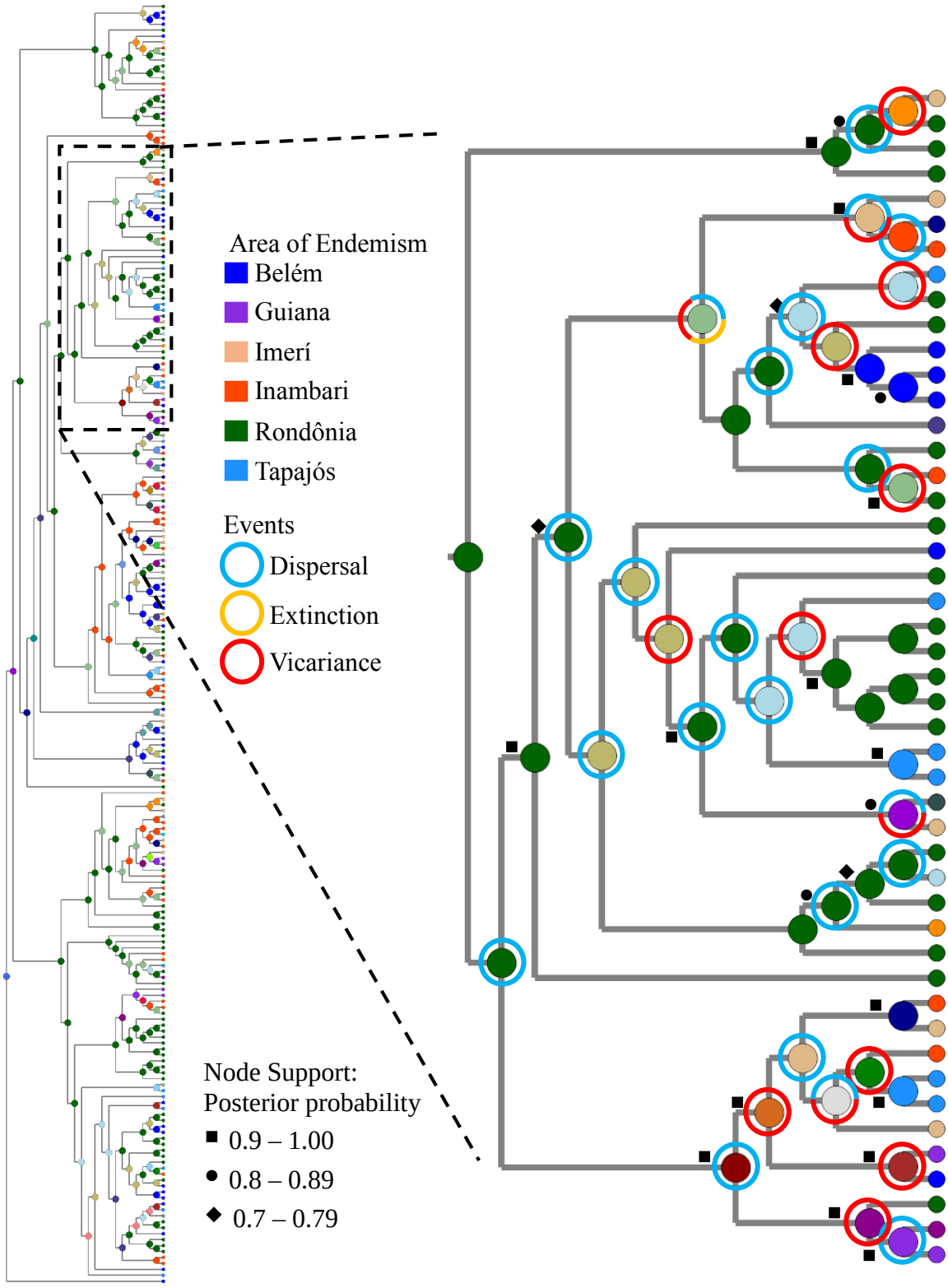


Figure 17. S-DIVA on the impact of area of endemism on *Plasmodium* phylogeny within Amazonia. Colors represent sampling location with parent nodes shaded to represent the most likely ancestral area. The speciation events (dispersal, extinction, vicariance) responsible for lineage divergence are shown. Indicated subtree shown in detail.

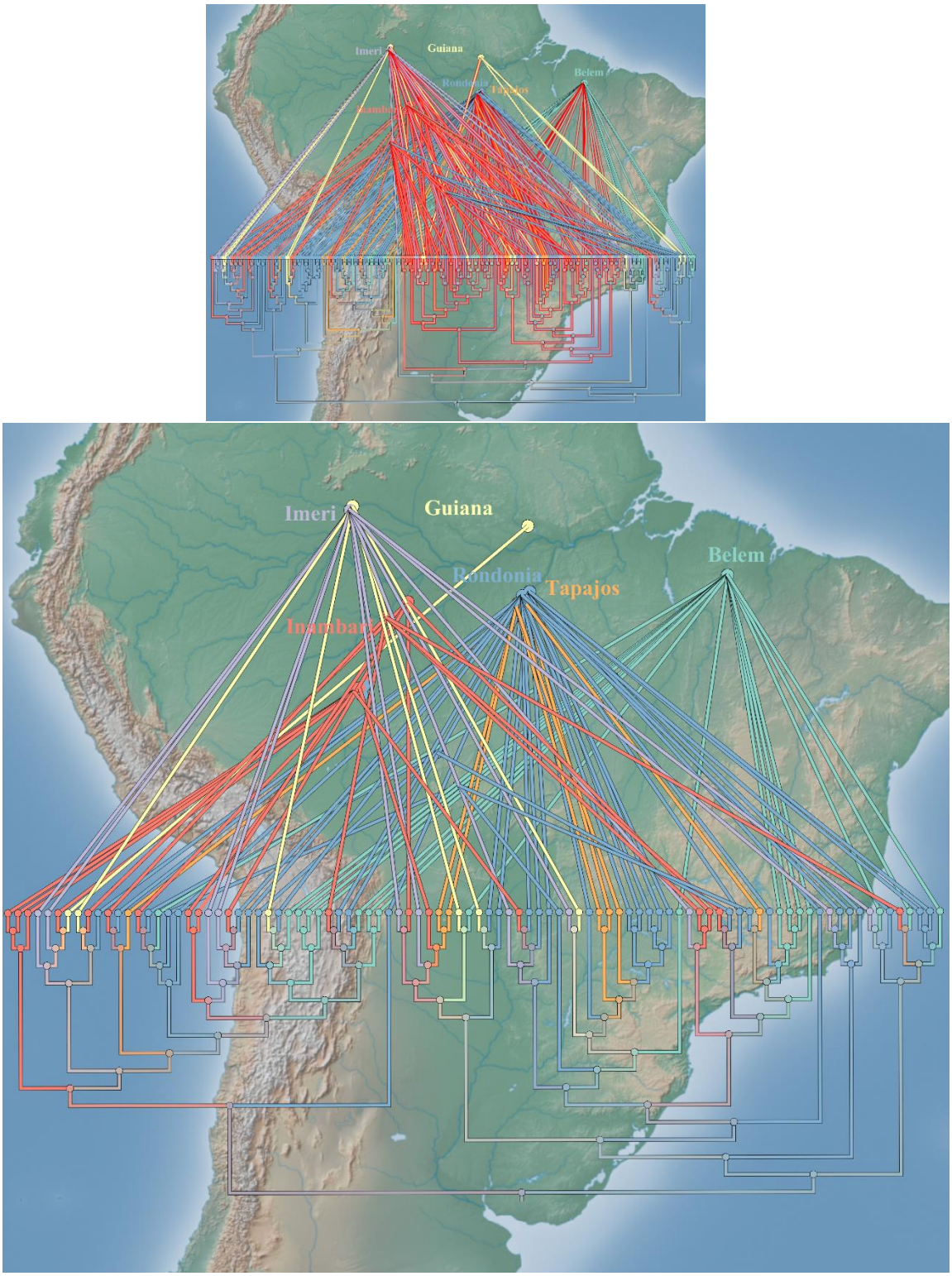


Figure 18. Visualization of the impact of geographic distribution (area of endemism) within the phylogeny of *Plasmodium* from Amazonia. Same Bayesian phylogenetic tree used for S-DIVA analysis (Figure 17), with the same subtree shown in detail, highlighted in red in upper image.

the lower host specificity than *Haemoproteus* (Beadell *et al.* 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al.* 2010), low rates of cospeciation between *Plasmodium* parasites and their avian hosts (Ricklefs and Fallon 2004, de Vienne *et al.* 2013, Lauron *et al.* 2015) and extremely high mosquito diversity (Rueda 2008) coupled with a generally low mosquito feeding specificity (Kilpatrick *et al.* 2006, Ejiri *et al.* 2008, 2011, Gager *et al.* 2008, Hamer *et al.* 2008, 2009,). Increased exposure of avian hosts to generalist mosquito vectors would not only increase *Plasmodium* prevalence (Medeiros *et al.* 2015), but also facilitate host switching (Kim and Tsuda 2012). However, one cannot assume that all mosquito species lack host specificity, because in other systems mosquito host specificity impacts distribution patterns and host associations of *Plasmodium* (Besansky *et al.* 2004, Njabo *et al.* 2011, Medeiros *et al.* 2013) and West Nile virus (Venkatesan and Rasgon 2010, Hamer *et al.* 2011). Host specificity has also been shown in other dipteran vectors of haemosporidian parasites (Besansky *et al.* 2004, Hellgren *et al.* 2008, Martinez-de la Puente *et al.* 2011). Even if host generality is more common, it alone does not explain the higher diversity of *Plasmodium*, since the development of *Plasmodium* parasites differ significantly among different parasite-vector combinations (Ghosh *et al.* 2000, Habtewold *et al.* 2008). Rather, the success of host dispersal/colonization and subsequent diversification more likely depend on coevolutionary relationships between *Plasmodium* parasites and their avian hosts (Apanius *et al.* 2000, Fallon *et al.* 2005, Bonneaud *et al.* 2006, Agosta *et al.* 2010, Ricklefs 2010, Ellis *et al.* 2015, Medeiros *et al.* 2015).

While diversity was high, overall haemosporidian prevalence was low which conforms to the well documented pattern in the Neotropics previously (Gabaldon *et al.*

1974, 1975, Bennett and Borrero 1976, White *et al.* 1978, Bennett and Lopes 1980, Sousa and Herman 1982, Woodworth-Lynas *et al.* 1989, Bennett *et al.* 1991, Young *et al.* 1993, Rodriguez and Matta 2001, Valkiūnas *et al.* 2003, 2004, Ribeiro *et al.* 2005, Basto *et al.* 2006, Fecchio *et al.* 2007, Londoño *et al.* 2007, Benedikt *et al.* 2009, Sebaio *et al.* 2012, González *et al.* 2014). The pattern of high haemosporidian diversity, but low prevalence may be explained by the dilution effect. High host diversity decreases the number of susceptible hosts (Keesing *et al.* 2006), thus decreasing transmission opportunities for haemosporidian parasites (Matta *et al.* 2014). Additionally, stronger immune defenses in long lived tropical bird species (Ricklefs 1992) may reduce overall parasite prevalence, causing haemosporidian parasites to trade increased host breadth for decreased prevalence (Medeiros *et al.* 2014, Moens and Pérez-Tris 2015).

Avian haemosporidians in South America seem to be more host generalist compared to other regions of the world (Moens and Pérez-Tris 2015). The host specificity index values found in this study are higher (more host generalist) than areas outside of South America (Moens and Pérez-Tris 2015), however the majority of all lineages were recovered from a single host (76%). This may be an artifact of poor sampling in many host species, where only a few individuals were sampled (Appendix B). One expectation is that high host diversity would support a more generalist haemosporidian community since generalists would benefit from higher host encounter rates and increased transmission (Dobson 2004, Keesing *et al.* 2006). The higher mean host specificity values for Amazonian haemosporidian lineages (2.28 for *Haemoproteus*, 2.34 for *Plasmodium*), where host diversity is highest, support this hypothesis.

Habitat is also known to affect host specificity (Loiseau *et al.* 2012b, Moens and Pérez-Tris 2016) with geographical barriers limiting the movement of specialist lineages (Mata *et al.* 2015). The unique habitats within Brazilian biomes also have affected host specificity, with more host specific *Plasmodium* lineages (lower $S_{TD}$* values) restricted to individual biomes. Although not statistically significant (p = 0.097), *Plasmodium* lineages restricted to a single Amazonian area of endemism were more host specific than those found in multiple areas of endemism. *Haemoproteus* lineages showed no effect of habitat on host specificity, potentially due to overall higher host specificity in this genus (Beadell *et al.* 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al.* 2010). Further study including denser sampling in non-passerine hosts will help to better understand host specificity within Brazil, especially since haemosporidian prevalence varied between host families.

Not only was haemosporidian diversity high, the majority of lineages (90.7%) were novel. This high untapped haemosporidian diversity within Brazil warrants additional sampling, especially in under represented host groups, such as non-passerines and under sampled regions. Haemosporidian prevalence varied widely between passerine and non-passerine families. For example in Columbidae, *Haemoproteu*s was more prevalent than *Plasmodium*, which differs from the general pattern for Brazil. Non-passerines are known to be infected by novel haemosporidian lineages (Valkiūnas 2005), which have unique coevolutionary relationships with their hosts (Santiago-Alarcon 2014). Additional sampling is needed to understand the unique host-parasite interactions between non-passerines and their haemosporidian parasites.

Haemosporidian lineages recovered from Brazil formed many distinct clades interspersed within the phylogenetic tree of all known haemosporidian lineages. These both the *Haemoproteus* and *Plasmodium* phylogenies. This suggests an evolutionary history of multiple introduction events into what is now Brazil with subsequent speciation events producing the high diversity of lineages seen. Results from the Amazon region of Ecuador support this history of multiple introductions followed by adaptive radiation of unique generalist parasites (Moens and Pérez-Tris 2015). The hyper diverse host community of the Ecuadorian Amazon led to the evolution of endemic, host generalist lineages from introduced specialist lineages (Moens and Pérez-Tris 2015). The diversity of Brazilian lineages, along with phylogenetic and host specificity analysis support these conclusions and demonstrate that Brazilian birds support a uniquely endemic and host generalist community of haemosporidian parasites.

Analysis of host specificity for Brazilian lineages further supports this evolutionary history towards host generalization. Research is needed to determine what role the unique biogeography of Brazil, especially the areas of endemism within Amazonia, have played in the diversification of avian haemosporidians.

**Impact of Amazonian Areas of Endemism on Avian Haemosporidians**

This study is the first PCR based avian haemosporidian survey from the Brazilian Amazon. Haemosporidian diversity was high with 303 haemosporidian lineages identified in samples from 372 host species. The biogeography of the Amazonian biome with its eight unique areas of endemism defined haemosporidian diversity and distribution. Areas of endemism contained unique parasite communities that not only differed in parasite prevalence (higher south of the Amazon River), but also in

community structure. Parasite communities in each area of endemism differed, presumably due to differences in host communities, with areas with more similar host communities harboring more similar parasite communities. Avian community structure in Amazonia closely matches areas of endemism (Cracraft 1985, Silva *et al.* 2002, 2005, Wesselingh *et al.* 2009), but this study provides the first example of avian parasites also matching these areas. Avian haemosporidian distribution is affected by host distribution, which in turn is due to the unique biogeography of Amazonia. This pattern of host distribution determining haemosporidian distribution matches what is known for haemosporidians parasites within North America (Ellis *et al.* 2015).

Phylogenetic and phylogeographical analyses support the unique role of areas of endemism in shaping avian haemosporidian communities. As shown above, individual areas of endemism supported genetically more similar haemosporidian lineages. As with parasite communities this can be attributed to host effects, with individual host families infected by genetically more similar haemosporidian parasites. These effects were much stronger in *Haemoproteus* for both area of endemism and host family variables, likely a consequence of higher host specificity in this genus (Beadell *et al.* 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al.* 2010). Olsson-Pons *et al*. (2015) saw similar biogeographical effects on haemosporidians distributed among islands in Melanesia, showing the ability of areas of endemism to work as strong isolating mechanisms for haemosporidian movement and subsequent speciation. Although there was no significant phylogenetic signal for geographic effects in *Haemoproteus* or *Plasmodium*, phylogeographical patterns were seen in S-DIVA analysis. Clades composed only of lineages from a single area of endemism occurred in both genera. A

stronger pattern was seen in *Haemoproteus* where many lineages were contained within a larger Rondônia specific clade. The patterns seen in phylogeographical analysis support an effect of area of endemism on the phylogeny of *Haemoproteus*. The lack of a phylogenetic signal ($p = 0.11$) potentially representing a type II error, due to overall low prevalence of *Haemoproteus*. Since areas of endemism constrain host distribution (Cracraft 1985, Silva *et al.* 2002, 2005, Wesselingh *et al.* 2009) and *Haemoproteus* has higher host specificity (Beadell *et al.* 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al.* 2010) one would expect a strong phylogeographic pattern for this genus within Amazonia. This is also present in host family associations within the *Haemoproteus* phylogeny. *Plasmodium* with its lower host specificity and general lack of host cospeciation (Ricklefs and Fallon 2004, de Vienne *et al.* 2013, Lauron *et al.* 2015) would not be expected to show strong phylogeographic effects. However, although the effects on *Plasmodium* are weaker than seen in *Haemoproteus,* an effect of area of endemism on *Plasmodium* phylogeography within Amazonia does exist.

Dispersal between areas of endemism was the most common evolutionary pattern reconstructed within *Haemoproteus* and *Plasmodium* phylogenies. Movement between areas of endemism can occur either with parasites moving with infected hosts, by hosting switching, or by a combination of the two. The major river tributaries that delineated areas of endemism in Amazonia generally restrict bird movement, with bird communities within Amazonia being more similar to areas outside of Amazonian than areas within (Cracraft and Prum 1988, Prum 1988, Amorim 2001). A more likely mechanism is through colonization/dispersal between uninfected Amazonian hosts and migratory hosts that come to Amazonia from outside areas, then distribute haemosporidian lineages

within Amazonia. This hypothesis could be explained by vicariance events, with parasite loss in areas of endemism due to movement of parasites by migratory hosts, and subsequent parasite loss due to lack of suitable hosts within the new avian communities. The stronger phylogeographical pattern for *Haemoproteus* with more frequent vicariance events further supports this hypothesis, due to its known higher host specificity (Beadell *et al.* 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al.* 2010). *Plasmodium* with its lower host specificity, could move more freely between hosts and areas of endemism. This suggestion is supported by phylogenetic and phylogeographical analyses.

The unique areas of endemism within Amazonia have shaped not only the avian communities, but also their haemosporidian parasites. Dispersal of avian hosts between areas of endemism was not only a major force in their diversification (Smith *et al.* 2014), but also in the diversification of their haemosporidian parasites. Colonization of haemosporidians amongst dispersing hosts within a highly diverse yet geographically fragmented habitat would provide the isolating mechanisms needed for speciation. High avian diversity in Amazonia (Mittermeier *et al*. 2003, Marini and Garcia 2005, Grenyer *et al.* 2006) would function to increase the potential of successful host switching (colonization and diversification) due to increased numbers of closely related avian hosts (Hayakawa *et al.* 2008, Poulin 2011). Within Amazonia, avian hosts with high levels of niche partitioning would also promote retention of newly evolved lineages, thus maintaining or even increasing overall haemosporidian diversity (MacArthur and MacArthur 1961, Hechinger and Lafferty 2005, Sheratt and Wilkinson 2009).

Although unknown, it can be assumed that the different areas of endemism support different vector communities, which would enhance potential isolation mechanisms for speciation. Matching vector biology to haemosporidian parasitism in avian hosts is missing from most avian haemosporidian research, but is essential for completely understanding the evolutionary mechanisms responsible for the diversity and distribution patterns of these parasites (Medeiros *et al*. 2015). This is especially true for Amazonia with its unique biogeography and extremely high avian, parasite, and vector diversity (Foley *et al.* 2007, Rueda 2008), yet basic vector research is lacking.

## CHAPTER V

## HOST LIFE HISTORY CHARATERISTICS PREDICT INFECTION PROBABILITY OF *HAEMOPROTEUS* AND *PLASMODIUM* IN AMAZONIAN BIRDS

### Results

Haemosporidian prevalence was 18.6% in the 1759 samples used for life history analysis. *Plasmodium* prevalence (15.9%) was significantly higher than *Haemoproteus* prevalence (3.2%) ($\chi^2 = 165.1$, df = 1, $p < 0.001$). Total haemosporidian prevalence ($\chi^2 = 44.04$, df = 5, $p < 0.001$), *Haemoproteus* prevalence ($\chi^2 = 25.98$, df = 5, $p < 0.001$), and *Plasmodium* prevalence ($\chi^2 = 27.86$, df = 5, $p < 0.001$) varied significantly between areas of endemism (Table 29). *Haemoproteus* prevalence was highest in Belém, Rondônia, and Tapajós, with Rondônia yielding 57% of all *Haemoproteus* positive samples. *Haemoproteus* prevalence was very low in Guiana, Imerí, and Inambari (Table 29). Belém, Rondônia, and Tapajós also showed the highest prevalence for *Plasmodium*, with more than 20% prevalence in both Rondônia and Tapajós (Table 29).

Table 29. Prevalence of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections in avian hosts used for life history analysis among areas of endemism.

| Biome | Area of Endemism | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|
| Amazonia | Belém | 323 | 11 (3.4) | 48 (14.9) | 58 (18.0)[a] |
| | Guiana | 178 | 1 (0.6) | 12 (6.7) | 13 (7.3) |
| | Imerí | 164 | 2 (1.2) | 22 (13.4) | 24 (14.6) |
| | Inambari | 419 | 4 (1.0) | 57 (13.6) | 61 (14.6) |
| | Rondônia | 575 | 32 (5.6) | 116 (20.2) | 140 (24.3)[b] |
| | Tapajós | 100 | 6 (6.0) | 25 (25.0) | 31 (31.0) |
| Total | | 1759 | 56 (3.2) | 280 (15.9) | 327 (18.6)[c] |

Number of *Haemoproteus*/*Plasmodium* coinfections: [a] 1, [b] 8, [c] 9

Samples were collected from 17 avian orders, 43 host families, and 294 host species (Table 30). 88.8% of all samples collected were from passerine birds (Table 30). Many orders were poorly sampled, representing only opportunistic collections, with 13 orders having less than 10 samples each (Table 30). Eight orders contained no haemosporidian infections. For orders with more than 20 samples overall prevalence was highest in Columbiformes (33.3%), Piciformes (23.4%), and Passeriformes (18.6%), although *Haemoproteus* and *Plasmodium* prevalence varied among these orders. *Haemoproteus* prevalence was highest in Columbiformes (29.6%) and Piciformes (6.3%) and lowest in Passeriformes (1.8%) (Table 30), whereas *Plasmodium* prevalence was low in Columbiformes (7.4%), but high in Piciformes (17.2%), and Passeriformes (16.5%) (Table 30).

**Impact of Host Life History on Haemosporidian Infection Probability in Amazonia**

For all Amazonian samples the best explanatory model for *Haemoproteus* prevalence included the categorical variables (fixed effects) of foraging height and area of endemism, whereas for *Plasmodium* the best explanatory model included nest type, foraging height, and area of endemism (Table 6). Host phylogenetic constraints were included in all models as nested random effects. For *Haemoproteus*, host family had a significant effect on parasitism, whereas for *Plasmodium* host family and host species significantly affected parasitism (Table 7). For *Haemoproteu*s host species was marginally significant ($p = 0.06$) (Table 7). Across all 15 candidate models foraging height was the best supported fixed effect for explaining both *Haemoproteu*s and *Plasmodium* prevalence (Table 8). Model average regression coefficients for the different categorical variables (nest height, nest type, foraging height, flocking behavior, and area

of endemism) showed that only area of endemism was significantly correlated with infection probability, but only for *Plasmodium (*Table 9). Least squared means were used to determine the probability of *Plasmodium* infection by area of endemism (Figure 19). The probability of *Plasmodium* parasitism was significantly lowest in Guiana relative to all other areas of endemism, with Belém, Rondônia, and Tapajós having increased rates of *Plasmodium* parasitism (Figure 19). Area of endemism had the strongest predictive value of any categorical variable, so additional analyses were conducted for each area of endemism separately to assess the effect of host life history characteristics.

Table 30. Prevalence of *Haemoproteus* (H), *Plasmodium* (P), and total haemosporidian infections among avian host taxonomic groups used for life history analysis, collapsed by host order.

| Order | Families | Species | Samples | H (%) | P (%) | Total (%) |
|---|---|---|---|---|---|---|
| Accipitriformes | 1 | 3 | 3 | 0 (0) | 0 (0) | 0 (0) |
| Anseriformes | 1 | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Apodiformes | 2 | 17 | 49 | 1 (2.0) | 2 (4.1) | 3 (6.1) |
| Caprimulgiformes | 2 | 5 | 8 | 0 (0) | 0 (0) | 0 (0) |
| Ciconiiformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Columbiformes | 1 | 5 | 27 | 8 (29.6) | 2 (7.4) | 9 (33.3)[a] |
| Coraciiformes | 2 | 5 | 9 | 1 (11.1) | 0 (0) | 1 (11.1) |
| Cuculiformes | 1 | 3 | 4 | 0 (0) | 1 (25.0) | 1 (25.0) |
| Falconiformes | 1 | 3 | 5 | 0 (0) | 2 (40.0) | 2 (40.0) |
| Galliformes | 1 | 1 | 2 | 0 (0) | 0 (0) | 0 (0) |
| Gruiformes | 2 | 3 | 5 | 0 (0) | 2 (40.0) | 2 (40.0) |
| Passeriformes | 20 | 210 | 1562 | 42 (1.8) | 257 (16.5) | 291 (18.6)[b] |
| Piciformes | 4 | 23 | 64 | 4 (6.3) | 11 (17.2) | 15 (23.4) |
| Psittaciformes | 1 | 4 | 7 | 0 (0) | 3 (42.9) | 3 (42.9) |
| Strigiformes | 1 | 5 | 6 | 0 (0) | 0 (0) | 0 (0) |
| Tinamiformes | 1 | 1 | 1 | 0 (0) | 0 (0) | 0 (0) |
| Trogoniformes | 1 | 4 | 4 | 0 (0) | 0 (0) | 0 (0) |
| Total (17 Orders) | 43 | 294 | 1759 | 56 (3.2) | 280 (15.9) | 327 (18.6)[d] |

Number of *Haemoproteus/Plasmodium* coinfections: [a] 1, [b] 8, [c] 9

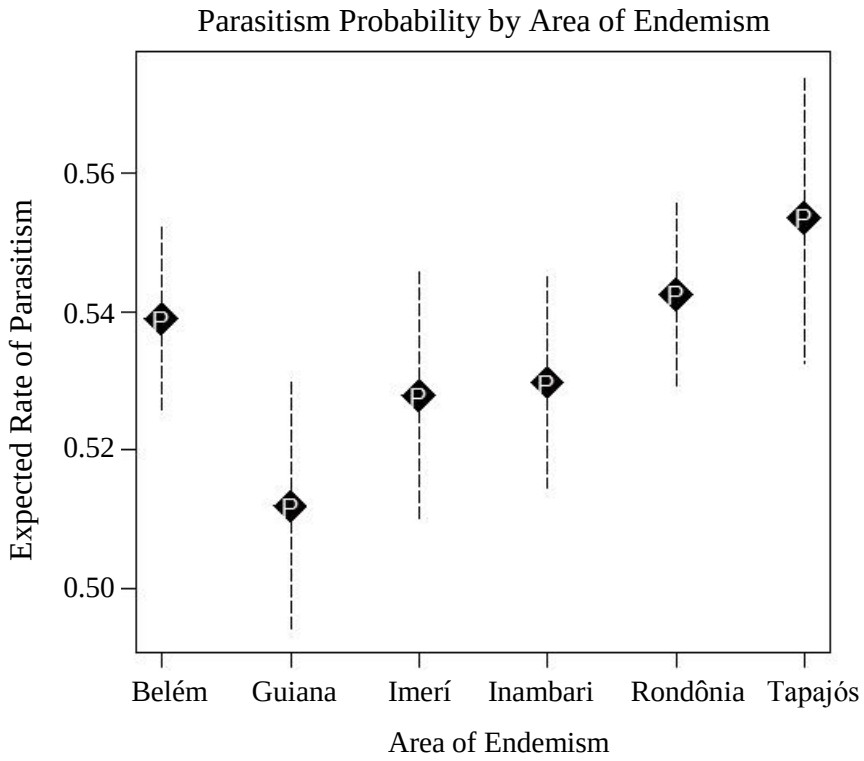


Figure 19. Predicted (least-square means) probabilities of *Plasmodium* parasitism by area of endemism within Amazonia and associated 95% confidence intervals.

**Impact of Host Life History on Haemosporidian Infection Probability for each Area of Endemism Individually**

For the Belém area of endemism the best candidate model for *Haemoproteus* prevalence included only the categorical variable of flocking behavior, whereas for *Plasmodium* the best candidate model included only foraging height (Table 10). In Belém host species was a significant factor influencing *Haemoproteus* prevalence whereas host family had a significant constraint on *Plasmodium* prevalence (Table 11). Across all candidate models flocking behavior for *Haemoproteus* and foraging height for *Plasmodium* were the best supported explanatory variables (Table 12). For both genera, flocking behavior significantly predicted parasitism (Tables 13, 14), with higher probability of infection for species that formed single-species flocks (Figure 20).

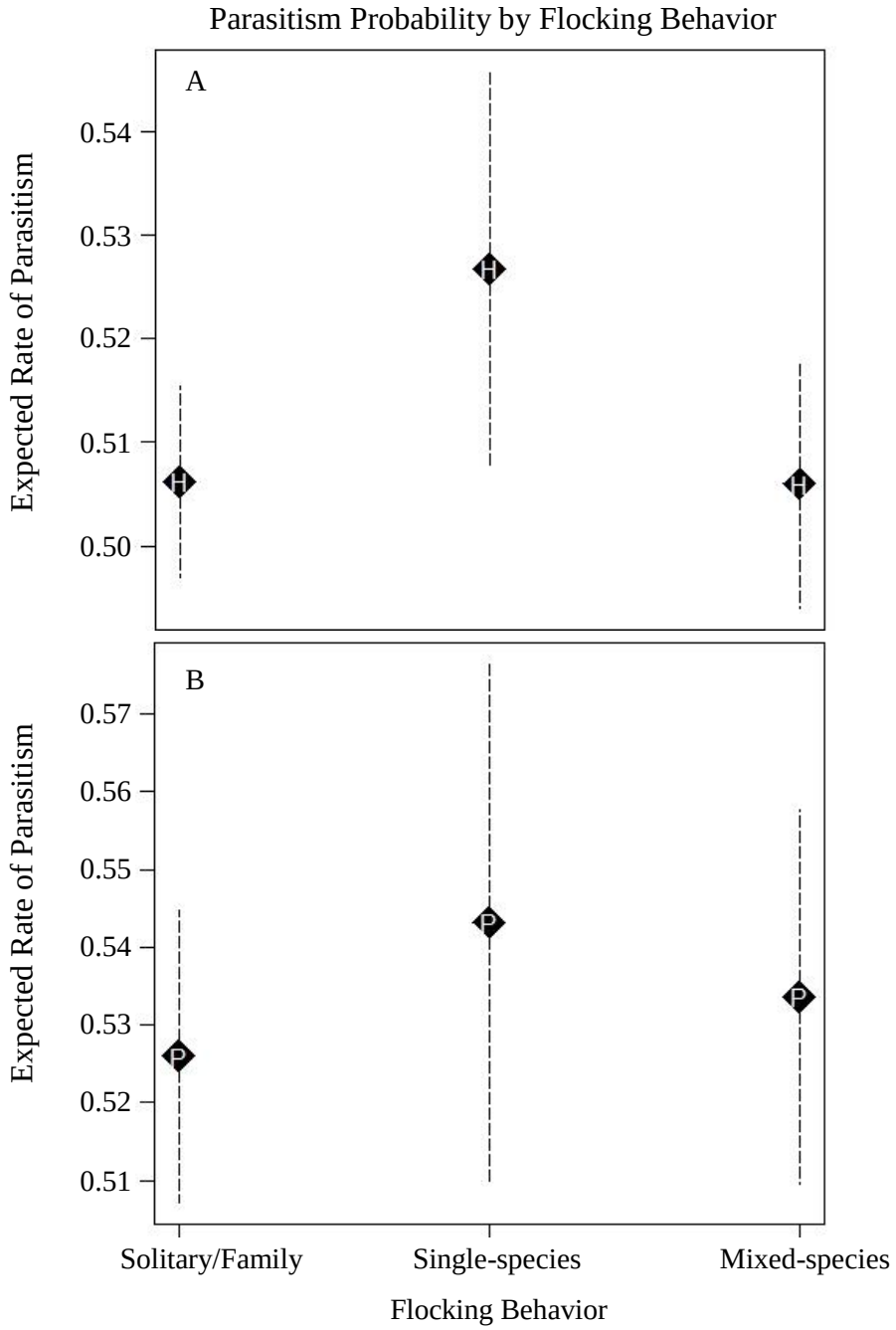


Figure 20. Predicted (least-square means) probabilities of parasitism by flocking behavior for (A) *Haemoproteus* and (B) *Plasmodium* within the Belém area of endemism and associated 95% confidence intervals.

In Guiana the best candidate model for *Haemoproteus* prevalence included only nesting height, whereas for *Plasmodium* the best candidate model included only foraging height (Table 10). For both haemosporidian genera in Guiana host family had a

significant constraint on prevalence (Table 11), and foraging height was the best supported explanatory variable (Table 12). For *Haemoproteus*, nest height significantly predicted parasitism (Table 13), with cliff/bank nesters having significantly higher probability of *Haemoproteus* infection (Figure 21A). However, since there was only one *Haemoproteu*s infection found in Guiana (Table 29) these results must be taken with caution. Foraging height significantly predicted *Plasmodium* parasitism probability (Table 14), with significantly higher infection probability for ground foraging birds (Figure 22B).

In Imerí the best candidate model for *Haemoproteus* prevalence included only nesting height, whereas for *Plasmodium* the best candidate model included only foraging height (Table 10). There were no significant host phylogenetic constraints for either genus (Table 11). Nest height was the best supported explanatory variable for *Haemoproteus*, and foraging height was best supported for *Plasmodium* (Table 12). There were no significant predictors for *Haemoproteus* prevalence (Table 13), potentially due to only two positive samples for this parasite genus (Table 29). Foraging height significantly predicted *Plasmodium* parasitism probability (Table 14), with significantly higher probability of infection for ground foraging birds (Figure 22C).

In Inambari flocking behavior was the best candidate model for *Haemoproteus* prevalence, where for *Plasmodium* nest type was the best candidate model (Table 10). There was not any significant host phylogenetic effect in *Haemoproteus,* yet host family had a significant constraint on *Plasmodium* prevalence (Table 11). Flocking behavior and nest type were the best supported explanatory variables for *Haemoproteus* and *Plasmodium* respectively (Table 12). There were no significant predictors for

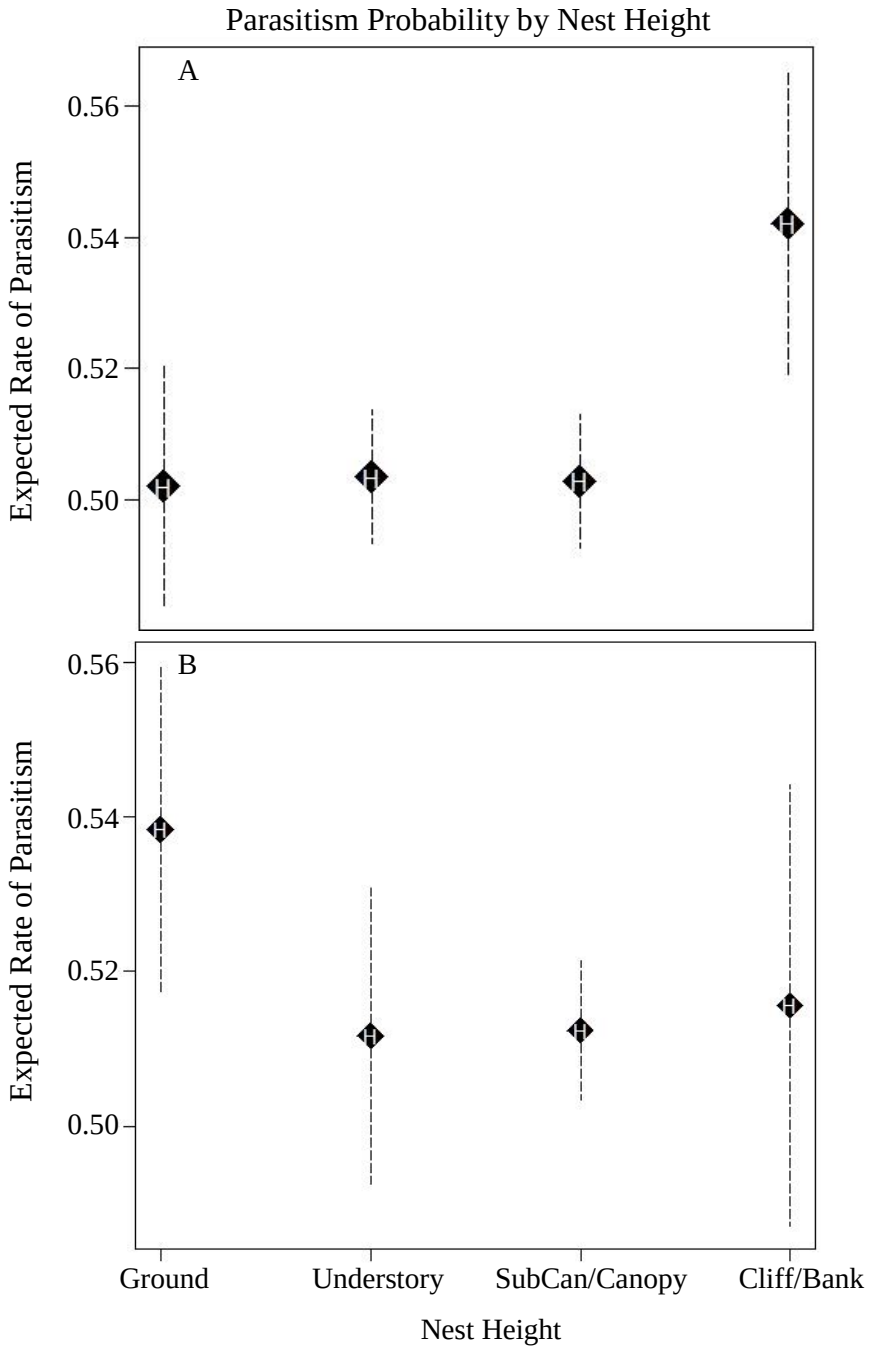


Figure 21. Predicted (least-square means) probabilities of *Haemoproteus* parasitism by nest height in the (A) Guiana area of endemism and the (B) Rondônia area of endemism and associated 95% confidence intervals.

*Haemoproteus* prevalence (Table 13). Nest type significantly predicted *Plasmodium* parasitism probability in Inambari (Table 14), with significantly lower probability of infection for open cup nesters and cavity nesters showing the highest parasitism

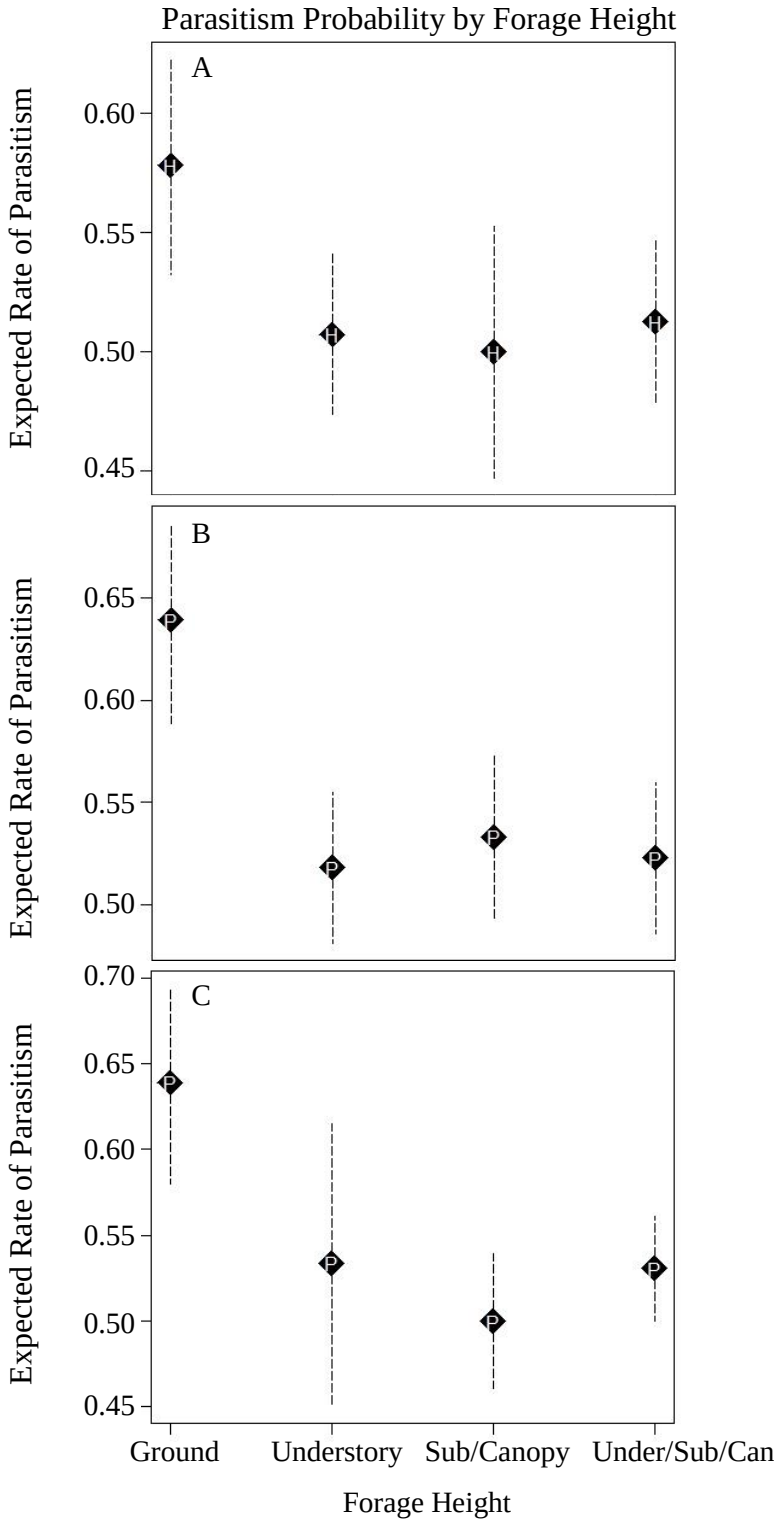


Figure 22. Predicted (least-square means) probabilities of parasitism by forage height in (A) *Haemoproteus* from the Tapajós area of endemism, (B) *Plasmodium* from the Guiana, area of endemism, and (C) *Plasmodium* from the Imerí area of endemism and associated 95% confidence intervals.

probability (Figure 23).

In Rondônia the best candidate model for *Haemoproteus* prevalence included nest height and flocking behavior, whereas for *Plasmodium* the best candidate model included flocking behavior alone (Table 10). There was no significant host phylogenetic effect for *Haemoproteus*, whereas host family had a significant influence on *Plasmodium* prevalence (Table 11). Nest height was the best supported explanatory variable for *Haemoproteus*, and flocking behavior was best supported for *Plasmodium* (Table 12). Nest height significantly predicted *Haemoproteus* parasitism probability (Table 13), with significantly higher probability of infection for ground nesting birds (Figure 22C). There were no significant predictors for *Plasmodium* prevalence (Table 14).

In Tapajós foraging height was the best candidate model for *Haemoproteus* prevalence, whereas flocking behavior was the best candidate model for *Plasmodium* (Table 10). There were no significant host phylogenetic constraints for either genus (Table 11). Foraging height was the best supported explanatory variable for *Haemoproteus*, and flocking behavior was best supported for *Plasmodium* (Table 12). Foraging height significantly predicted *Haemoproteus* parasitism probability (Table 13), with significantly higher probability of infection for ground foraging birds (Figure 22A). There were no significant predictors for *Plasmodium* prevalence (Table 14).

In comparing all analyses for *Haemoproteus*, nest height (Guiana, Rondônia) foraging height (Tapajós), and flocking behavior (Belém) signficantly predicted parasitism probability (Figures 20, 21, 22, Table 13). Probability of infection by *Haemoproteus* was higher for birds that formed single-species flocks in Belém (Figure 20), nested in cliff/banks in Guiana (Figure 21), nested on the ground in Rondônia

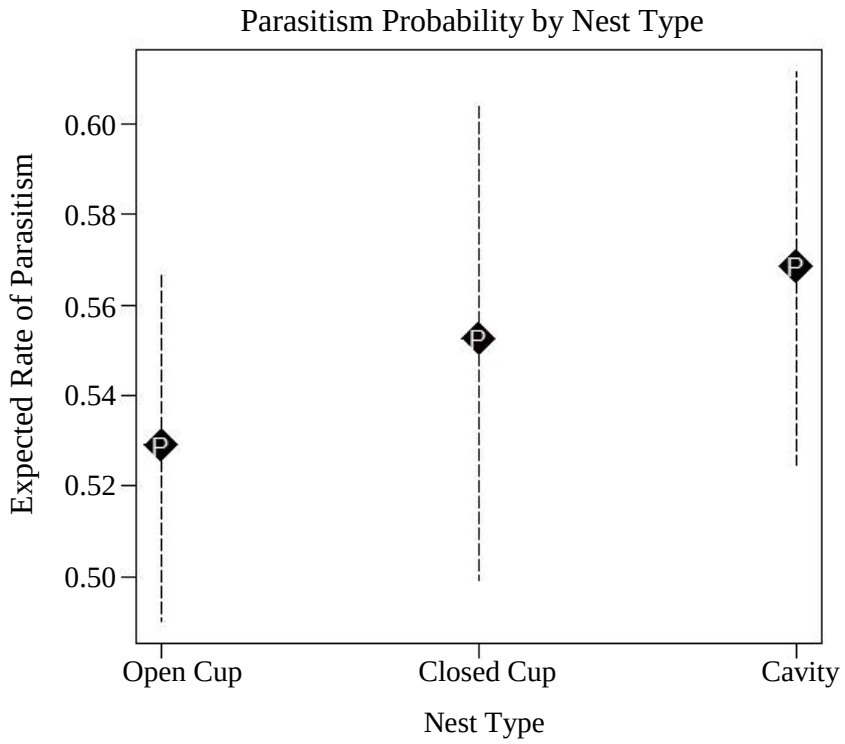


Figure 23. Predicted (least-square means) probabilities of *Plasmodium* parasitism by nest type in the Inambari area of endemism and associated 95% confidence intervals.

(Figure 21), or foraged on the ground in Tapajós (Figure 22). Host family had a significant phylogenetic constraint on *Haemoproteus* prevalence for all Amazonian samples (Table 7) and in Guiana (Table 11). Host species was a significant constraint in Belém (Table 11), while only marginally significant ($p = 0.06$) for all Amazonian samples (Table 7).

For *Plasmodium*, area of endemism (all Amazonian samples), nest type (Inambari), foraging height (Guiana, Imerí), and flocking behavior (Belém) signficantly predicted parasitism probability (Figures 19, 20, 22, 23, Tables 9, 14). Probability of infection by *Plasmodium* was higher for birds that lived in Belém, Rondônia, or Tapajós (Figure 19), formed single-species flocks in Belém (Figure 20), nested in closed cups or cavities in Inambari (Figure 23), or foraged on the ground in Guiana and Imerí (Figure 22). *Plasmodium* parasitism probability was significantly lower for birds living in Guiana

(Figure 19) or nested in open cup nests in Inambari (Figure 23). Host family had a significant phylogenetic constraint on *Plasmodium* prevalence for all Amazonian samples (Table 7) and in Belém, Guiana, Inambari, and Rondônia (Table 11). Host species was a significant constraint only for all the combined Amazonian samples analyzed together (Table 7).

## Discussion

Avian life history characteristics can influence haemosporidian parasitism rates (Ricklefs 1992, Young *et al.* 1993, Tella 2002, Fecchio *et al.* 2011, 2013, Svensson-Coelho *et al.* 2013, González *et al.* 2014, Lutz *et al.* 2015, Matthews *et al.* 2016). The mechanism behind these effects is hypothesized to be differential exposure to suitable vectors due to host life history variation. Association with habitats that harbor more suitable vectors can increase haemosporidian prevalence (van Riper *et al.* 1986, Super and van Riper 1995, Tella *et al.* 1999, Mendes *et al.* 2005, Ejiri *et al.* 2008, Hellgren *et al.* 2008, Svensson and Ricklefs 2009, Yohannes *et al.* 2009, González *et al.* 2014, Krama *et al.* 2015). Since vector abundance is vertically stratified (Bennett and Fallis 1960) with *Plasmodium* vectors primarily distributed near the ground and *Haemoproteus* vectors distributed in midstory and canopy regions (Garvin and Greiner 2003, Swanson and Adler 2010, Cerńy *et al.* 2011, Lassen *et al.* 2012, Swanson *et al.* 2012) variation in life history that vertically stratifies hosts, such as nest and foraging height, can alter the risk of parasitism.

Vectors rely on a number of chemical and visual cues to locate hosts (Khan 1977, Takken 1991, Muir *et al.* 1992, Bidlingmayer 1994, Bernier *et al.* 1999). Thus life history characteristics that alter these cues can affect host-vector encounter rates (Withers 1978,

Wickler and Marsh 1980, Gibson and Torr 1999). Mosquitoes generally rely on chemical cues produced by hosts (kairomones) such as ammonia, 1-octen-3-ol, and $CO_2$ (Gibson and Torr 1999, Logan *et al.* 2010). Life history variation that increases kairomone accumulation, such as nesting and flocking behavior, may increase mosquito attraction and the potential for *Plasmodium* transmission. Visual cues are thought to be more important than kairomones in the host seeking behavior of biting midges (Muller 1991, Bishop 2002, Bishop *et al.* 2008). Therefore behaviors that increase visual cues for host seeking midges may increase *Haemoproteus* transmission. Although the majority of *Haemoproteus* parasites are transmitted by biting midges (*Culicoides*), a small group within the sub-genus *Haemoproteus* are transmitted by hippoboscid flies (Valkiūnas 2005). The impact of host life history on these hippoboscid flies is not well known, although these vectors are generally host specific and do not travel long distances (Petersen *et al*. 2007). Therefore it is less likely that host life history variation would be a major factor in variation in transmission of *Haemoproteus* parasites by hippoboscid flies.

In Amazonia, the unique biogeography and its expected effects on avian and vector communities constrains the ability to detect parasitism effects due to host life history. For Amazonian samples, area of endemism was the only variable that could significantly predict parasitism, but only for *Plasmodium.* The impact of area of endemism on avian and vector communities is the most likely factor that explains these results, but the exact mechanisms are unknown. Area of endemism was the strongest, albeit not significant, predictor variable for *Haemoproteus*, which may be due to either generally low *Haemoproteus* prevalence, higher host specificity (Beadell et al. 2004, 2009, Valkiūnas 2005, Ishtiaq *et al.* 2007, 2010, Dimitrov *et al*. 2010), or unique

attributes of the *Haemoproteus* vector communities which are yet unknown. Each area of endemism supports a unique avian community (Cracraft 1985, Silva *et al.* 2002, 2005, Wesselingh *et al.* 2009) and most likely a unique vector community, so the most appropriate way to understand the impact of life history on parasite prevalence in Amazonia was to treat each area of endemism separately.

Nest height predicted infection probability for *Haemoproteus* in Guiana and Rondônia. Probability of infection was highest for cliff/bank nesters in Guiana and ground nesters in Rondônia. Since there was only one *Haemoproteus* infection in Guiana these results do not truly represent life history affects but rather are an artifact of lack of *Haemoproteus* infections. The results from Rondônia are opposite of what has been previously reported elsewhere. For instance, *Haemoproteus* parasitism rates were highest for mid canopy nesters in Tennessee (Matthews *et al.* 2016) and Colombia (González *et al.* 2014) and canopy nesters in Malawi (Lutz *et al.* 2015). Lutz *et al.* (2015) actually found the lowest infection probability for ground nesting hosts. High infection probability for *Haemoproteus* for ground nesting birds is opposite of what would be expected based on biting midge stratification, with higher vector abundance above the ground (Garvin and Greiner 2003, Swanson and Adler 2010, Cerńy *et al.* 2011, Lassen *et al.* 2012, Swanson *et al.* 2012). Work in other areas has shown no impact of nest height on haemosporidian prevalence (Garvin and Remsen 1997, Ricklefs *et al.* 2005, Fecchio *et al.* 2013, Svensson-Coelho *et al.* 2013). These contrasting patterns do not support a general pattern of nest height impact on haemosporidian prevalence, but rather that unique ecological, behavioral, or geographical factors most likely impact parasitism by altering host-vector encounter rates.

Nest type predicted the probability of *Plasmodium* infection in Inambari, with birds that nest in open cup nests having the lowest infection probability, with higher probability for closed cup and cavity nesting birds. Similar results for *Plasmodium* have been shown for both the Brazilian Cerrado (Fecchio *et al.* 2011), Colombia (González *et al.* 2015), and Malawi (Lutz *et al.* 2015). Increase in kairomones in closed cup and cavities (Withers 1978, Wickler and Marsh 1980, Gibson and Torr 1999) may explain the higher parasitism rates, as they may increase mosquito encounter rates. However, open cup nests with presumably lower kairomone concentration have been shown to increase *Plasmodium* parasitism rates (Ribeiro *et al*. 2005, González *et al.* 2014, Matthews *et al.* 2016). Ribeiro *et al.* (2005) suggested that vectors would come into contact with species that nest in open-cup nests more often due to increased exposure, making them more susceptible to transmission. The association between nest type and haemosporidian prevalence is most certainly more complex, and would include host susceptibility, host defense behaviors, and variation in other host life characteristics. For many hosts in Amazonia such information is lacking, making more detailed analyses difficult if not impossible. A detailed understanding of variations in nesting and vector defense behaviors may uncover the mechanisms involved in nest type effects on haemosporidian parasitism.

Foraging height predicted parasitism probability for *Haemoproteus* in Tapajós and *Plasmodium* in Guiana and Imerí. In all cases the probability of infection was higher for ground foraging birds. Higher mosquito abundance closer to the ground (Garvin and Greiner 2003, Swanson and Adler 2010, Cerńy *et al.* 2011, Lassen *et al.* 2012, Swanson *et al.* 2012) may explain the results for *Plasmodium*. However, the results from some

other areas do not support higher rates of *Plasmodium* parasitism for ground foraging hosts (Astudillo *et al*. 2013, Svensson-Coelho *et al.* 2013, Matthews *et al*. 2016). This discrepancy may be due to habitat or climatic variations and their impact on host and vector communities. Higher rates of *Haemoproteus* parasitism in ground foragers is in conflict with the known stratification of biting midges (Garvin and Greiner 2003, Swanson and Adler 2010, Cerńy *et al.* 2011, Lassen *et al.* 2012, Swanson *et al.* 2012) and the results from others that have shown higher parasitism rates for mid-level foraging hosts (Astudillo *et al*. 2013, González *et al.* 2014, Matthews *et al*. 2016). It is possible that biting midges in Amazonia show different stratification patterns as supported by higher probability of *Haemoproteus* infection for ground nesting birds in Rondônia. Additionally, as with nesting height, the association between vertical stratification and haemosporidian parasitism most certainly involves many interrelated factors and warrants further study.

Flocking is known to increase transmission of both contact transmitted (Poulin 1991, Pennycott *et al.* 2002, Ellis *et al.* 2004) and vector transmitted (Brown *et al.* 2001, Fecchio *et al.* 2011 2013, González *et al.* 2014, Lutz *et al.* 2015) pathogens. In Belém, flocking behavior predict parasitism rates for both *Haemoproteus* and *Plasmodium*, with higher rates for species that formed single-species flocks. Single-species flocks have shown high rates of *Haemoproteus* parasitism in the Brazilian Cerrado (Fecchio *et al.* 2011, 2013) and Malawi (Lutz *et al.* 2015). However, *Haemoproteus* parasitism rates were equally high in mixed-species flocks in the Brazilian Cerrado (Fecchio *et al.* 2011, 2013) and higher in mixed-species flocks than in single-species flocks in Colombia (González *et al.* 2014). Lutz *et al.* (2015) found higher *Plasmodium* parasitism rates for

species in mixed flocks, and others have found no effect of flocking behavior on *Plasmodium* parasitism (González *et al.* 2014, Matthews *et al.* 2016). The higher parasitism rates found for single-species flocks in this study may be a consequence of peculiarities of haemosporidian transmission in Amazonia. Host switching is an important mechanism in avian haemosporidian transmission with switching among closely related hosts occurring commonly during the evolutionary history of these parasites (Ricklefs and Fallon 2002, Ricklefs *et al.* 2004, Križanauskiené *et al.* 2006, Ricklefs *et al.* 2014, Ellis *et al.* 2015). Host switching between closely related hosts should facilitate transmission due to similarities in host immune defenses (Woolhouse *et al.* 2005, Poulin 2011). Therefore, the influence of flocking behavior on haemosporidian prevalence may be related to the phylogenetic relationship between flock members. In Amazonia, host phylogeny significantly correlated with the prevalence of both *Plasmodium* and *Haemoproteus*, with phylogenetic effects at both the family and species level. This underlying host phylogenetic effect on haemosporidian parasitism may be responsible for the higher rates of parasitism in single-species flocks than in mixed-species flocks that are composed of more phylogenetically distant members.

Host life history characteristics of Amazonian birds have impacts on haemosporidian prevalence, when host communities in each area of endemism are analyzed individually. Host-vector encounter rates are thought to be the main mechanism driving variations in haemosporidian prevalence across host life history characteristics, however few studies have related the distribution of haemosporidian parasites directly to host-vector encounter rates (Gager *et al.* 2008, Hellgren *et al.* 2008, Medeiros *et al.* 2013, Carlson *et al.* 2015). Associations between vector and host populations alone cannot

solely explain haemosporidian prevalence. It is more likely to be explained by host compatibility mechanisms involving differential susceptibility to different vector species (Gager *et al*. 2008, Medeiros *et al*. 2013, 2015). Additional research on all aspects of vector biology within Amazonia is needed to determine the relationships between vectors, their avian hosts, and haemosporidian parasite transmission. For analyzing the impact of host life history on avian haemosporidian prevalence without specifically measuring differences in vector exposure and host susceptibility will fail to explain the complex patterns of avian haemosporidian transmission within Amazonia.

# CHAPTER VI

# COEVOLUTIONARY HISTORY OF AVIAN HAEMOSPORIDIANS AND THEIR HOSTS FROM GURUPI, BRAZIL

## Results

Fifty eight total haemosporidian infections, 10 *Haemoproteus*, 47 *Plasmodium*, and one *Haemoproteus/Plasmodium* coinfection, were found among the 323 samples collected in Gurupi (Table 18). These 58 infections represented 48 unique genetic lineages, 9 *Haemoproteus* (Table 31) and 39 *Plasmodium* lineages (Table 32). Most lineages were only recovered from a single sample, making it impossible to calculate host specificity indices. Host specificity indices, $S_{TD}$*, could only be determined for three lineages of *Haemoproteus* and sixteen *Plasmodium* lineages. The mean $S_{TD}$* was 2.85 for *Haemoproteus* and 2.44 for *Plasmodium* (Tables 31, 32). Due to the large sample size disparity, $S_{TD}$* values for the two genera were not compared statistically.

Bayesian consensus host and parasite trees were used to construct a tanglegram showing host-parasite associations (Figure 24). These associations were used to construct cost-event analyses (CoRe-PA), using the events of codivergence, duplication, sorting (extinction), and host switching. Based on 100 randomizations of host-parasite associations, total event costs between 39 and 108 were statistically well supported (Table 5). These analyses support a coevolutionary history dominated by host switching with occasional codivergence and duplication, with sorting (extinction) having far lesser influence on the coevolutionary pattern. When host switching was made very costly (event costs 0213 and 0012) it was still identified as the most frequent mechanism within

Table 31. *Haemoproteu*s lineages used in cophylogeny analysis including host specificity index $S_{TD}$*.

| Parasite Code | Lineage Name | Hosts | $S_{TD}$* |
|---|---|---|---|
| H1 | TACCRI01 | *Tachyphonus luctuosus* | 1.16 |
| H2 | COLPAS06 | *Columbina passerina* | - |
| H3 | COLPAS03[a] | *Columbina passerina, Thamnophilus doliatus* | 4.00 |
| H4 | TACCRI03 | *Tachyphonus cristatus* | - |
| H5 | THAFUR01 | *Thalurania furcata* | - |
| H6 | PSABIF02 | *Psarocolius bifasciatus* | - |
| H7 | CAMRUB01 | *Campephilus rubricollis* | - |
| H8 | CAMRUB02 | *Campephilus rubricollis* | - |
| H9 | COLPAS04 | *Myiophobus fasciatus* | 3.38 |
| | | Mean | 2.85 |

[a] *Haemoproteus paramultipigmentatus*

the coevolutionary history of haemosporidians and their avian hosts (Table 5).

Within both *Haemoproteus* and *Plasmodium* host switches occurred most frequently at higher taxonomic levels (family, order). Host switches above the genus level occurred twice as frequently as those at the level of host genus (Figure 24). There were as many switches between hosts of different orders, as there were between hosts within different genera. No host switches occurred between hosts within the same genus (Figure 24).

Global cophylogenetic analysis (PaCO) detected a significant global signal of cospeciation between haemosporidians and their avian hosts ($m^2_{xy} = 0.89$, $p < 0.001$). The global cospeciation signal is mostly due to host-parasite links involving: 1) *Haemoproteus* lineages infecting *Campephilus rubricollis, Columbina passerina, Tachyphonus cristatus,* and *Tachyphonus luctuosus,* 2) *Plasmodium* lineages infecting various Thamnophilidae host species, most notably all lineages found in *Formicivora grisea* and *Willisornis poecilinotus*, 3) *Plasmodium* lineage PADOM11 infecting *Coereba flaveola* and *Tachyphonus rufus* (Figure 25). Overall *Haemoproteus* had a stronger parasite cospeciation signal than *Plasmodium*, with 66.7% (6 of 9) of

*Haemoproteus* lineages having squared residuals well below the median (Figure 25).

Table 32. *Plasmodium* lineages used in cophylogeny analysis including host specificity index $S_{TD}$*.

| Parasite Code | Lineage Name | Hosts | $S_{TD}$* |
|---|---|---|---|
| P1 | THAAMA01 | *Thamnophilus amazonicus* | - |
| P2 | DYSMEN01 | *Dysithamnus mentalis* | - |
| P3 | THACAE01 | *Dysithamnus mentalis* | 2.30 |
| P4 | THAMAE01 | *Phlegopsis nigromaculata* | 2.23 |
| P5 | MYRAXI03 | *Myrmotherula axillaris* | 1.00 |
| P6 | XENMIN03 | *Xenops minutus* | - |
| P7 | PHLNIG03 | *Phlegopsis nigromaculata, Thamnophilus aethiops* | - |
| P8 | THACAE08 | *Thamnomanes caesius* | 1.00 |
| P9 | WILPOE15 | *Piaya cayana, Piprites chloris, Willisornis poecilinotus, Xiphorhynchus elegans* | 3.65 |
| P10 | PICFLA01 | *Piculus flavigula* | 1.00 |
| P11 | MYRAXI09 | *Myrmotherula axillaris* | - |
| P12 | WILPOE16 | *Willisornis poecilinotus* | - |
| P13 | THAAET01 | *Thamnophilus aethiops* | - |
| P14 | PHIERY01 | *Philydor erythrocerum* | - |
| P15 | PHIERY02 | *Philydor erythrocerum* | - |
| P16 | MYITYR01 | *Campylorhynchus turdinus, Rhytipterna simplex* | 2.94 |
| P17 | WILPOE17 | *Poecilotriccus sylvia, Willisornis poecilinotus* | 3.00 |
| P18 | WILPOE18 | *Willisornis poecilinotus* | - |
| P19 | PYRLEU03 | *Pyriglena leuconota* | - |
| P20 | PSABIF01 | *Psarocolius bifasciatus* | - |
| P21 | MICMIN01 | *Micrastur gilvicollis* | - |
| P22 | CERCIN01 | *Cercomacra cinerascens* | - |
| P23 | PADOM09[a] | *Pheugopedius coraya, Tachyphonus cristatus* | 2.82 |
| P24 | RAMCAR01 | *Ramphocelus carbo, Thraupis episcopus* | 2.00 |
| P25 | PADOM11 | *Coereba flaveola, Tachyphonus rufus* | 2.32 |
| P26 | TACRUB04 | *Tachyphonus rufus* | 3.00 |
| P27 | ARAJAN01 | *Aratinga jandaya* | - |
| P28 | VOLJAC03 | *Poliptila guianensis* | 3.00 |
| P29 | AUTPAR01 | *Rhynchocyclus olivaceus* | 2.80 |
| P30 | PYRLEP01 | *Pyrrhura lepida* | - |
| P31 | GRW06[a] | *Sporophila americana* | 3.00 |
| P32 | SPOAME01 | *Sporophila americana* | - |
| P33 | PYRLEP02 | *Pyrrhura lepida* | - |
| P34 | ATTCIN01 | *Attila cinnamoneus* | - |
| P35 | PACRUF01 | *Pachyramphus rufus* | - |
| P36 | FORGRI01 | *Formicivora grisea* | - |
| P37 | FORGRI02 | *Formicivora grisea* | - |
| P38 | TOFLA01 | *Tolmomyias flaviventris* | 3.00 |
| P39 | FORGRI03 | *Formicivora grisea* | - |
| | | Mean | 2.44 |

[a] *Plasmodium elongatum*

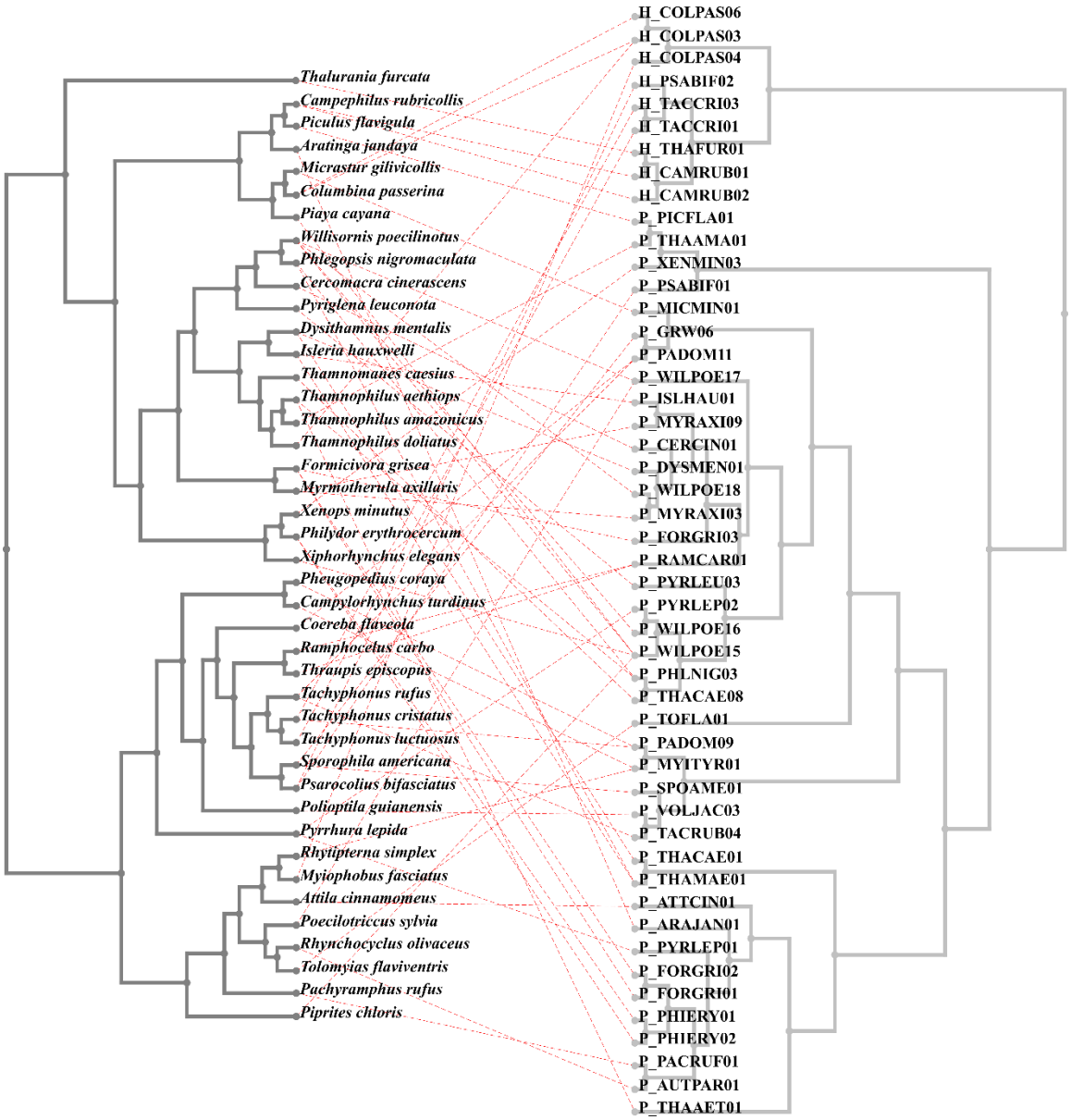


Figure 24. Tanglegram showing associations between haemosporidian parasites and their avian hosts from the Belém area of endemism. Bayesian majority-rule consensus trees produced in Beast. For haemosporidian parasites lineage names are given, see Appendix A for complete information on lineages. The letter in front of each lineage denotes the parasite genus, *Haemoproteus* (H) or *Plasmodium* (P).

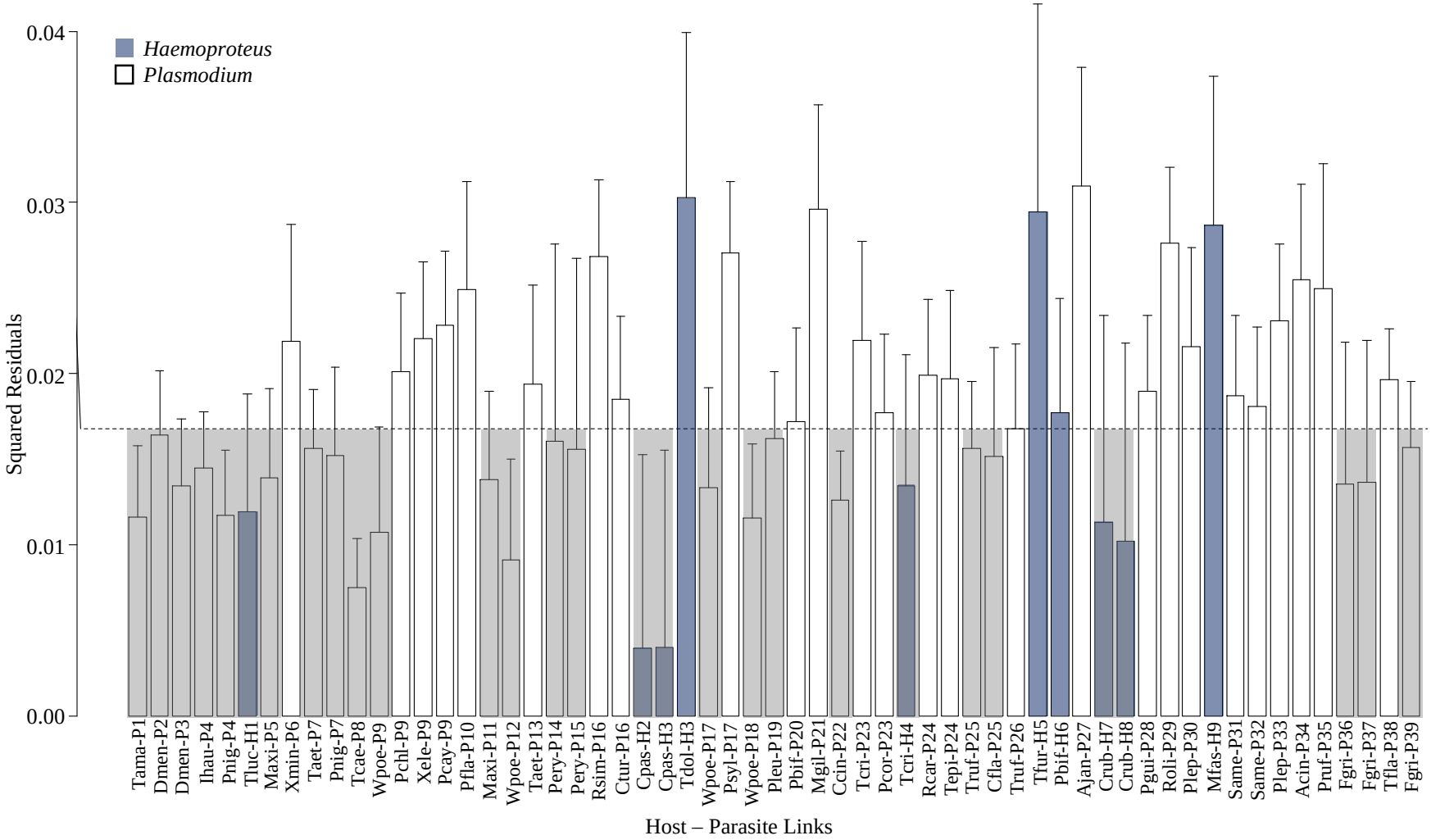


Figure 25. Jackknifed squared residuals and upper 95% confidence intervals showing the contribution of individual host-parasite links to the global cospeciation fit. The median squared residual value is shown (dotted line) for comparison. Links with low squared residuals likely represent coevolutionary relationships (gray boxes). For explanation of host and parasite codes see Tables 4, 31, 32

## Discussion

Host switching is an important evolutionary mechanism in avian haemosporidians with closely related haemosporidian lineages conserved within higher host taxa (Bensch *et al.* 2000, Ricklefs and Fallon 2002, Waldenström 2002, Ricklefs *et al.* 2004, Križanauskiené *et al.* 2006, Ricklefs *et al.* 2014, Ellis *et al.* 2015). Dispersal followed by isolation and specialization in a particular host can lead to host switching which involves the formation of new haemosporidian lineages after dispersal (Zarlenga *et al.* 2006, Janz and Nylin 2007, Waltari *et al.* 2007, Hoberg and Brook 2008, Loiseau *et al.* 2012b, Santiago-Alarcon *et al*. 2014, Ricklefs *et al*. 2014). Ricklefs *et al.* (2014) postulates that species formation is predominantly allopatric involving host expansion (dispersal) followed by secondary sympatric speciation due to host-parasite coevolution leading to reproductive incompatibility between closely related haemosporidian lineages. This would shift parasite lineage across hosts and increase local parasite diversity (Ricklefs *et al*. 2014).

Host switching was the most frequent event in the evolutionary history of avian haemosporidians in Gurupi, regardless of event costs. Only when host switching was made costly did the other evolutionary events (codivergence, duplication, sorting) increase in prevalence, codivergence being the second most common event. Ricklefs *et al.* (2004) found duplication (within host speciation) to be a frequent event for avian haemosporidians when event costs were low, but this is not supported by the data from Gurupi. Duplication was not affected by differences in the duplication event costs, and only increased when host switching was costly. The dominance of host switching in Gurupi, occurring at higher taxonomic levels both matches what is known for

evolutionary history of avian haemosporidians (Ricklefs *et al.* 2014, Ellis 2015) and supports similar analyses on *Haemoproteus* lineages (Galen and Witt 2014, Santiago-Alarcon *et al.* 2014). Galen and Witt (2014) found that *Haemoproteus* lineages that infected Andean house wrens (*Troglodytes aedon*) in Peru diversified by host switches between distantly related avian species within this region. In *Plasmodium* the generally poor matching of host and parasite phylogenies is attributed to the high proportion of host switching compared to other evolutionary events (Ricklefs and Fallon 2004, de Vienne *et al.* 2013, Lauron *et al.* 2015).

When barriers do not prevent haemosporidians from switching hosts, lineages can infect distantly related hosts (Levin *et al.* 2011, Ricklefs *et al.* 2014). Even extremely phylogenetically distant hosts can become infected, as seen in the successful infection of mice with avian *Plasmodium lophurae* (McGhee 1951) and the susceptibility of erythrocytes from several mammalian species to avian *Plasmodium* parasites (McGhee 1957). Since most vector species are not sufficiently specialized to prevent gene flow (Gager *et al.* 2008, Hamer *et al.* 2008, 2009, Medeiros *et al.* 2013) and often come in contact with a diverse array of haemosporidian parasites (Martinez-de la Puente *et al.* 2011, Santiago-Alarcon *et al.* 2012a, 2012b, Medeiros *et al.* 2013, Valkiūnas *et al.* 2013) host switching is promoted while cospeciation between avian hosts and their haemosporidian parasites is reduced. In the absence of host switching opportunities resulting from behavioral or geographic host isolation, cospeciation between avian haemosporidians and their hosts may become more likely (Desdevises *et al.* 2002, Desdevises 2007).

The global cospeciation analysis detected significant cospeciation signal among avian haemosporidian parasites and their hosts from Gurupi, which goes against the generally accepted evolutionary history of these parasites (Ricklefs *et al.* 2014). Taken together with the presence of codivergence (cospeciation) events, the global cospeciation analysis supports cospeciation as an important factor in the diversification of haemosporidians from Gurupi. The significant cospeciation signal was due mainly to avian hosts and *Haemoproteus* lineages, especially those infecting non-passerine hosts (*Campephilus rubricollis* and *Columbina passerina*). Santiago-Alarcon *et al.* (2014) found a similar strong cospeciation signal in *Haemoproteus* lineages infecting non-passerine hosts.

The strongest cospeciation signal was found between two lineages of *Haemoproteu*s that infected *Columbina passerina.* These lineages belong to the sub-genus *Haemoproteus,* which are highly host and vector specific, confined to the host families Columbidae, Frigatidae, and Laridae (Valkiūnas 2005, Levin *et al.* 2011, 2012) and only transmitted by hippoboscid flies (Valkiūnas 2005). Although hippoboscid flies are thought to be highly host specific due to limited dispersion ability (Petersen *et al.* 2007), recent work in the Galapagos Islands (Santiago-Alarcon *et al.* 2010, Valkiūnas *et al.* 2010, Levin *et al*. 2011) has shown hippoboscid fly species sharing endemic dove and sea bird hosts. The evolutionary history of hippoboscid flies also includes at least two host switches from mammals to birds (Petersen *et al.* 2007). Therefore it is more likely that the coevolution between parasites, hosts, and vectors has led to reproductive isolation of parasites of the sub-genus *Haemoproteus*. Ookinete structure is markedly different between *Haemoproteus* parasites that are transmitted by hippoboscid flies (sub-genus

*Haemoproteus*) and those transmitted by *Culicoides* (sub-genus *Parahaemoproteus*), which along with internal environmental differences in these two vector groups restricts vector usage of these two sub-genera (Valkiūnas 2005). These vector restrictions have diminished the host range for hippoboscid transmitted *Haemoproteus* parasites, allowing cospeciation to occur between parasites and their avian hosts. Even when these parasites infect passerine hosts they do not develop past the tissue stage, representing abortive infections (Valkiūnas 2005, Valkiūnas *et al.* 2013).

Due to high levels of host switching and dispersal, cospeciation is not thought to have played a large role in the evolutionary history of *Plasmodium* (Ricklefs and Fallon 2004, de Vienne *et al.* 2013, Lauron *et al.* 2015). However, the results of this study showed significant cospeciation within lineages of *Plasmodium* that infect species within the avian families of Thamnophilidae and Thraupidae. Thamnophilidae and Thraupidae are highly diverse families within South America, with Thamnophilidae being endemic to the Neotropics (Ridgely and Tudor 1989a, 1989b). This high host diversity may have allowed for cospeciation between specific host species and their *Plasmodium* parasites. Hyper-diverse regions like Amazonia are ideal systems to study coevolutionary patterns between parasites and hosts, and the results of this study support a strong coevolutionary history between avian hosts and their haemosporidian parasites in Amazonia.

# CHAPTER VII

# CONCLUSIONS

The diversity of tropical ecosystems is widely recognized for a wide range of taxa including many different parasite groups. However, we are only starting to understand haemosporidian diversity and related host-parasite relationships in the tropics due to historical sampling bias towards the temperate regions of North America and Europe. Based on data from various regions, avian haemosporidian diversity should be function of avian and vector host diversity, both of which are high in tropics. This study represents the one of largest sampling efforts within the tropics, with 4521 avian samples collected from throughout Brazil, and the first large scale sampling of the Brazilian Amazon. From the results of this study, specific conclusions can be drawn, as detailed below, on the haemosporidian communities of this hyper diverse region.

**Conclusion 1**

Brazil supports a diverse community of avian haemosporidians, with 365 unique haemosporidian lineages found in samples from 447 avian host species. No other study has described as many lineages from one region, the next largest being the 248 lineages reported from Malawi. Not only is this community diverse, it is also highly endemic, with 331 lineages (more than 90%) described for the first time. This study builds on the growing data set of South American avian haemosporidians and also increases the known worldwide diversity of avian haemosporidian genetic lineages by more than 15 percent.

**Conclusion 2**

Geographic barriers are known to affect haemosporidian host specificity by limiting the movement of specialist parasites. The river tributaries that delineate areas of endemism seem to have had the same affect in Amazonia, creating its diverse and endemic haemosporidian community containing many host generalist lineages. The evolutionary history of haemosporidians in Brazil shows multiple instances of lineage introduction followed by speciation. In Amazonia these introduced lineages represent host specialists that through host switching diverged into many endemic generalist lineages. The data from the Brazilian Amazon shows this same evolutionary pattern with dispersal between areas of endemism being the main type of event in the phylogeographical history of avian haemosporidians. Within the Brazilian Amazon specialist lineages are generally confined to individual areas of endemism.

**Conclusion 3**

The long geological history of Amazonia's eight areas of endemism has shaped its avian communities. Each area of endemism supports a unique avian community that is more similar to areas outside of Amazonia then to adjacent Amazonian areas of endemism. The areas of endemism essentially serving as islands, isolated by major river tributaries of the Amazon River. The dispersal between these areas of endemism serving as the major speciation force in Amazonian birds. Island biogeography is known to affect avian haemosporidian community structure and distribution and similar patterns were found in Amazonia, with areas of endemism affecting community structure, genetic diversity, and phylogeny of haemosporidian parasites. As avian hosts dispersed and diversified within Amazonia, their haemosporidian parasites did as well as they switched

amongst diverse avian hosts. Host switching being the major force within haemosporidian diversification as shown for the Belém area endemism, and certainly throughout Amazonia.

**Conclusion 4**

Niche partitioning within rich tropical ecosystems most certainly aided in avian speciation through life history diversification between related host species. This variation in host life history can influence haemosporidian parasitism, by altering host-vector encounter rates. In Amazonia host life history could predict rates of haemosporidian parasitism, although the importance of any specific characteristic was not universal, differing between areas of endemism. Although host life history impacts haemosporidian parasitism, it is a local effect, restricted to specific areas of endemism and not universal across the whole Amazonia, again showing the overriding importance of the biogeography of this region.

**Conclusion 5**

The Miocene formation of Amazonian areas of endemism provided sufficient evolutionary time for cospeciation to occur between some specialist lineages and their avian hosts from the Belém area of endemism (the only one for which such an analysis was conducted). The cospeciation signal seen within some *Plasmodium* lineages indicates the existence of unique coevolutionary relationships with avian hosts. *Plasmodium* is not expected to undergo cospeciation with its avian hosts. However, the presence of distinct areas of endemism has created relative isolation and formation of diverse host and parasite communities. In turn, this allowed for cospeciation to occur.

**Need for Future Research**

Denser sampling of both geographical areas and certain avian groups is necessary to produce a more detailed, clearer picture of diversity and distribution patterns of avian haemosporidians in the Amazon and South America in general. Molecular work needs to be accompanied by a greater effort of matching sequences with morphotypes using microscopy, ideally resulting in delineation and formal description of new species in place of current lineages.

The diversity and distribution patterns of avian haemosporidians are known to be related to the presence of suitable vectors yet very little is known about vector communities from this region. Much remains to be known on how vector-host relationships impact haemosporidian distribution and diversification and how these relationships are impacted by biogeographical forces. Research on all aspects of vector biology is needed to determine the relationships that exist between vectors, their haemosporidian parasites, and avian hosts in hyper-diverse areas like the Brazilian Amazon and the other biomes surveyed as part of this study.

# APPENDICES

## Appendix A

**All Haemosporidian Sequences Collected from Brazilian Birds. New Lineages are in Bold and Host Specificity Indices ($S_{TD}$*) are given for all Lineages with More than One Occurrence.**

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| AFR122 | *Haemoproteus* | *Hypocnemis striata* | Amazonia - Tapajós I | Tapajós | 2 | KU562121 |
| AFR122 | *Haemoproteus* | *Hypocnemoides maculicauda* | Amazonia - Tapajós H | Rondônia | 2 | KU562122 |
| AFR122 | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós B | Rondônia | 2 | KU562123 |
| **ARAJAN01** | *Plasmodium* | *Aratinga jandaya* | Amazonia - Gurupi | Belém | - | KU562740 |
| **ARRTAC01** | *Plasmodium* | *Arremon taciturnus* | Amazonia - Tapajós D | Rondônia | 3 | KU562435 |
| **ARRTAC01** | *Plasmodium* | *Arremon taciturnus* | Amazonia - Tapajós D | Rondônia | 3 | KU562436 |
| **ARRTAC01** | *Plasmodium* | *Megastictus margaritatus* | Amazonia - Purus 02 | Inambari | 3 | KU562437 |
| **ARRTAC01** | *Plasmodium* | *Arremon taciturnus* | Amazonia - Madeira 09 | Rondônia | 3 | KU562438 |
| **ARRTAC02** | *Plasmodium* | *Arremon taciturnus* | Amazonia - Madeira 09 | Rondônia | - | KU562789 |
| **ARRTAC03** | *Plasmodium* | *Arremon taciturnus* | Amazonia - CICRA | Inambari | - | KU562821 |
| **ARRTAC04** | *Plasmodium* | *Arremon taciturnus* | Amazonia - CICRA | Inambari | - | KU562842 |
| **ATTCIN01** | *Plasmodium* | *Attila cinnamoneus* | Amazonia - Gurupi | Belém | - | KU562744 |
| **AUTINF01** | *Plasmodium* | *Automolus infuscatus* | Amazonia - Negro 02 | Imerí | - | KU562594 |
| **AUTINF02** | *Plasmodium* | *Automolus infuscatus* | Amazonia - Negro 02 | Imerí | - | KU562603 |
| **AUTINF03** | *Plasmodium* | *Automolus infuscatus* | Amazonia - Negro 02 | Imerí | - | KU562610 |
| **AUTINF04** | *Plasmodium* | *Automolus infuscatus* | Amazonia - CICRA | Inambari | - | KU562817 |
| **AUTINF05** | *Haemoproteus* | *Automolus infuscatus* | Amazonia - CICRA | Inambari | - | KU562247 |
| **AUTOCH01** | *Haemoproteus* | *Automolus ochrolaemus* | Amazonia - Tapajós B | Rondônia | - | KU562133 |
| **AUTOCH02** | *Plasmodium* | *Automolus ochrolaemus* | Amazonia - Tapajós D | Rondônia | - | KU562410 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **AUTOCH03** | *Plasmodium* | *Automolus ochrolaemus* | Amazonia - Tapajós A | Rondônia | 2.7 | KU562460 |
| **AUTOCH03** | *Plasmodium* | *Automolus infuscatus* | Amazonia - CICRA | Inambari | 2.7 | KU562461 |
| **AUTOCH03** | *Plasmodium* | *Cercomacra nigrescens* | Amazonia - CICRA | Inambari | 2.7 | KU562462 |
| **AUTOCH03** | *Plasmodium* | *Automolus rufipileatus* | Amazonia - CICRA | Inambari | 2.7 | KU562463 |
| **AUTOCH04** | *Haemoproteus* | *Automolus ochrolaemus* | Amazonia - CICRA | Inambari | - | KU562249 |
| **AUTOCH05** | *Plasmodium* | *Automolus ochrolaemus* | Amazonia - CICRA | Inambari | - | KU562841 |
| **AUTOCH06** | *Plasmodium* | *Automolus orchrolaemus* | Amazonia - CICRA | Inambari | - | KU562822 |
| **AUTOCH07** | *Plasmodium* | *Automolus orchrolaemus* | Amazonia - CICRA | Inambari | - | KU562823 |
| **AUTPAR01** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós H | Rondônia | 2.8 | KU562259 |
| **AUTPAR01** | *Plasmodium* | *Thamnophilus amazonicus* | Amazonia - Tapajós H | Rondônia | 2.8 | KU562260 |
| **AUTPAR01** | *Plasmodium* | *Automolus paraensis* | Amazonia - Tapajós I | Tapajós | 2.8 | KU562261 |
| **AUTPAR01** | *Plasmodium* | *Attila spadiceus* | Amazonia - Tapajós I | Tapajós | 2.8 | KU562262 |
| **AUTPAR01** | *Plasmodium* | *Thamnophilus schistaceus* | Amazonia - Tapajós I | Tapajós | 2.8 | KU562263 |
| **AUTPAR01** | *Plasmodium* | *Epinecrophylla haematonota* | Amazonia - Tapajós B | Rondônia | 2.8 | KU562264 |
| **AUTPAR01** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | 2.8 | KU562265 |
| **AUTPAR01** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós J | Tapajós | 2.8 | KU562266 |
| **AUTPAR01** | *Plasmodium* | *Thamnophilus schistaceus* | Amazonia - Tapajós I | Tapajós | 2.8 | KU562267 |
| **AUTPAR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós J | Tapajós | 2.8 | KU562268 |
| **AUTPAR01** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós A | Rondônia | 2.8 | KU562269 |
| **AUTPAR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | 2.8 | KU562270 |
| **AUTPAR01** | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Purus 02 | Inambari | 2.8 | KU562271 |
| **AUTPAR01** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 2.8 | KU562272 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **AUTPAR01** | *Plasmodium* | *Rhynchocyclus olivaceus* | Amazonia - Gurupi | Belém | 2.8 | KU562273 |
| **AUTRUF01** | *Haemoproteus* | *Automolus rufipileatus* | Amazonia - Tapajós I | Tapajós | - | KU562136 |
| BAFLA04 | *Plasmodium* | *Thamnophilus pelzelni* | Caatinga - Aiuaba | | 2.87 | KU562527 |
| BAFLA04 | *Plasmodium* | *Coereba flaveola* | Caatinga - Aiuaba | | 2.87 | KU562528 |
| BAFLA04 | *Plasmodium* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 2.87 | KU562529 |
| BAFLA04 | *Plasmodium* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 2.87 | KU562530 |
| BAFLA04 | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.87 | KU562531 |
| **CAMRUB01** | *Haemoproteus* | *Campephilus rubricollis* | Amazonia - Gurupi | Belém | - | KU562234 |
| **CAMRUB02** | *Haemoproteus* | *Campephilus rubricollis* | Amazonia - Gurupi | Belém | - | KU562235 |
| **CANLEU01** | *Plasmodium* | *Cantorchilus leucotis* | Amazonia - Tapajós H | Rondônia | 2 | KU562255 |
| **CANLEU01** | *Plasmodium* | *Pheugopedius genibarbis* | Amazonia - Tapajós H | Rondônia | 2 | KU562256 |
| **CANLEU01** | *Plasmodium* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | 2 | KU562257 |
| **CANLEU02** | *Plasmodium* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | - | KU562512 |
| **CANLEU03** | *Haemoproteus* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | - | KU562162 |
| **CANLEU04** | *Haemoproteus* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | - | KU562163 |
| **CANLEU05** | *Haemoproteus* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | - | KU562164 |
| **CASFUS01** | *Plasmodium* | *Casiornis fuscus* | Caatinga - Aiuaba | | - | KU562542 |
| **CERCIN01** | *Plasmodium* | *Cercomacra cinerascens* | Amazonia - Gurupi | Belém | - | KU562736 |
| **CERCIN02** | *Plasmodium* | *Cercomacra cinerascens* | Amazonia - Madeira 07 | Inambari | - | KU562790 |
| **CERCIN03** | *Plasmodium* | *Cercomacra cinerascens* | Amazonia - Madeira 07 | Inambari | - | KU562791 |
| **CERCIN04** | *Haemoproteus* | *Cercomacra cinerascens* | Amazonia - Madeira 06 | Inambari | - | KU562243 |
| **CERERY01** | *Plasmodium* | *Ceratopipra erythroptera* | Amazonia - Negro 02 | Imerí | 1 | KU562612 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **CERERY01** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - Purus 01 | Inambari | 1 | KU562613 |
| **CERRUB01** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - Madeira 09 | Rondônia | - | KU562806 |
| **CERSER01** | *Plasmodium* | *Cercomacra serva* | Amazonia - Purus 01 | Inambari | - | KU562651 |
| **CHLAEN01** | *Plasmodium* | *Chloroceryle aenea* | Amazonia - Madeira 09 | Rondônia | - | KU562792 |
| **CHLIND01** | *Haemoproteus* | *Chloroceryle inda* | Amazonia - Negro 01 | Guiana | - | KU562203 |
| **CLAPRE01** | *Haemoproteus* | *Claravis pretiosa* | Amazonia - COM | Rondônia | - | KU562242 |
| COLBUC01[a] | *Haemoproteus* | *Columbina talpacoti* | Pantanal - Corumbá | | - | KU562218 |
| COLPAS03[b] | *Haemoproteus* | *Columbina passerina* | Amazonia - Gurupi | Belém | 4 | KU562227 |
| COLPAS03[b] | *Haemoproteus* | *Columbina passerina* | Amazonia - Gurupi | Belém | 4 | KU562228 |
| COLPAS03[b] | *Haemoproteus* | *Thamnophilus doliatus* | Amazonia - Gurupi | Belém | 4 | KU562229 |
| COLPAS03[b] | *Haemoproteus* | *Columbina passerina* | Amazonia - Gurupi | Belém | 4 | KU562230 |
| COLPAS04 | *Haemoproteus* | *Automolus infuscatus* | Amazonia - Negro 02 | Imerí | 3.38 | KU562204 |
| COLPAS04 | *Haemoproteus* | *Columbina talpacoti* | Pantanal - Corumbá | | 3.38 | KU562205 |
| COLPAS04 | *Haemoproteus* | *Myiophobus fasciatus* | Amazonia - Gurupi | Belém | 3.38 | KU562206 |
| **COLPAS06** | *Haemoproteus* | *Columbina passerina* | Amazonia - Gurupi | Belém | - | KU562226 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Aiuaba | | 2.35 | KU562513 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Aiuaba | | 2.35 | KU562514 |
| **CORPIL01** | *Plasmodium* | *Veniliornis affinis* | Caatinga - Aiuaba | | 2.35 | KU562515 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Aiuaba | | 2.35 | KU562516 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562517 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562518 |
| **CORPIL01** | *Plasmodium* | *Veniliornis passerinus* | Caatinga - Serido | | 2.35 | KU562519 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562520 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562521 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562522 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562523 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562524 |
| **CORPIL01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 2.35 | KU562525 |
| **CORPIL02** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 1 | KU562701 |
| **CORPIL02** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 1 | KU562702 |
| **CORPIL03** | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562220 |
| **CORPIL04** | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562221 |
| **CORPIL05** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562704 |
| **CORPIL06** | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562222 |
| **CORPIL07** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562705 |
| CORPIL08 | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562223 |
| **CORPIL09** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562706 |
| **CORPIL10** | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562224 |
| **CORPIL11** | *Haemoproteus* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562225 |
| **CORPIL12** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562707 |
| **CORPIL13** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562708 |
| **CORPIL14** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562709 |
| **CORPIL15** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | - | KU562710 |
| **CRAVUL01** | *Plasmodium* | *Cranioleuca vulpina* | Amazonia- Tapajós IL | Rondônia | - | KU562510 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| CYACYA01 | *Haemoproteus* | *Cyanocompsa cyanoides* | Amazonia - Tapajós H | Rondônia | - | KU562119 |
| CYACYA02 | *Haemoproteus* | *Cyanocompsa cyanoides* | Amazonia - Tapajós H | Rondônia | - | KU562120 |
| CYACYA03 | *Plasmodium* | *Cyanocompsa cyanoides* | Amazonia - Purus 02 | Inambari | - | KU562676 |
| CYACYA04 | *Plasmodium* | *Cyanocompsa cyanoides* | Amazonia - Madeira 09 | Rondônia | 3.04 | KU562793 |
| CYCYA01 | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Tapajós J | Tapajós | 3.04 | KU562328 |
| CYCYA01 | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Tapajós A | Rondônia | 3.04 | KU562329 |
| CYCYA01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós B | Rondônia | 3.04 | KU562330 |
| CYCYA01 | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós I | Tapajós | 3.04 | KU562331 |
| CYCYA01 | *Plasmodium* | *Myrmornis torquata* | Amazonia - Tapajós D | Rondônia | 3.04 | KU562332 |
| CYCYA01 | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós D | Rondônia | 3.04 | KU562333 |
| CYCYA01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Negro 02 | Imerí | 3.04 | KU562334 |
| CYCYA01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Negro 02 | Imerí | 3.04 | KU562335 |
| CYCYA01 | *Plasmodium* | *Sclerurus caudacutus* | Amazonia - Negro 02 | Imerí | 3.04 | KU562336 |
| CYCYA01 | *Plasmodium* | *Microbates collaris* | Amazonia - Negro 02 | Imerí | 3.04 | KU562337 |
| CYCYA01 | *Plasmodium* | *Geotrygon montana* | Amazonia - PTB | Imerí | 3.04 | KU562338 |
| CYCYA01 | *Plasmodium* | *Dixiphia pipra* | Amazonia - PTB | Imerí | 3.04 | KU562339 |
| CYCYA01 | *Plasmodium* | *Dixiphia pipra* | Amazonia - PTB | Imerí | 3.04 | KU562340 |
| CYCYA01 | *Plasmodium* | *Dixiphia pipra* | Amazonia - PTB | Imerí | 3.04 | KU562341 |
| CYCYA01 | *Plasmodium* | *Hylexetastes perrotii* | Amazonia - PTB | Imerí | 3.04 | KU562342 |
| CYCYA01 | *Plasmodium* | *Dixiphia pipra* | Amazonia - PTB | Imerí | 3.04 | KU562343 |
| CYCYA01 | *Plasmodium* | *Pithys albifrons* | Amazonia - PTB | Imerí | 3.04 | KU562344 |
| CYCYA01 | *Plasmodium* | *Pithys albifrons* | Amazonia - PTB | Imerí | 3.04 | KU562345 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **CYCYAN01** | *Haemoproteus* | *Cyanerpes cyaneus* | Amazonia - COM | Rondônia | - | KU562241 |
| **CYMSAN01** | *Plasmodium* | *Cymbilaimus sanctaemariae* | Amazonia - CICRA | Inambari | - | KU562840 |
| **CYPARA01** | *Plasmodium* | *Cyphorhinus arada* | Amazonia - CICRA | Inambari | - | KU562824 |
| **CYPHIR01** | *Haemoproteus* | *Cypsnagra hirundinacea* | Cerrado - CER | | - | KU562197 |
| **CYPHIR02** | *Haemoproteus* | *Cypsnagra hirundinacea* | Cerrado - CER | | - | KU562198 |
| **CYPHIR03** | *Haemoproteus* | *Cypsnagra hirundinacea* | Cerrado - CER | | 1 | KU562199 |
| **CYPHIR03** | *Haemoproteus* | *Cypsnagra hirundinacea* | Cerrado - CER | | 1 | KU562200 |
| **DACCAY01** | *Plasmodium* | *Dacnis cayana* | Amazonia - CICRA | Inambari | - | KU562836 |
| **DECLONG01** | *Haemoproteus* | *Deconychura longicauda* | Amazonia - CICRA | Inambari | - | KU562248 |
| **DENCER01** | *Plasmodium* | *Dendrocolaptes certhia* | Amazonia - Tapajós I | Tapajós | - | KU562277 |
| **DENCER02** | *Haemoproteus* | *Dendrocolaptes certhia* | Amazonia - Tapajós D | Rondônia | 3.62 | KU562151 |
| **DENCER02** | *Haemoproteus* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | 3.62 | KU562152 |
| **DENCER02** | *Haemoproteus* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | 3.62 | KU562153 |
| **DENCER02** | *Haemoproteus* | *Malacoptila rufa* | Amazonia - Tapajós A | Rondônia | 3.62 | KU562154 |
| **DENCER02** | *Haemoproteus* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | 3.62 | KU562155 |
| **DENCER02** | *Haemoproteus* | *Saltator coerolescens* | Amazonia- Tapajós IL | Rondônia | 3.62 | KU562156 |
| **DENFUL01** | *Haemoproteus* | *Dendrocincla fuliginosa* | Amazonia - Tapajós H | Rondônia | 3 | KU562125 |
| **DENFUL01** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós B | Rondônia | 3 | KU562126 |
| **DENFUL02** | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - PTB | Imerí | - | KU562768 |
| **DENFUL03** | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - CICRA | Inambari | - | KU562830 |
| **DENMER01** | *Plasmodium* | *Dendrocincla merula* | Amazonia - Purus 01 | Inambari | 3 | KU562624 |
| **DENMER01** | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Purus 02 | Inambari | 3 | KU562625 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **DENMER02** | *Plasmodium* | *Dendrocincla merula* | Amazonia - Madeira 07 | Inambari | - | KU562794 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Amazonia- Tapajós IL | Rondônia | 2.86 | KU562464 |
| DENPET03[c] | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | 2.86 | KU562465 |
| DENPET03[c] | *Plasmodium* | *Hemithraupis guira* | Cerrado - CER | | 2.86 | KU562466 |
| DENPET03[c] | *Plasmodium* | *Cypsnagra hirundinacea* | Cerrado - CER | | 2.86 | KU562467 |
| DENPET03[c] | *Plasmodium* | *Cypsnagra hirundinacea* | Cerrado - CER | | 2.86 | KU562468 |
| DENPET03[c] | *Plasmodium* | *Cypsnagra hirundinacea* | Cerrado - CER | | 2.86 | KU562469 |
| DENPET03[c] | *Plasmodium* | *Mimus saturninus* | Cerrado - CER | | 2.86 | KU562470 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Purus 02 | Inambari | 2.86 | KU562471 |
| DENPET03[c] | *Plasmodium* | *Cacicus solitarius* | Atlantic Forest - Natal | | 2.86 | KU562472 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Pantanal - Corumbá | | 2.86 | KU562473 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Pantanal - Corumbá | | 2.86 | KU562474 |
| DENPET03[c] | *Plasmodium* | *Saltator coerulescens* | Pantanal - Corumbá | | 2.86 | KU562475 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Pantanal - Corumbá | | 2.86 | KU562476 |
| DENPET03[c] | *Plasmodium* | *Arremon taciturnus* | Amazonia - CICRA | Inambari | 2.86 | KU562477 |
| DENPET03[c] | *Plasmodium* | *Hypocnemis subflava* | Amazonia - CICRA | Inambari | 2.86 | KU562478 |
| DENPET03[c] | *Plasmodium* | *Automolus rufipileatus* | Amazonia - CICRA | Inambari | 2.86 | KU562479 |
| DENPET03[c] | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 2.86 | KU562480 |
| DENPET03[c] | *Plasmodium* | *Turdus hauxwelli* | Amazonia - CICRA | Inambari | 2.86 | KU562481 |
| DENPET03[c] | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 2.86 | KU562482 |
| DENPET03[c] | *Plasmodium* | *Cyphorhinus arada* | Amazonia - CICRA | Inambari | 2.86 | KU562483 |
| DENPET03[c] | *Plasmodium* | *Cyphorhinus arada* | Amazonia - CICRA | Inambari | 2.86 | KU562484 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| DENPET03[c] | *Plasmodium* | *Xiphorhynchus ocellatus* | Amazonia - CICRA | Inambari | 2.86 | KU562485 |
| DENPET03[c] | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 2.86 | KU562486 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Amazonia - CICRA | Inambari | 2.86 | KU562487 |
| DENPET03[c] | *Plasmodium* | *Turdus hauxwelli* | Amazonia - CICRA | Inambari | 2.86 | KU562488 |
| DENPET03[c] | *Plasmodium* | *Arremon taciturnus* | Amazonia - CICRA | Inambari | 2.86 | KU562489 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Amazonia - CICRA | Inambari | 2.86 | KU562490 |
| DENPET03[c] | *Plasmodium* | *Ramphocelus carbo* | Amazonia - CICRA | Inambari | 2.86 | KU562491 |
| DETUR01 | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós A | Rondônia | 3.76 | KU562451 |
| DETUR01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós A | Rondônia | 3.76 | KU562452 |
| DETUR01 | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | 3.76 | KU562453 |
| DETUR01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Maderia 03 | Inambari | 3.76 | KU562454 |
| DETUR01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Madeira 04 | Inambari | 3.76 | KU562455 |
| **DICCIN01** | *Plasmodium* | *Dichrozona cincta* | Amazonia - Tapajós A | Rondônia | - | KU562502 |
| **DIXPIP01** | *Plasmodium* | *Dixiphia pipra* | Amazonia - Negro 01 | Guiana | - | KU562589 |
| **DYSMEN01** | *Plasmodium* | *Dysithamnus mentalis* | Amazonia - Gurupi | Belém | - | KU562712 |
| **EUPXAN01** | *Plasmodium* | *Euphonia xanthogaster* | Amazonia - CICRA | Inambari | - | KU562835 |
| **FORCOL01** | *Plasmodium* | *Formicarius colma* | Amazonia - Tapajós A | Rondônia | - | KU562449 |
| **FORCOL02** | *Plasmodium* | *Formicarius colma* | Amazonia - Negro 01 | Guiana | 1 | KU562577 |
| **FORCOL02** | *Plasmodium* | *Formicarius colma* | Amazonia - Negro 01 | Guiana | 1 | KU562578 |
| **FORCOL03** | *Plasmodium* | *Formicarius colma* | Amazonia - Negro 01 | Guiana | - | KU562591 |
| **FORCOL04** | *Plasmodium* | *Formicarius colma* | Amazonia - Negro 01 | Guiana | - | KU562593 |
| **FORCOL05** | *Plasmodium* | *Formicarius colma* | Amazonia - Purus 01 | Inambari | - | KU562645 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **FORCOL06** | *Plasmodium* | *Formicarius colma* | Amazonia - Purus 01 | Inambari | 1 | KU562646 |
| **FORCOL06** | *Plasmodium* | *Formicarius colma* | Amazonia - Purus 01 | Inambari | 1 | KU562647 |
| **FORCOL06** | *Plasmodium* | *Formicarius colma* | Amazonia - Madeira 07 | Inambari | 1 | KU562648 |
| **FORCOL06** | *Plasmodium* | *Formicarius colma* | Amazonia - Madeira 07 | Inambari | 1 | KU562649 |
| **FORCOL06** | *Plasmodium* | *Formicarius colma* | Amazonia - Madeira 05 | Inambari | 1 | KU562650 |
| **FORCOL07** | *Plasmodium* | *Formicarius colma* | Amazonia - PTB | Imerí | 1 | KU562763 |
| **FORCOL07** | *Plasmodium* | *Formicarius colma* | Amazonia - PTB | Imerí | 1 | KU562764 |
| **FORCOL07** | *Plasmodium* | *Formicarius colma* | Amazonia - PTB | Imerí | 1 | KU562765 |
| **FORCOL08** | *Plasmodium* | *Formicarius colma* | Amazonia - Madeira 07 | Inambari | - | KU562795 |
| **FORGRI01** | *Plasmodium* | *Formicivora grisea* | Amazonia - Gurupi | Belém | - | KU562746 |
| **FORGRI02** | *Plasmodium* | *Formicivora grisea* | Amazonia - Gurupi | Belém | - | KU562747 |
| **FORGRI03** | *Plasmodium* | *Formicivora grisea* | Amazonia - Gurupi | Belém | - | KU562748 |
| FOSER01 | *Plasmodium* | *Formicivora melanogaster* | Caatinga - Serido | | - | KU562703 |
| **FURLEU01** | *Plasmodium* | *Furnarius leucopus* | Caatinga - Aiuaba | | 3.08 | KU562532 |
| **FURLEU01** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 3.08 | KU562533 |
| **FURLEU01** | *Plasmodium* | *Automolus infuscatus* | Amazonia - Purus 02 | Inambari | 3.08 | KU562534 |
| **FURLEU01** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Purus 02 | Inambari | 3.08 | KU562535 |
| **FURLEU01** | *Plasmodium* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 3.08 | KU562536 |
| **FURLEU01** | *Plasmodium* | *Ramphocelus carbo* | Pantanal - Corumbá | | 3.08 | KU562537 |
| **FURLEU01** | *Plasmodium* | *Ramphocelus carbo* | Pantanal - Corumbá | | 3.08 | KU562538 |
| **FURLEU01** | *Plasmodium* | *Coryphospingus pileatus* | Caatinga - Serido | | 3.08 | KU562539 |
| **FURLEU01** | *Plasmodium* | *Arremon taciturnus* | Amazonia - Madeira 09 | Rondônia | 3.08 | KU562540 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **FURLEU01** | *Plasmodium* | *Gymnopithys salvini* | Amazonia - CICRA | Inambari | 3.08 | KU562541 |
| **GALALB01** | *Plasmodium* | *Galbula albirostris* | Amazonia - Negro 01 | Guiana | - | KU562579 |
| **GALCYA01** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | - | KU562360 |
| **GALCYA02** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | 1 | KU562396 |
| **GALCYA02** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Purus 01 | Inambari | 1 | KU562397 |
| **GALCYA02** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Madeira 07 | Inambari | 1 | KU562398 |
| **GALCYA03** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | - | KU562399 |
| **GALCYA04** | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | - | KU562497 |
| **GEOMON01** | *Haemoproteus* | *Geotrygon montana* | Amazonia - Purus 01 | Inambari | - | KU562207 |
| GEOTRY01 | *Haemoproteus* | *Geotrygon montana* | Amazonia - Tapajós I | Tapajós | 1 | KU562129 |
| GEOTRY01 | *Haemoproteus* | *Geotrygon montana* | Amazonia - Tapajós J | Tapajós | 1 | KU562130 |
| GEOTRY01 | *Haemoproteus* | *Geotrygon montana* | Amazonia - Purus 01 | Inambari | 1 | KU562131 |
| **GLYSPI03** | *Haemoproteus* | *Glyphorynchus spirurus* | Amazonia - Tapajós A | Rondônia | - | KU562142 |
| **GLYSPI04** | *Plasmodium* | *Glyphorynchus spirurus* | Amazonia - Negro 02 | Imerí | - | KU562611 |
| **GLYSPI05** | *Plasmodium* | *Glyphorynchus spirurus* | Amazonia - Purus 02 | Inambari | - | KU562663 |
| **GLYSPI06** | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - PTB | Imerí | 2.91 | KU562749 |
| **GLYSPI06** | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - PTB | Imerí | 2.91 | KU562750 |
| **GLYSPI06** | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - PTB | Imerí | 2.91 | KU562751 |
| **GLYSPI06** | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | 2.91 | KU562752 |
| **GLYSPI06** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - COM | Rondônia | 2.91 | KU562753 |
| **GLYSPI06** | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - COM | Rondônia | 2.91 | KU562754 |
| **GLYSPI06** | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - COM | Rondônia | 2.91 | KU562755 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **GLYSPI06** | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - COM | Rondônia | 2.91 | KU562756 |
| **GLYSPI06** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - COM | Rondônia | 2.91 | KU562757 |
| **GLYSPI06** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - COM | Rondônia | 2.91 | KU562758 |
| **GLYSPI06** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - COM | Rondônia | 2.91 | KU562759 |
| **GLYSPI06** | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - COM | Rondônia | 2.91 | KU562760 |
| **GLYSPI06** | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | 2.91 | KU562761 |
| **GLYSPI06** | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | 2.91 | KU562762 |
| **GLYSPI07** | *Plasmodium* | *Glyphorynchus spirurus* | Amazonia - CICRA | Inambari | - | KU562839 |
| GRW06[d] | *Plasmodium* | *Dendrocincla merula* | Amazonia - Purus 02 | Inambari | 3 | KU562666 |
| GRW06[d] | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - Purus 02 | Inambari | 3 | KU562667 |
| GRW06[d] | *Plasmodium* | *Donacobius atricapilla* | Pantanal - Corumbá | | 3 | KU562668 |
| GRW06[d] | *Plasmodium* | *Sporophila americana* | Amazonia - Gurupi | Belém | 3 | KU562669 |
| GRW06[d] | *Plasmodium* | *Dendrocincla merula* | Amazonia - Maderia 03 | Inambari | 3 | KU562670 |
| GRW06[d] | *Plasmodium* | *Dendrocincla merula* | Amazonia - Madeira 04 | Inambari | 3 | KU562671 |
| **GYMLEU02** | *Plasmodium* | *Gymnopithys leucaspis* | Amazonia - Negro 02 | Imerí | - | KU562614 |
| **GYMSAL01** | *Haemoproteus* | *Gymnopithys salvini* | Amazonia - Purus 01 | Inambari | 2.65 | KU562209 |
| **GYMSAL01** | *Haemoproteus* | *Dendrocincla merula* | Amazonia - Purus 02 | Inambari | 2.65 | KU562210 |
| **GYMSAL01** | *Haemoproteus* | *Myrmoborus myotherinus* | Amazonia - Purus 02 | Inambari | 2.65 | KU562211 |
| **GYMSAL02** | *Haemoproteus* | *Gymnopithys salvini* | Amazonia - Purus 01 | Inambari | - | KU562212 |
| **GYMSAL03** | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Madeira 07 | Inambari | - | KU562796 |
| **GYMSAL04** | *Plasmodium* | *Gymnopithys salvini* | Amazonia - CICRA | Inambari | - | KU562819 |
| **HABRUB01** | *Plasmodium* | *Habia rubica* | Amazonia - CICRA | Inambari | - | KU562818 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **HEMGRI01** | *Plasmodium* | *Hemitriccus griseipectus* | Amazonia - CICRA | Inambari | - | KU562834 |
| **HYLNAE01** | *Plasmodium* | *Hylophylax naevius* | Amazonia - Negro 02 | Imerí | - | KU562600 |
| **HYLNAE02** | *Plasmodium* | *Hylophylax naevius* | Amazonia - Madeira 05 | Inambari | - | KU562797 |
| **HYLOCH01** | *Plasmodium* | *Hylophilus ochraceiceps* | Amazonia - Tapajós J | Tapajós | - | KU562348 |
| **HYLPUN01** | *Plasmodium* | *Hylophylax punctulatus* | Amazonia - Tapajós A | Rondônia | - | KU562458 |
| **HYLPUN02** | *Haemoproteus* | *Hylophylax punctulatus* | Amazonia - Tapajós A | Rondônia | - | KU562143 |
| **HYPCAN02** | *Plasmodium* | *Hypocnemis cantator* | Amazonia - COM | Rondônia | - | KU562782 |
| **HYPOCH01** | *Plasmodium* | *Hypocnemis ochrogyna* | Amazonia - Madeira 10 | Rondônia | - | KU562798 |
| **HYPSTR01** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós H | Rondônia | - | KU562274 |
| **HYPSTR02** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós H | Rondônia | - | KU562275 |
| **HYPSTR03** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós I | Tapajós | - | KU562371 |
| **HYPSTR04** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós A | Rondônia | 2 | KU562391 |
| **HYPSTR04** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2 | KU562392 |
| **HYPSTR04** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós D | Rondônia | 2 | KU562393 |
| **HYPSTR04** | *Plasmodium* | *Schistocichla leucostigma* | Amazonia - Tapajós D | Rondônia | 2 | KU562394 |
| **HYPSTR05** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | - | KU562409 |
| **HYPSTR06** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | - | KU562428 |
| **HYPSTR07** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2 | KU562429 |
| **HYPSTR07** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós D | Rondônia | 2 | KU562430 |
| **HYPSTR07** | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Purus 02 | Inambari | 2 | KU562431 |
| **HYPSTR08** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | - | KU562445 |
| **HYPSTR09** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562498 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **HYPSTR10** | *Haemoproteus* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562160 |
| **HYPSTR11** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562501 |
| **HYPSTR12** | *Haemoproteus* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562161 |
| **HYPSTR13** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562503 |
| **HYPSTR14** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562504 |
| **HYPSTR15** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562506 |
| **HYPSTR16** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562507 |
| **HYPSTR17** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | - | KU562508 |
| ICTCAY01 | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 1 | KU562685 |
| ICTCAY01 | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 1 | KU562686 |
| ICTCAY01 | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 1 | KU562687 |
| **ISLGUT01** | *Plasmodium* | *Isleria guttata* | Amazonia - Negro 01 | Guiana | - | KU562590 |
| **ISLGUT02** | *Plasmodium* | *Isleria guttata* | Amazonia - Negro 01 | Guiana | - | KU562592 |
| **ISLHAU01** | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Tapajós A | Rondônia | 3 | KU562357 |
| **ISLHAU01** | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Gurupi | Belém | 3 | KU562358 |
| **ISLHAU01** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 3 | KU562359 |
| **ISLHAU02** | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Tapajós A | Rondônia | - | KU562395 |
| **ISLHAU03** | *Haemoproteus* | *Isleria hauxwelli* | Amazonia - Tapajós A | Rondônia | - | KU562144 |
| **KNIPOE01** | *Haemoproteus* | *Knipolegus poecilocercus* | Amazonia- Tapajós IL | Rondônia | - | KU562165 |
| **LATEUL01** | *Haemoproteus* | *Lathrotriccus euleri* | Amazonia - CICRA | Inambari | - | KU562246 |
| **LEPAMA01** | *Plasmodium* | *Leptopogon amaraucephalus* | Amazonia - Tapajós I | Tapajós | - | KU562376 |
| **LEPANG01** | *Plasmodium* | *Lepidocolaptes angustirostris* | Caatinga - Aiuaba | | - | KU562526 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| LEPCOR01 | *Plasmodium* | *Dendrocincla fuliginosa* | Amazonia - CICRA | Inambari | 3 | KU562828 |
| LEPCOR01 | *Plasmodium* | *Corythopis torquatus* | Amazonia - CICRA | Inambari | 3 | KU562829 |
| LEPCOR03 | *Haemoproteus* | *Ceratopipra rubrocapilla* | Amazonia - COM | Rondônia | 2.94 | KU562237 |
| LEPCOR03 | *Haemoproteus* | *Elaenia parvirostris* | Amazonia - COM | Rondônia | 2.94 | KU562238 |
| LEPCOR03 | *Haemoproteus* | *Elaenia parvirostris* | Amazonia - COM | Rondônia | 2.94 | KU562239 |
| LEPCOR03 | *Haemoproteus* | *Machaeropterus pyrocephalus* | Amazonia - COM | Rondônia | 2.94 | KU562240 |
| LEPCOR04 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Negro 01 | Guiana | 2.76 | KU562581 |
| LEPCOR04 | *Plasmodium* | *Ceratopipra erythrocephala* | Amazonia - Negro 01 | Guiana | 2.76 | KU562582 |
| LEPCOR04 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 2.76 | KU562583 |
| LEPCOR04 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - Madeira 09 | Rondônia | 2.76 | KU562584 |
| LEPCOR04 | *Plasmodium* | *Pipra fasciicauda* | Amazonia - Madeira 08 | Inambari | 2.76 | KU562585 |
| LEPCOR04 | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Madeira 09 | Rondônia | 2.76 | KU562586 |
| LEPCOR04 | *Plasmodium* | *Turdus hauxwelli* | Amazonia - CICRA | Inambari | 2.76 | KU562587 |
| LEPCOR04 | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 2.76 | KU562588 |
| **LEPCOR05** | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 01 | Inambari | - | KU562644 |
| **LEPCOR06** | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Madeira 07 | Inambari | - | KU562799 |
| **LEPNAT01** | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - COM | Rondônia | 1 | KU562773 |
| **LEPNAT01** | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - COM | Rondônia | 1 | KU562774 |
| **LEPNAT02** | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - Madeira 10 | Rondônia | - | KU562800 |
| **LEPNAT03** | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - Madeira 10 | Rondônia | - | KU562801 |
| LEPRUF02 | *Haemoproteus* | *Leptotila rufaxilla* | Amazonia- Tapajós IL | Rondônia | 1 | KU562145 |
| LEPRUF02 | *Haemoproteus* | *Leptotila rufaxilla* | Caatinga - Aiuaba | | 1 | KU562146 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **LEPVIL01** | *Plasmodium* | *Lepidothrix vilasboasi* | Amazonia - Tapajós I | Tapajós | - | KU562284 |
| **MACPYR01** | *Plasmodium* | *Machaeropterus pyrocephalus* | Amazonia - COM | Rondônia | 2.88 | KU562775 |
| **MACPYR01** | *Plasmodium* | *Hypocnemis cantator* | Amazonia - COM | Rondônia | 2.88 | KU562776 |
| **MACPYR01** | *Plasmodium* | *Xenopipo atronitens* | Amazonia - COM | Rondônia | 2.88 | KU562777 |
| **MACPYR01** | *Plasmodium* | *Hemitriccus margaritaceiventer* | Amazonia - COM | Rondônia | 2.88 | KU562778 |
| **MACPYR02** | *Plasmodium* | *Machaeropterus pyrocephalus* | Amazonia - COM | Rondônia | - | KU562784 |
| **MALRUF01** | *Plasmodium* | *Malacoptila rufa* | Amazonia - Madeira 07 | Inambari | - | KU562802 |
| **MICGIL01** | *Haemoproteus* | *Micrastur gilvicollis* | Amazonia - PTB | Imerí | - | KU562236 |
| **MICMIN01** | *Plasmodium* | *Micrastur mintoni* | Amazonia - Gurupi | Belém | - | KU562735 |
| **MIOMAC01** | *Plasmodium* | *Mionectes macconnelli* | Amazonia - Tapajós I | Tapajós | - | KU562372 |
| **MIOMAC02** | *Plasmodium* | *Mionectes macconnelli* | Amazonia - Tapajós A | Rondônia | - | KU562459 |
| **MONNIG01** | *Plasmodium* | *Monasa nigrifrons* | Amazonia- Tapajós IL | Rondônia | 3.12 | KU562492 |
| **MONNIG01** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós D | Rondônia | 3.12 | KU562493 |
| **MONNIG01** | *Plasmodium* | *Ramphastos tucanus* | Amazonia - Negro 01 | Guiana | 3.12 | KU562494 |
| MYCAME02 | *Plasmodium* | *Micrastur semitorquatus* | Amazonia - Tapajós B | Rondônia | - | KU562365 |
| **MYIFER01** | *Plasmodium* | *Myiarchus ferox* | Amazonia - Tapajós H | Rondônia | - | KU562258 |
| **MYISWA01** | *Haemoproteus* | *Myiarchus swainsoni* | Cerrado - CER | | 2 | KU562174 |
| **MYISWA01** | *Haemoproteus* | *Myiarchus swainsoni* | Cerrado - CER | | 2 | KU562175 |
| **MYISWA01** | *Haemoproteus* | *Phaeomyias murina* | Cerrado - CER | | 2 | KU562176 |
| **MYISWA01** | *Haemoproteus* | *Elaenia chiriquensis* | Cerrado - CER | | 2 | KU562177 |
| **MYISWA01** | *Haemoproteus* | *Elaenia chiriquensis* | Cerrado - CER | | 2 | KU562178 |
| MYITYR01 | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Purus 02 | Inambari | 2.94 | KU562654 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| MYITYR01 | *Plasmodium* | *Myiarchus tyrannulus* | Caatinga - Serido | | 2.94 | KU562655 |
| MYITYR01 | *Plasmodium* | *Myiarchus tyrannulus* | Caatinga - Serido | | 2.94 | KU562656 |
| MYITYR01 | *Plasmodium* | *Myiarchus tyrannulus* | Caatinga - Serido | | 2.94 | KU562657 |
| MYITYR01 | *Plasmodium* | *Rhytipterna simplex* | Amazonia - Gurupi | Belém | 2.94 | KU562658 |
| MYITYR01 | *Plasmodium* | *Campylorhynchus turdinus* | Amazonia - Gurupi | Belém | 2.94 | KU562659 |
| **MYRAXI01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Tapajós I | Tapajós | 2.12 | KU562278 |
| **MYRAXI01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Tapajós J | Tapajós | 2.12 | KU562279 |
| **MYRAXI01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Tapajós J | Tapajós | 2.12 | KU562280 |
| **MYRAXI01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Tapajós D | Rondônia | 2.12 | KU562281 |
| **MYRAXI01** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós A | Rondônia | 2.12 | KU562282 |
| **MYRAXI01** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 2.12 | KU562283 |
| **MYRAXI02** | *Haemoproteus* | *Myrmotherula axillaris* | Amazonia - Tapajós I | Tapajós | - | KU562132 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós D | Rondônia | 1 | KU562439 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós D | Rondônia | 1 | KU562440 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Gurupi | Belém | 1 | KU562441 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Gurupi | Belém | 1 | KU562442 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - CHU | Rondônia | 1 | KU562443 |
| **MYRAXI03** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - CHU | Rondônia | 1 | KU562444 |
| **MYRAXI04** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós A | Rondônia | - | KU562505 |
| **MYRAXI05** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | - | KU562661 |
| **MYRAXI06** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | - | KU562662 |
| **MYRAXI07** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | - | KU562674 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **MYRAXI08** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | - | KU562675 |
| **MYRAXI09** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Gurupi | Belém | 1 | KU562723 |
| **MYRAXI09** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Gurupi | Belém | 1 | KU562724 |
| **MYRAXI09** | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Gurupi | Belém | 1 | KU562725 |
| **MYRFOR01** | *Plasmodium* | *Myrmeciza fortis* | Amazonia - Purus 01 | Inambari | - | KU562626 |
| **MYRFOR02** | *Plasmodium* | *Myrmeciza fortis* | Amazonia - Purus 01 | Inambari | - | KU562627 |
| **MYRFOR03** | *Plasmodium* | *Myrmeciza fortis* | Amazonia - Purus 01 | Inambari | - | KU562653 |
| **MYRHEM01** | *Plasmodium* | *Myrmeciza hemimelaena* | Amazonia - Madeira 10 | Rondônia | - | KU562803 |
| **MYRHEM02** | *Plasmodium* | *Myrmeciza hemimelaena* | Amazonia - CICRA | Inambari | - | KU562814 |
| **MYRLEU01** | *Plasmodium* | *Myrmoborus leucophrys* | Amazonia - CICRA | Inambari | 3 | KU562825 |
| **MYRLEU01** | *Plasmodium* | *Turdus hauxwelli* | Amazonia - CICRA | Inambari | 3 | KU562826 |
| **MYRLON01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Tapajós A | Rondônia | - | KU562457 |
| **MYRLON02** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Negro 02 | Imerí | 2 | KU562608 |
| **MYRLON02** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Purus 02 | Inambari | 2 | KU562609 |
| **MYRLON03** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Negro 02 | Imerí | - | KU562623 |
| **MYRLON04** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Purus 02 | Inambari | - | KU562672 |
| **MYRLON05** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Purus 02 | Inambari | - | KU562673 |
| MYRMAX01 | *Plasmodium* | *Rhegmatorhina berlepschi* | Amazonia - Tapajós B | Rondônia | 1.98 | KU562285 |
| MYRMAX01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Negro 01 | Guiana | 1.98 | KU562286 |
| MYRMAX01 | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Purus 01 | Inambari | 1.98 | KU562287 |
| MYRMAX01 | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Purus 01 | Inambari | 1.98 | KU562288 |
| MYRMAX01 | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Purus 01 | Inambari | 1.98 | KU562289 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| MYRMAX01 | *Plasmodium* | *Myrmeciza fortis* | Amazonia - Purus 01 | Inambari | 1.98 | KU562290 |
| MYRMAX01 | *Plasmodium* | *Schistocichla humaythae* | Amazonia - Purus 01 | Inambari | 1.98 | KU562291 |
| MYRMAX01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 01 | Inambari | 1.98 | KU562292 |
| MYRMAX01 | *Plasmodium* | *Gymnopithys rufigula* | Amazonia - PTB | Imerí | 1.98 | KU562293 |
| MYRMAX01 | *Plasmodium* | *Gymnopithys rufigula* | Amazonia - PTB | Imerí | 1.98 | KU562294 |
| MYRMAX01 | *Plasmodium* | *Gymnopithys rufigula* | Amazonia - PTB | Imerí | 1.98 | KU562295 |
| MYRMAX01 | *Plasmodium* | *Gymnopithys salvini* | Amazonia- Madeira 01 | Inambari | 1.98 | KU562296 |
| **MYRMEN01** | *Plasmodium* | *Myrmotherula menetriesii* | Amazonia - Tapajós J | Tapajós | - | KU562321 |
| **MYRMY006** | *Plasmodium* | *Myrmoborus myoterhinus* | Amazonia - Tapajós A | Rondônia | - | KU562456 |
| MYRMYO01 | *Plasmodium* | *Conopophaga aurita* | Amazonia - Tapajós J | Tapajós | 2.93 | KU562322 |
| MYRMYO01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2.93 | KU562323 |
| MYRMYO01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2.93 | KU562324 |
| MYRMYO01 | *Plasmodium* | *Glyphorhynchus spirurus* | Amazonia - Tapajós A | Rondônia | 2.93 | KU562325 |
| MYRMYO01 | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós A | Rondônia | 2.93 | KU562326 |
| MYRMYO01 | *Plasmodium* | *Mionectes oleagineus* | Amazonia - Tapajós A | Rondônia | 2.93 | KU562327 |
| **MYRMYO03** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós B | Rondônia | - | KU562367 |
| **MYRMYO04** | *Plasmodium* | *Myrmoborus myoterhinus* | Amazonia - Tapajós B | Rondônia | - | KU562402 |
| **MYRMYO05** | *Plasmodium* | *Myrmoborus myoterhinus* | Amazonia - Tapajós D | Rondônia | - | KU562432 |
| **MYRMYO07** | *Haemoproteus* | *Myrmoborus myotherinus* | Amazonia - Purus 01 | Inambari | - | KU562208 |
| **MYRMYO08** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Purus 01 | Inambari | - | KU562652 |
| **MYRMYO09** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Madeira 07 | Inambari | - | KU562804 |
| **MYRMYO10** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - CICRA | Inambari | - | KU562815 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **MYRMYO11** | *Haemoproteus* | *Myrmoborus myotherinus* | Amazonia - CICRA | Inambari | - | KU562245 |
| **MYRMYO12** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - CICRA | Inambari | - | KU562820 |
| **NEOFAS01** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 1 | KU562571 |
| **NEOFAS01** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 1 | KU562572 |
| **NEOFAS01** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 1 | KU562573 |
| **NEOFAS02** | *Haemoproteus* | *Neothraupis fasciata* | Cerrado - CER | | - | KU562201 |
| **NEOFAS03** | *Haemoproteus* | *Neothraupis fasciata* | Cerrado - CER | | - | KU562202 |
| **NEOFAS04** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | - | KU562574 |
| **NEOFAS05** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | - | KU562575 |
| **NEOFAS06** | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | - | KU562576 |
| **NYSCHA01** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | - | KU562196 |
| **NYSCHA02** | *Plasmodium* | *Nystalus chacuru* | Cerrado - CER | | - | KU562553 |
| **NYSMAC01** | *Haemoproteus* | *Nystalus maculatus* | Cerrado - CER | | 1 | KU562179 |
| **NYSMAC01** | *Haemoproteus* | *Nystalus maculatus* | Cerrado - CER | | 1 | KU562180 |
| **NYSMAC01** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | 1 | KU562181 |
| **NYSMAC02** | *Haemoproteus* | *Nystalus maculatus* | Cerrado - CER | | - | KU562190 |
| **NYSMAC03** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | 1 | KU562191 |
| **NYSMAC03** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | 1 | KU562192 |
| **NYSMAC03** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | 1 | KU562193 |
| **NYSMAC03** | *Haemoproteus* | *Nystalus chacuru* | Cerrado - CER | | 1 | KU562194 |
| **NYSMAC03** | *Haemoproteus* | *Nystalus maculatus* | Caatinga - Serido | | 1 | KU562195 |
| **NYSMAC04** | *Haemoproteus* | *Nystalus maculatus* | Caatinga - Serido | | - | KU562219 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **PACMAR01** | *Haemoproteus* | *Pachyrhamphus marginatus* | Caatinga - Aiuaba | | - | KU562171 |
| **PACRUF01** | *Plasmodium* | *Pachyramphus rufus* | Amazonia - Gurupi | Belém | - | KU562745 |
| PADOM09[d] | *Plasmodium* | *Elaenia cristata* | Cerrado - CER | | 2.82 | KU562554 |
| PADOM09[d] | *Plasmodium* | *Elaenia cristata* | Cerrado - CER | | 2.82 | KU562555 |
| PADOM09[d] | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 2.82 | KU562556 |
| PADOM09[d] | *Plasmodium* | *Myiarchus tyrannulus* | Atlantic Forest - Natal | | 2.82 | KU562557 |
| PADOM09[d] | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 2.82 | KU562558 |
| PADOM09[d] | *Plasmodium* | *Saltator coerulescens* | Pantanal - Corumbá | | 2.82 | KU562559 |
| PADOM09[d] | *Plasmodium* | *Donacobius atricapilla* | Pantanal - Corumbá | | 2.82 | KU562560 |
| PADOM09[d] | *Plasmodium* | *Tachyphonus cristatus* | Amazonia - Gurupi | Belém | 2.82 | KU562561 |
| PADOM09[d] | *Plasmodium* | *Pheugopedius genibarbis* | Amazonia - Gurupi | Belém | 2.82 | KU562562 |
| PADOM09[d] | *Plasmodium* | *Dendrocolaptes certhia* | Amazonia - PTB | Imerí | 2.82 | KU562563 |
| PADOM09[d] | *Plasmodium* | *Cnemotriccus fuscatus* | Amazonia - CHU | Rondônia | 2.82 | KU562564 |
| PADOM09[d] | *Plasmodium* | *Rhytipterna simplex* | Amazonia - CHU | Rondônia | 2.82 | KU562565 |
| PADOM09[d] | *Plasmodium* | *Ramphotrigon ruficauda* | Amazonia - Madeira 05 | Inambari | 2.82 | KU562566 |
| PADOM11 | *Plasmodium* | *Volatinia jacarina* | Cerrado - CER | | 2.32 | KU562548 |
| PADOM11 | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 2.32 | KU562549 |
| PADOM11 | *Plasmodium* | *Neothraupis fasciata* | Cerrado - CER | | 2.32 | KU562550 |
| PADOM11 | *Plasmodium* | *Tachyphonus rufus* | Amazonia - Gurupi | Belém | 2.32 | KU562551 |
| PADOM11 | *Plasmodium* | *Coereba flaveola* | Amazonia - Gurupi | Belém | 2.32 | KU562552 |
| PAPOL03 | *Haemoproteus* | *Pachyramphus polychopterus* | Caatinga - Aiuaba | | 1 | KU562172 |
| PAPOL03 | *Haemoproteus* | *Pachyramphus marginatus* | Cerrado - CER | | 1 | KU562173 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **PARCAP01** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562682 |
| **PARCAP02** | *Haemoproteus* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562217 |
| **PARCAP03** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562683 |
| **PARCAP04** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562684 |
| **PARCAP05** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562688 |
| **PARCAP06** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562689 |
| **PARCAP07** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562690 |
| **PARCAP08** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | - | KU562691 |
| **PARCAP09** | *Plasmodium* | *Paroaria capitata* | Pantanal - Corumbá | | 3 | KU562692 |
| **PARCAP09** | *Plasmodium* | *Habia rubica* | Amazonia - CICRA | Inambari | 3 | KU562693 |
| **PARCON01** | *Plasmodium* | *Paroaria coronata* | Pantanal - Corumbá | | - | KU562698 |
| **PHAE01** | *Plasmodium* | *Phaethornis sp.* | Amazonia - Tapajós B | Rondônia | - | KU562400 |
| **PHAMAL02** | *Plasmodium* | *Phaethornis malaris* | Amazonia - Tapajós H | Rondônia | 3.92 | KU562250 |
| **PHAMAL02** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós B | Rondônia | 3.92 | KU562251 |
| **PHAMAL02** | *Plasmodium* | *Myiobius barbatus* | Amazonia - Tapajós A | Rondônia | 3.92 | KU562252 |
| **PHEGEN01** | *Plasmodium* | *Pheugopedius genibarbis* | Amazonia - CICRA | Inambari | - | KU562811 |
| **PHIERY01** | *Plasmodium* | *Philydor erythropterum* | Amazonia - Gurupi | Belém | - | KU562728 |
| **PHIERY02** | *Plasmodium* | *Philydor erythropterum* | Amazonia - Gurupi | Belém | - | KU562729 |
| **PHIERY03** | *Plasmodium* | *Phlegopsis erythroptera* | Amazonia - Negro 02 | Imerí | - | KU562595 |
| **PHLNIG01** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós B | Rondônia | - | KU562135 |
| **PHLNIG02** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós J | Tapajós | - | KU562347 |
| **PHLNIG03** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós A | Rondônia | 2.38 | KU562349 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **PHLNIG03** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós A | Rondônia | 2.38 | KU562350 |
| **PHLNIG03** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós B | Rondônia | 2.38 | KU562351 |
| **PHLNIG03** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós B | Rondônia | 2.38 | KU562352 |
| **PHLNIG03** | *Plasmodium* | *Synallaxis rutilans* | Amazonia - Purus 01 | Inambari | 2.38 | KU562353 |
| **PHLNIG03** | *Plasmodium* | *Pyriglena leuconota* | Pantanal - Caceres | | 2.38 | KU562354 |
| **PHLNIG03** | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Gurupi | Belém | 2.38 | KU562355 |
| **PHLNIG03** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Gurupi | Belém | 2.38 | KU562356 |
| **PHLNIG04** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós D | Rondônia | - | KU562148 |
| **PHLNIG05** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós D | Rondônia | 1 | KU562149 |
| **PHLNIG05** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós D | Rondônia | 1 | KU562150 |
| **PHLNIG06** | *Haemoproteus* | *Phlegopsis nigromaculata* | Amazonia - Tapajós D | Rondônia | - | KU562157 |
| **PICFLA01** | *Plasmodium* | *Piculus flavigula* | Amazonia - Gurupi | Belém | - | KU562722 |
| **PIPCHL01** | *Plasmodium* | *Piprites chloris* | Amazonia - COM | Rondônia | - | KU562787 |
| **PIPFAS02** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - Tapajós H | Rondônia | 2 | KU562368 |
| **PIPFAS02** | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 02 | Inambari | 2 | KU562369 |
| **PIPFAS02** | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - Purus 02 | Inambari | 2 | KU562370 |
| **PIPFAS03** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - Tapajós D | Rondônia | - | KU562427 |
| **PIPFAS04** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - Madeira 04 | Inambari | - | KU562805 |
| **PIPFAS05** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | - | KU562812 |
| **PIPFAS06** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | - | KU562816 |
| **PIPFAS07** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | - | KU562827 |
| **PIPFAS08** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | - | KU562831 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **PIPFAS09** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | - | KU562832 |
| **PSABIF01** | *Plasmodium* | *Psarocolius bifasciatus* | Amazonia - Gurupi | Belém | - | KU562734 |
| **PSABIF02** | *Haemoproteus* | *Psarocolius bifasciatus* | Amazonia - Gurupi | Belém | - | KU562233 |
| **PSOOCH01** | *Plasmodium* | *Psophia ochroptera* | Amazonia - Negro 02 | Imerí | 1 | KU562606 |
| **PSOOCH01** | *Plasmodium* | *Psophia ochroptera* | Amazonia - Negro 02 | Imerí | 1 | KU562607 |
| **PYRLEP01** | *Plasmodium* | *Pyrrhura lepida* | Amazonia - Gurupi | Belém | - | KU562741 |
| **PYRLEP02** | *Plasmodium* | *Pyrrhura lepida* | Amazonia - Gurupi | Belém | - | KU562743 |
| **PYRLEU01** | *Plasmodium* | *Pyriglena leuconota* | Pantanal - Caceres | | - | KU562694 |
| **PYRLEU02** | *Plasmodium* | *Pyriglena leuconota* | Pantanal - Caceres | | 1 | KU562695 |
| **PYRLEU02** | *Plasmodium* | *Pyriglena leuconota* | Pantanal - Caceres | | 1 | KU562696 |
| **PYRLEU02** | *Plasmodium* | *Pyriglena leuconota* | Pantanal - Caceres | | 1 | KU562697 |
| **PYRLEU03** | *Plasmodium* | *Pyriglena leuconota* | Amazonia - Gurupi | Belém | - | KU562733 |
| RAMCAR01 | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Gurupi | Belém | 2 | KU562737 |
| RAMCAR01 | *Plasmodium* | *Thraupis episcopus* | Amazonia - Gurupi | Belém | 2 | KU562738 |
| **RAMCAR02** | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Tapajós H | Rondônia | - | KU562276 |
| **RAMCAR03** | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Purus 02 | Inambari | - | KU562677 |
| **RAMCAR04** | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Purus 02 | Inambari | - | KU562678 |
| **RAMCAR05** | *Plasmodium* | *Ramphocelus carbo* | Amazonia - Purus 02 | Inambari | - | KU562679 |
| **RHYSIM01** | *Plasmodium* | *Rhytipterna simplex* | Amazonia - CHU | Rondônia | 3 | KU562769 |
| **RHYSIM01** | *Plasmodium* | *Chlorophanes spiza* | Amazonia - CICRA | Inambari | 3 | KU562770 |
| **SALATR01** | *Plasmodium* | *Saltator atricollis* | Cerrado - CER | | 1 | KU562567 |
| **SALATR01** | *Plasmodium* | *Saltator atricollis* | Cerrado - CER | | 1 | KU562568 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **SALMAX01** | *Plasmodium* | *Saltator maximus* | Amazonia - CICRA | Inambari | - | KU562837 |
| **SALMAX02** | *Plasmodium* | *Saltator maximus* | Amazonia - CICRA | Inambari | - | KU562838 |
| **SCHRUF01** | *Plasmodium* | *Schistochlamys ruficapillus* | Atlantic Forest - Natal | | 3 | KU562680 |
| **SCHRUF01** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - CICRA | Inambari | 3 | KU562681 |
| **SCHTUR01** | *Haemoproteus* | *Schiffornis turdina* | Amazonia - Tapajós B | Rondônia | - | KU562134 |
| **SCHTUR02** | *Plasmodium* | *Schiffornis turdina* | Amazonia - Tapajós D | Rondônia | - | KU562434 |
| **SCLCAU01** | *Haemoproteus* | *Sclerurus caudacutus* | Amazonia - Tapajós I | Tapajós | - | KU562128 |
| **SPOAME01** | *Plasmodium* | *Sporophila americana* | Amazonia - Gurupi | Belém | - | KU562742 |
| **SUISUI01** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | - | KU562183 |
| **SUISUI02** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | 1 | KU562184 |
| **SUISUI02** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | 1 | KU562185 |
| **SUISUI02** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | 1 | KU562186 |
| **SUISUI02** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | 1 | KU562187 |
| **SUISUI03** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | - | KU562188 |
| **SUISUI04** | *Haemoproteus* | *Suiriri suiriri* | Cerrado - CER | | - | KU562189 |
| **TACCRI01** | *Haemoproteus* | *Tachyphonus cristatus* | Amazonia - Tapajós B | Rondônia | 1.16 | KU562138 |
| **TACCRI01** | *Haemoproteus* | *Dendrocincla fuliginosa* | Amazonia - Negro 02 | Imerí | 1.16 | KU562139 |
| **TACCRI01** | *Haemoproteus* | *Tachyphonus luctuosus* | Amazonia - Gurupi | Belém | 1.16 | KU562140 |
| **TACCRI01** | *Haemoproteus* | *Tachyphonus cristatus* | Amazonia - PTB | Imerí | 1.16 | KU562141 |
| **TACCRI02** | *Plasmodium* | *Tachyphonus cristatus* | Amazonia - Tapajós D | Rondônia | 3 | KU562446 |
| **TACCRI02** | *Plasmodium* | *Dendrocincla merula* | Amazonia - CICRA | Inambari | 3 | KU562447 |
| **TACCRI02** | *Plasmodium* | *Leptopogon amaurocephalus* | Amazonia - CICRA | Inambari | 3 | KU562448 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **TACCRI03** | *Haemoproteus* | *Tachyphonus cristatus* | Amazonia - Gurupi | Belém | - | KU562231 |
| **TACPHO01** | *Plasmodium* | *Tachyphonus phoenicius* | Amazonia - COM | Rondônia | - | KU562779 |
| **TACPHO01** | *Plasmodium* | *Xenopipo atronitens* | Amazonia - COM | Rondônia | - | KU562780 |
| TACRUB01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 01 | Inambari | 1.37 | KU562628 |
| TACRUB01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 02 | Inambari | 1.37 | KU562629 |
| TACRUB01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 02 | Inambari | 1.37 | KU562630 |
| TACRUB01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 02 | Inambari | 1.37 | KU562631 |
| TACRUB01 | *Plasmodium* | *Lepidothrix coronata* | Amazonia - Purus 02 | Inambari | 1.37 | KU562632 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562633 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562634 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562635 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562636 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562637 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562638 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - COM | Rondônia | 1.37 | KU562639 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - CHU | Rondônia | 1.37 | KU562640 |
| TACRUB01 | *Plasmodium* | *Lepidothrix nattereri* | Amazonia - COM | Rondônia | 1.37 | KU562641 |
| TACRUB01 | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - Madeira 07 | Inambari | 1.37 | KU562642 |
| TACRUB01 | *Plasmodium* | *Ceratopipra rubrocapilla* | Amazonia - Madeira 09 | Rondônia | 1.37 | KU562643 |
| **TACRUB02** | *Haemoproteus* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 1 | KU562213 |
| **TACRUB02** | *Haemoproteus* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 1 | KU562214 |
| **TACRUB02** | *Haemoproteus* | *Tachyphonus rufus* | Atlantic Forest - Natal | | 1 | KU562215 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **TACRUB03** | *Haemoproteus* | *Tachyphonus rufus* | Atlantic Forest - Natal | | - | KU562216 |
| **TACRUB04** | *Plasmodium* | *Tachyphonus rufus* | Amazonia - Gurupi | Belém | - | KU562739 |
| **TANCAY01** | *Haemoproteus* | *Tangara cayana* | Cerrado - CER | | - | KU562182 |
| **TANSCH01** | *Plasmodium* | *Tangara schrankii* | Amazonia - CICRA | Inambari | - | KU562833 |
| **TERERY01** | *Plasmodium* | *Terenotriccus erythrurus* | Amazonia - Tapajós H | Rondônia | - | KU562253 |
| **TERERY02** | *Plasmodium* | *Terenotriccus erythrurus* | Amazonia - Tapajós H | Rondônia | - | KU562254 |
| **THAAET01** | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Gurupi | Belém | - | KU562727 |
| **THAAET02** | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Madeira 07 | Inambari | - | KU562807 |
| **THAAMA01** | *Plasmodium* | *Thamnophilus amazonicus* | Amazonia - Gurupi | Belém | - | KU562711 |
| THACAE01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2.3 | KU562412 |
| THACAE01 | *Plasmodium* | *Leptotila rufaxilla* | Amazonia- Tapajós IL | Rondônia | 2.3 | KU562413 |
| THACAE01 | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós A | Rondônia | 2.3 | KU562414 |
| THACAE01 | *Plasmodium* | *Thamnophilus aethiops* | Amazonia - Tapajós A | Rondônia | 2.3 | KU562415 |
| THACAE01 | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | 2.3 | KU562416 |
| THACAE01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 01 | Inambari | 2.3 | KU562417 |
| THACAE01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | 2.3 | KU562418 |
| THACAE01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Purus 02 | Inambari | 2.3 | KU562419 |
| THACAE01 | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | 2.3 | KU562420 |
| THACAE01 | *Plasmodium* | *Dysithamnus mentalis* | Amazonia - Gurupi | Belém | 2.3 | KU562421 |
| THACAE01 | *Plasmodium* | *Thamnophilus murinus* | Amazonia - PTB | Imerí | 2.3 | KU562422 |
| THACAE01 | *Plasmodium* | *Thamnophilus murinus* | Amazonia - PTB | Imerí | 2.3 | KU562423 |
| THACAE01 | *Plasmodium* | *Thamnophilus murinus* | Amazonia - PTB | Imerí | 2.3 | KU562424 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| THACAE01 | *Plasmodium* | *Myrmeciza goeldii* | Amazonia - CICRA | Inambari | 2.3 | KU562425 |
| THACAE01 | *Plasmodium* | *Myrmeciza hemimelaena* | Amazonia - CICRA | Inambari | 2.3 | KU562426 |
| **THACAE02** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Tapajós J | Tapajós | - | KU562346 |
| **THACAE03** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Tapajós A | Rondônia | - | KU562450 |
| **THACAE04** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Tapajós D | Rondônia | - | KU562495 |
| **THACAE05** | *Haemoproteus* | *Thamnomanes caesius* | Amazonia - Tapajós D | Rondônia | - | KU562147 |
| **THACAE06** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Negro 02 | Imerí | - | KU562601 |
| **THACAE07** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Negro 02 | Imerí | - | KU562602 |
| **THACAE08** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Gurupi | Belém | 1 | KU562714 |
| **THACAE08** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - Gurupi | Belém | 1 | KU562715 |
| **THACAE09** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - PTB | Imerí | 1 | KU562766 |
| **THACAE09** | *Plasmodium* | *Thamnomanes caesius* | Amazonia - PTB | Imerí | 1 | KU562767 |
| **THAFUR01** | *Haemoproteus* | *Thalurania furcata* | Amazonia - Gurupi | Belém | - | KU562232 |
| THAMAE01 | *Plasmodium* | *Myrmotherula menetriesii* | Amazonia - Tapajós J | Tapajós | 2.23 | KU562298 |
| THAMAE01 | *Plasmodium* | *Myrmotherula axillaris* | Amazonia - Tapajós J | Tapajós | 2.23 | KU562299 |
| THAMAE01 | *Plasmodium* | *Rhegmatorhina gymnops* | Amazonia - Tapajós J | Tapajós | 2.23 | KU562300 |
| THAMAE01 | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós A | Rondônia | 2.23 | KU562301 |
| THAMAE01 | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós A | Rondônia | 2.23 | KU562302 |
| THAMAE01 | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós B | Rondônia | 2.23 | KU562303 |
| THAMAE01 | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós D | Rondônia | 2.23 | KU562304 |
| THAMAE01 | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós D | Rondônia | 2.23 | KU562305 |
| THAMAE01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós D | Rondônia | 2.23 | KU562306 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| THAMAE01 | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Tapajós B | Rondônia | 2.23 | KU562307 |
| THAMAE01 | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós D | Rondônia | 2.23 | KU562308 |
| THAMAE01 | *Plasmodium* | *Galbula cyanicollis* | Amazonia - Tapajós A | Rondônia | 2.23 | KU562309 |
| THAMAE01 | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | 2.23 | KU562310 |
| THAMAE01 | *Plasmodium* | *Dichrozona cincta* | Amazonia - Tapajós A | Rondônia | 2.23 | KU562311 |
| THAMAE01 | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | 2.23 | KU562312 |
| THAMAE01 | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Purus 02 | Inambari | 2.23 | KU562313 |
| THAMAE01 | *Plasmodium* | *Dichrozona cincta* | Amazonia - Purus 02 | Inambari | 2.23 | KU562314 |
| THAMAE01 | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Gurupi | Belém | 2.23 | KU562315 |
| THAMAE01 | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Gurupi | Belém | 2.23 | KU562316 |
| THAMAE01 | *Plasmodium* | *Dichrozona cincta* | Amazonia - Madeira 04 | Inambari | 2.23 | KU562317 |
| THAMAE01 | *Plasmodium* | *Gymnopithys salvini* | Amazonia - Madeira 04 | Inambari | 2.23 | KU562318 |
| THAMAE01 | *Plasmodium* | *Rhegmatorhina hoffmannsi* | Amazonia - Madeira 09 | Rondônia | 2.23 | KU562319 |
| THAMAE01 | *Plasmodium* | *Xiphorhynchus elegans* | Amazonia - Madeira 05 | Inambari | 2.23 | KU562320 |
| THAMB01 | *Plasmodium* | *Hypocnemis hypoxanta* | Amazonia - Negro 02 | Imerí | - | KU562605 |
| **THAMUR01** | *Plasmodium* | *Thamnophilus murinus* | Amazonia - Negro 02 | Imerí | 2 | KU562615 |
| **THAMUR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 01 | Inambari | 2 | KU562616 |
| **THAMUR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 01 | Inambari | 2 | KU562617 |
| **THAMUR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | 2 | KU562618 |
| **THAMUR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | 2 | KU562619 |
| **THAMUR01** | *Plasmodium* | *Isleria hauxwelli* | Amazonia - Purus 02 | Inambari | 2 | KU562620 |
| **THAMUR01** | *Plasmodium* | *Myrmotherula longipennis* | Amazonia - Purus 02 | Inambari | 2 | KU562621 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **THAMUR01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Maderia 03 | Inambari | 2 | KU562622 |
| **THANIG01** | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | - | KU562509 |
| **THANIG02** | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | - | KU562511 |
| **THASAT01** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | 2 | KU562361 |
| **THASAT01** | *Plasmodium* | *Myrmornis torquata* | Amazonia - Tapajós B | Rondônia | 2 | KU562362 |
| **THASAT01** | *Plasmodium* | *Hylophylax naevius* | Amazonia - Tapajós D | Rondônia | 2 | KU562363 |
| **THASAT01** | *Plasmodium* | *Myrmotherula hauxwelli* | Amazonia - Tapajós D | Rondônia | 2 | KU562364 |
| **THASAT02** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | - | KU562366 |
| **THASAT03** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | - | KU562378 |
| **THASAT04** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | - | KU562379 |
| **THASAT05** | *Haemoproteus* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | - | KU562137 |
| **THASAT06** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós A | Rondônia | 2.84 | KU562381 |
| **THASAT06** | *Plasmodium* | *Myrmoborus myotherinus* | Amazonia - Tapajós A | Rondônia | 2.84 | KU562382 |
| **THASAT06** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós A | Rondônia | 2.84 | KU562383 |
| **THASAT06** | *Plasmodium* | *Hypocnemis striata* | Amazonia - Tapajós A | Rondônia | 2.84 | KU562384 |
| **THASAT06** | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | 2.84 | KU562385 |
| **THASAT06** | *Plasmodium* | *Turdus fumigatus* | Amazonia- Tapajós IL | Rondônia | 2.84 | KU562386 |
| **THASAT06** | *Plasmodium* | *Cantorchilus leucotis* | Amazonia- Tapajós IL | Rondônia | 2.84 | KU562387 |
| **THASAT06** | *Plasmodium* | *Turdus fumigatus* | Amazonia- Tapajós IL | Rondônia | 2.84 | KU562388 |
| **THASAT06** | *Plasmodium* | *Thamnophilus nigrocinereus* | Amazonia- Tapajós IL | Rondônia | 2.84 | KU562389 |
| **THASAT06** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - CICRA | Inambari | 2.84 | KU562390 |
| **THASAT07** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós D | Rondônia | - | KU562496 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **THASAT08** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós A | Rondônia | - | KU562500 |
| **THASAT09** | *Haemoproteus* | *Thamnomanes saturninus* | Amazonia - Tapajós A | Rondônia | - | KU562159 |
| **THASAT10** | *Plasmodium* | *Thamnomanes saturninus* | Amazonia - Tapajós B | Rondônia | 2.32 | KU562403 |
| **THASAT10** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - CHU | Rondônia | 2.32 | KU562404 |
| **THASAT10** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - CHU | Rondônia | 2.32 | KU562405 |
| **THASAT10** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - COM | Rondônia | 2.32 | KU562406 |
| **THASAT10** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - CHU | Rondônia | 2.32 | KU562407 |
| **THASAT10** | *Plasmodium* | *Pipra fasciicauda* | Amazonia - Maderia 02 | Rondônia | 2.32 | KU562408 |
| **THASCH02** | *Plasmodium* | *Thamnophilus schistaceus* | Amazonia - CICRA | Inambari | - | KU562813 |
| **THASCH03** | *Haemoproteus* | *Thamnophilus schistaceus* | Amazonia - CICRA | Inambari | - | KU562244 |
| TOFLA01 | *Plasmodium* | *Pachyrhamphus validus* | Caatinga - Aiuaba | | 3 | KU562543 |
| TOFLA01 | *Plasmodium* | *Tolmomyias flaviventris* | Amazonia - Gurupi | Belém | 3 | KU562544 |
| **TOLFLA01** | *Haemoproteus* | *Tolmomyias flaviventris* | Amazonia - Tapajós H | Rondônia | - | KU562127 |
| TUAMA01 | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - CHU | Rondônia | 1 | KU562771 |
| TUAMA01 | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | 1 | KU562772 |
| TULEU06 | *Plasmodium* | *Turdus leucomelas* | Cerrado - CER | | - | KU562570 |
| TUMIG03 | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | - | KU562788 |
| **TURALB01** | *Plasmodium* | *Turdus albicollis* | Amazonia - Maderia 03 | Inambari | - | KU562808 |
| **TURAMA01** | *Plasmodium* | *Turdus amaurochalinus* | Cerrado - CER | | - | KU562569 |
| **TURAMA03** | *Plasmodium* | *Turdus amaurochalinus* | Amazonia - COM | Rondônia | - | KU562783 |
| VIOLI06[e] | *Haemoproteus* | *Cyclharis guijanensis* | Caatinga - Aiuaba | | 3 | KU562167 |
| VIOLI06[e] | *Haemoproteus* | *Lepidocolaptes angustirostris* | Caatinga - Aiuaba | | 3 | KU562168 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| VIOLI06[e] | *Haemoproteus* | *Cyclharis guijanensis* | Caatinga - Aiuaba | | 3 | KU562169 |
| VIOLI06[e] | *Haemoproteus* | *Cyclharis guijanensis* | Caatinga - Serido | | 3 | KU562170 |
| VOLJAC02 | *Plasmodium* | *Zonotrichia capensis* | Caatinga - Serido | | 1 | KU562699 |
| VOLJAC02 | *Plasmodium* | *Zonotrichia capensis* | Caatinga - Serido | | 1 | KU562700 |
| **VOLJAC03** | *Plasmodium* | *Volatinia jacarina* | Cerrado - CER | | 3 | KU562545 |
| **VOLJAC03** | *Plasmodium* | *Volatinia jacarina* | Cerrado - CER | | 3 | KU562546 |
| **VOLJAC03** | *Plasmodium* | *Polioptila paraensis* | Amazonia - Gurupi | Belém | 3 | KU562547 |
| **WILPOE01** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós J | Tapajós | - | KU562297 |
| **WILPOE02** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós I | Tapajós | 2 | KU562373 |
| **WILPOE02** | *Plasmodium* | *Phlegopsis nigromaculata* | Amazonia - Tapajós I | Tapajós | 2 | KU562374 |
| **WILPOE03** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós I | Tapajós | - | KU562375 |
| **WILPOE04** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós I | Tapajós | - | KU562377 |
| **WILPOE05** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós J | Tapajós | - | KU562380 |
| **WILPOE06** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós B | Rondônia | - | KU562401 |
| **WILPOE07** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Tapajós D | Rondônia | - | KU562411 |
| **WILPOE08** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Negro 02 | Imerí | - | KU562596 |
| **WILPOE09** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Negro 02 | Imerí | 1 | KU562597 |
| **WILPOE09** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Negro 02 | Imerí | 1 | KU562598 |
| **WILPOE10** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Negro 02 | Imerí | - | KU562599 |
| **WILPOE11** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Negro 02 | Imerí | - | KU562604 |
| **WILPOE12** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | - | KU562660 |
| **WILPOE13** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | - | KU562664 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **WILPOE14** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Purus 02 | Inambari | - | KU562665 |
| **WILPOE15** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | 3.65 | KU562716 |
| **WILPOE15** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | 3.65 | KU562717 |
| **WILPOE15** | *Plasmodium* | *Piprites chloris* | Amazonia - Gurupi | Belém | 3.65 | KU562718 |
| **WILPOE15** | *Plasmodium* | *Xiphorhynchus spixii* | Amazonia - Gurupi | Belém | 3.65 | KU562719 |
| **WILPOE15** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | 3.65 | KU562720 |
| **WILPOE15** | *Plasmodium* | *Piaya cayana* | Amazonia - Gurupi | Belém | 3.65 | KU562721 |
| **WILPOE16** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | - | KU562726 |
| **WILPOE17** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | 3 | KU562730 |
| **WILPOE17** | *Plasmodium* | *Poecilotriccus fumifrons* | Amazonia - Gurupi | Belém | 3 | KU562731 |
| **WILPOE18** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Gurupi | Belém | - | KU562732 |
| **WILPOE19** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - COM | Rondônia | 1 | KU562785 |
| **WILPOE19** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - COM | Rondônia | 1 | KU562786 |
| **WILPOE20** | *Plasmodium* | *Willisornis poecilinotus* | Amazonia - Maderia 03 | Inambari | - | KU562809 |
| **XENATR01** | *Plasmodium* | *Xenopipo atronitens* | Amazonia - COM | Rondônia | - | KU562781 |
| **XENMIN01** | *Haemoproteus* | *Xenops minutus* | Amazonia - Tapajós H | Rondônia | - | KU562124 |
| **XENMIN02** | *Plasmodium* | *Xenops minutus* | Amazonia - Tapajós D | Rondônia | - | KU562433 |
| **XENMIN03** | *Plasmodium* | *Xenops minutus* | Amazonia - Gurupi | Belém | - | KU562713 |
| **XIPELE01** | *Haemoproteus* | *Xiphorhynchus elegans* | Amazonia - Tapajós A | Rondônia | - | KU562158 |
| **XIPELE02** | *Plasmodium* | *Xiphorhynchus elegans* | Amazonia - Tapajós A | Rondônia | - | KU562499 |
| **XIPOBS01** | *Haemoproteus* | *Xiphorhynchus obsoletus* | Amazonia- Tapajós IL | Rondônia | - | KU562166 |
| **XIPOCE01** | *Plasmodium* | *Xiphorhynchus ocellatus perplexo* | Amazonia - Madeira 07 | Inambari | - | KU562810 |

| Lineage | Genus | Host | Sample Location | Area of Endemism | $S_{TD}$* | Accession |
|---|---|---|---|---|---|---|
| **XIPPAR01** | *Plasmodium* | *Xiphorhynchus pardalotus* | Amazonia - Negro 01 | Guiana | - | KU562580 |

[a] *Haemoproteus multipigmentatus*, [b] *H. paramultipigmentatus*, [c] *Plasmodium nucleophilum*, [d] *P. elongatum*, [e] *H. vireonis*

## Appendix B

### Distribution of Avian Haemosporidian Parasites Among all Host Species Collected from Brazil. *Haem/Plas* Denotes Coinfection with both *Haemoproteus* and *Plasmodium*.

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Accipitriformes** | **4** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **4** | **100.0** |
| **Accipitridae** | **4** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **4** | **100.0** |
| *Accipiter superciliosus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Leucopternis kuhli* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Leucopternis melanops* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Anseriformes** | **1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **1** | **100.0** |
| **Anhimidae** | **1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **1** | **100.0** |
| *Anhima cornuta* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Apodiformes** | **69** | **1** | **1.4** | **2** | **2.9** | **0** | **0.0** | **3** | **4.3** | **66** | **95.7** |
| **Apodidae** | **1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **1** | **100.0** |
| *Tachornis squamata* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Trochilidae** | **68** | **1** | **1.5** | **2** | **2.9** | **0** | **0.0** | **3** | **4.4** | **65** | **95.6** |
| *Amazilia versicolor* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Avocettula recurvirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Calliphlox amethystina* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Campylopterus largipennis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Chlorostilbon aureoventris* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Colibri serrirostris* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Eupetomena macroura* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Florisuga mellivora* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Glaucis hirsutus* | 7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 |
| *Heliactin bilophus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Heliomaster longirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hylocharis cyanus* | 8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 100.0 |
| *Phaethornis bourcieri* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Phaethornis malaris* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Phaethornis ruber* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Phaethornis sp.* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Phaethornis superciliosus* | 7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 |
| *Polytmus theresiae* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Thalurania furcata* | 12 | 1 | 8.3 | 0 | 0.0 | 0 | 0.0 | 1 | 8.3 | 11 | 91.7 |
| *Threnetes leucurus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Caprimulgiformes** | **14** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **14** | **100.0** |
| **Caprimulgidae** | **11** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **11** | **100.0** |
| *Antrostomus sericocaudatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hydropsalis torquata* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Nyctidromus albicollis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Nyctiprogne leucopyga* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Setopagis parvula* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| **Nyctibiidae** | **3** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **3** | **100.0** |
| *Nyctibius aethereus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Nyctibius bracteatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Charadriiformes** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| **Jacanidae** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| *Jacana jacana* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| **Columbiformes** | **49** | **11** | **22.4** | **1** | **2.0** | **2** | **4.1** | **14** | **28.6** | **35** | **71.4** |
| **Columbidae** | **49** | **11** | **22.4** | **1** | **2.0** | **2** | **4.1** | **14** | **28.6** | **35** | **71.4** |
| *Claravis pretiosa* | 3 | 1 | 33.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 66.7 |
| *Columbina minuta* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Columbina passerina* | 5 | 4 | 80.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 20.0 |
| *Columbina talpacoti* | 6 | 2 | 33.3 | 0 | 0.0 | 1 | 16.7 | 3 | 50.0 | 3 | 50.0 |
| *Geotrygon montana* | 19 | 3 | 15.8 | 1 | 5.3 | 0 | 0.0 | 0 | 0.0 | 15 | 78.9 |
| *Geotrygon violacea* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Leptotila rufaxilla* | 11 | 1 | 9.1 | 0 | 0.0 | 1 | 9.1 | 2 | 18.2 | 9 | 81.8 |
| *Leptotila verreauxi* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Patagioenas plumbea* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Coraciiformes** | **14** | **1** | **7.1** | **1** | **7.1** | **0** | **0.0** | **2** | **14.3** | **12** | **85.7** |
| **Cerylidae** | **10** | **1** | **10.0** | **1** | **10.0** | **0** | **0.0** | **2** | **20.0** | **8** | **80.0** |
| *Chloroceryle aenea* | 7 | 0 | 0.0 | 1 | 14.3 | 0 | 0.0 | 2 | 28.6 | 6 | 85.7 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Chloroceryle americana* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Chloroceryle inda* | 2 | 1 | 50.0 | 0 | 0.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| **Momotidae** | **4** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **4** | **100.0** |
| *Baryphthengus martii* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Momotus momota* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| **Cuculiformes** | **6** | **0** | **0.0** | **1** | **16.7** | **0** | **0.0** | **1** | **16.7** | **5** | **83.3** |
| **Cuculidae** | **6** | **0** | **0.0** | **1** | **16.7** | **0** | **0.0** | **1** | **16.7** | **5** | **83.3** |
| *Piaya cayana* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Tapera naevia* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Taraba major* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Falconiformes** | **8** | **1** | **12.5** | **2** | **25.0** | **0** | **0.0** | **3** | **37.5** | **5** | **62.5** |
| **Falconidae** | **8** | **1** | **12.5** | **2** | **25.0** | **0** | **0.0** | **3** | **37.5** | **5** | **62.5** |
| *Micrastur gilvicollis* | 3 | 1 | 33.3 | 0 | 0.0 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Micrastur mintoni* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Micrastur ruficollis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Micrastur semitorquatus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| **Galliformes** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| **Cracidae** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| *Penelope superciliaris* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| **Gruiformes** | **6** | **0** | **0.0** | **2** | **33.3** | **0** | **0.0** | **2** | **33.3** | **4** | **66.7** |
| **Psophiidae** | **3** | **0** | **0.0** | **2** | **66.7** | **0** | **0.0** | **2** | **66.7** | **1** | **33.3** |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Psophia ochroptera* | 3 | 0 | 0.0 | 2 | 66.7 | 0 | 0.0 | 2 | 66.7 | 1 | 33.3 |
| **Rallidae** | **3** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **3** | **100.0** |
| *Aramides cajanea* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Laterallus viridis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Laterallus exilis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Passeriformes** | **4151** | **118** | **2.8** | **525** | **12.6** | **19** | **0.5** | **662** | **15.9** | **3489** | **84.1** |
| **Cardinalidae** | **41** | **1** | **2.4** | **4** | **9.8** | **0** | **0.0** | **5** | **12.2** | **36** | **87.8** |
| *Caryothraustes canadensis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Cyanocompsa cyanoides* | 15 | 1 | 6.7 | 2 | 13.3 | 0 | 0.0 | 3 | 20.0 | 12 | 80.0 |
| *Granatellus pelzeni* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Habia rubica* | 23 | 0 | 0.0 | 2 | 8.7 | 0 | 0.0 | 2 | 8.7 | 21 | 91.3 |
| *Pheucticus aureoventris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Conopophagidae** | **22** | **0** | **0.0** | **1** | **4.5** | **0** | **0.0** | **1** | **4.5** | **21** | **95.5** |
| *Conopophaga aurita* | 12 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 1 | 8.3 | 11 | 91.7 |
| *Conopophaga peruviana* | 8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 100.0 |
| *Conopophaga roberti* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| **Corvidae** | **1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **1** | **100.0** |
| *Cyanocorax cyanopogon* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Cotingidae** | **7** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **7** | **100.0** |
| *Lipaugus vociferans* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Phoenicircus carnifex* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Xipholena lamellipennis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Dendrocolaptidae** | **547** | **8** | **1.5** | **31** | **5.7** | **2** | **0.4** | **41** | **7.5** | **506** | **92.5** |
| *Campylorhamphus procurvoides* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Campylorhamphus trochilirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Certhiasomus stictolaemus* | 23 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 23 | 100.0 |
| *Deconychura longicauda* | 18 | 1 | 5.6 | 0 | 0.0 | 0 | 0.0 | 1 | 5.6 | 17 | 94.4 |
| *Dendrexetastes rufigula* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Dendrocincla fuliginosa* | 39 | 2 | 5.1 | 5 | 12.8 | 0 | 0.0 | 7 | 17.9 | 32 | 82.1 |
| *Dendrocincla merula* | 78 | 1 | 1.3 | 6 | 7.7 | 0 | 0.0 | 7 | 9.0 | 71 | 91.0 |
| *Dendrocolaptes certhia* | 11 | 1 | 9.1 | 2 | 18.2 | 0 | 0.0 | 3 | 27.3 | 8 | 72.7 |
| *Dendrocolaptes medius* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Dendrocolaptes picumnus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Dendrocolaptes platyrostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Dendroplex kienerii* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Dendroplex picus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Glyphorhynchus spirurus* | 150 | 0 | 0.0 | 8 | 5.3 | 1 | 0.7 | 9 | 6.0 | 141 | 94.0 |
| *Gymnopithys salvini* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Hylexetastes perrotii* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Hylexetastes uniformis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Lepidocolaptes angustirostris* | 44 | 1 | 2.3 | 1 | 2.3 | 0 | 0.0 | 2 | 4.5 | 42 | 95.5 |
| *Lepidocolaptes layardi* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |

| Brazilian Avian Hosts | Samples | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Sittasomus griseicapillus* | 7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 |
| *Xenops minutus* | 40 | 1 | 2.5 | 2 | 5.0 | 0 | 0.0 | 3 | 7.5 | 37 | 92.5 |
| *Xenops sp.* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Xiphorhynchus elegans* | 56 | 0 | 0.0 | 1 | 1.8 | 1 | 1.8 | 2 | 3.6 | 54 | 96.4 |
| *Xiphorhynchus guttatus* | 10 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 10 | 100.0 |
| *Xiphorhynchus obsoletus* | 5 | 1 | 20.0 | 0 | 0.0 | 0 | 0.0 | 1 | 20.0 | 4 | 80.0 |
| *Xiphorhynchus ocellatus* | 13 | 0 | 0.0 | 1 | 7.7 | 0 | 0.0 | 1 | 7.7 | 12 | 92.3 |
| *Xiphorhynchus ocellatus perplexo* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Xiphorhynchus pardalotus* | 26 | 0 | 0.0 | 1 | 3.8 | 0 | 0.0 | 1 | 3.8 | 25 | 96.2 |
| *Xiphorhynchus spixii* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| **Donacobiidae** | **6** | **0** | **0.0** | **2** | **33.3** | **0** | **0.0** | **2** | **33.3** | **4** | **66.7** |
| *Donacobius atricapilla* | 6 | 0 | 0.0 | 2 | 33.3 | 0 | 0.0 | 2 | 33.3 | 4 | 66.7 |
| **Emberizidae** | **159** | **5** | **3.1** | **17** | **10.7** | **0** | **0.0** | **22** | **13.8** | **137** | **86.2** |
| *Ammodramus humeralis* | 42 | 3 | 7.1 | 0 | 0.0 | 0 | 0.0 | 3 | 7.1 | 39 | 92.9 |
| *Arremon taciturnus* | 20 | 0 | 0.0 | 9 | 45.0 | 0 | 0.0 | 9 | 45.0 | 11 | 55.0 |
| *Charitospiza eucosma* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Emberizoides herbicola* | 14 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 14 | 100.0 |
| *Sicalis citrina* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Sporophila albogularis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Sporophila americana* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Sporophila angolensis* | 22 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 22 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Sporophila minuta* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Sporophila nigricollis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Sporophila plumbea* | 18 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 18 | 100.0 |
| *Volatinia jacarina* | 30 | 2 | 6.7 | 5 | 16.7 | 0 | 0.0 | 7 | 23.3 | 23 | 76.7 |
| *Zonotrichia capensis* | 2 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 |
| **Formicariidae** | **36** | **0** | **0.0** | **14** | **38.9** | **0** | **0.0** | **14** | **38.9** | **22** | **61.1** |
| *Formicarius analis* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Formicarius colma* | 31 | 0 | 0.0 | 14 | 45.2 | 0 | 0.0 | 14 | 45.2 | 17 | 54.8 |
| **Fringillidae** | **6** | **0** | **0.0** | **1** | **16.7** | **0** | **0.0** | **1** | **16.7** | **5** | **83.3** |
| *Euphonia chlorotica* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Euphonia laniirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Euphonia plumbea* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Euphonia xanthogaster* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| **Furnariidae** | **241** | **5** | **2.1** | **17** | **7.1** | **1** | **0.4** | **23** | **9.5** | **218** | **90.5** |
| *Autolomus subulatus* | 12 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 12 | 100.0 |
| *Automolus infuscatus* | 29 | 1 | 3.4 | 5 | 17.2 | 1 | 3.4 | 7 | 24.1 | 22 | 75.9 |
| *Automolus melanopezus* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Automolus melanozenops* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Automolus ochrolaemus* | 24 | 2 | 8.3 | 4 | 16.7 | 0 | 0.0 | 6 | 25.0 | 18 | 75.0 |
| *Automolus paraensis* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Automolus rubiginosus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Automolus rufipileatus* | 10 | 1 | 10.0 | 2 | 20.0 | 0 | 0.0 | 3 | 30.0 | 7 | 70.0 |
| *Cranioleuca vulpina* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Crotophaga ani* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Furnarius leucopus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Microcerculus marginatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Phacellodomus ruber* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Phacellodomus rufifrons* | 62 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 62 | 100.0 |
| *Philydor erythrocercum* | 11 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 11 | 100.0 |
| *Philydor erythropterum* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Philydor pyrrhodes* | 9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 9 | 100.0 |
| *Philydor ruficaudatum* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Sclerurus caudacutus* | 6 | 1 | 16.7 | 1 | 16.7 | 0 | 0.0 | 2 | 33.3 | 4 | 66.7 |
| *Sclerurus mexicanus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Sclerurus rufigularis* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |
| *Synallaxis albescens* | 14 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 14 | 100.0 |
| *Synallaxis gujanensis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Synallaxis rutilans* | 26 | 0 | 0.0 | 1 | 3.8 | 0 | 0.0 | 1 | 3.8 | 25 | 96.2 |
| *Synallaxis scutata* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Syndactyla ucayalae* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Hirundinidae** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| *Stelgidopteryx ruficollis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| Brazilian Avian Hosts | Samples | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Icteridae** | **5** | **1** | **20.0** | **2** | **40.0** | **0** | **0.0** | **3** | **60.0** | **2** | **40.0** |
| *Cacicus solitarius* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Psarocolius bifasciatus* | 2 | 1 | 50.0 | 1 | 50.0 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 |
| **Melanopareiidae** | **4** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **4** | **100.0** |
| *Melanopareia torquata* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| **Mimidae** | **15** | **0** | **0.0** | **3** | **20.0** | **0** | **0.0** | **3** | **20.0** | **12** | **80.0** |
| *Mimus saturninus* | 15 | 0 | 0.0 | 3 | 20.0 | 0 | 0.0 | 3 | 20.0 | 12 | 80.0 |
| **Parulidae** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| *Basileuterus culicivorus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myiothlypis flaveola* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Pipridae** | **486** | **2** | **0.4** | **70** | **14.4** | **0** | **0.0** | **72** | **14.8** | **414** | **85.2** |
| *Ceratopipra chloromeros* | 10 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 10 | 100.0 |
| *Ceratopipra erythrocephala* | 7 | 0 | 0.0 | 3 | 42.9 | 0 | 0.0 | 3 | 42.9 | 4 | 57.1 |
| *Ceratopipra erythroptera* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Ceratopipra rubrocapilla* | 67 | 1 | 1.5 | 9 | 13.4 | 0 | 0.0 | 10 | 14.9 | 57 | 85.1 |
| *Chiroxiphia pareola* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Chiroxiphia pareola regina* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Dixiphia pipra* | 22 | 0 | 0.0 | 5 | 22.7 | 0 | 0.0 | 5 | 22.7 | 17 | 77.3 |
| *Heterocercus linteatus* | 13 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 |
| *Lepidothrix coronata* | 107 | 0 | 0.0 | 13 | 12.1 | 0 | 0.0 | 13 | 12.1 | 94 | 87.9 |
| *Lepidothrix iris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Lepidothrix nattereri* | 71 | 0 | 0.0 | 16 | 22.5 | 0 | 0.0 | 16 | 22.5 | 55 | 77.5 |
| *Lepidothrix vilasboasi* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Machaeropterus pyrocephalus* | 18 | 1 | 5.6 | 2 | 11.1 | 0 | 0.0 | 3 | 16.7 | 15 | 83.3 |
| *Manacus manacus* | 9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 9 | 100.0 |
| *Neopelma pallescens* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Pipra fasciicauda* | 128 | 0 | 0.0 | 16 | 12.5 | 0 | 0.0 | 16 | 12.5 | 112 | 87.5 |
| *Piprites chloris* | 8 | 0 | 0.0 | 2 | 25.0 | 0 | 0.0 | 2 | 25.0 | 6 | 75.0 |
| *Tyranneutes stolzmanni* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Xenopipo atronitens* | 7 | 0 | 0.0 | 2 | 28.6 | 0 | 0.0 | 2 | 28.6 | 5 | 71.4 |
| **Polioptilidae** | **10** | **0** | **0.0** | **2** | **20.0** | **0** | **0.0** | **2** | **20.0** | **8** | **80.0** |
| *Microbates collaris* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Polioptila paraensis* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Polioptila plumbea* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Ramphocaenus melanurus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Scleruridae** | **9** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **9** | **100.0** |
| *Sclerurus caudacutus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Sclerurus rufigularis* | 8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 100.0 |
| **Thamnophilidae** | **1168** | **16** | **1.4** | **221** | **18.9** | **5** | **0.4** | **242** | **20.7** | **926** | **79.3** |
| *Cercomacra cinerascens* | 5 | 1 | 20.0 | 3 | 60.0 | 0 | 0.0 | 4 | 80.0 | 1 | 20.0 |
| *Cercomacra nigrescens* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Cercomacra serva* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Cymbilaimus lineatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Cymbilaimus sanctaemariae* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Dichrozona cincta* | 11 | 0 | 0.0 | 4 | 36.4 | 0 | 0.0 | 4 | 36.4 | 7 | 63.6 |
| *Dysithamnus mentalis* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Epinecrophylla haematonota* | 50 | 0 | 0.0 | 1 | 2.0 | 0 | 0.0 | 1 | 2.0 | 49 | 98.0 |
| *Epinecrophylla leucophthalma* | 30 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 30 | 100.0 |
| *Epinecrophylla ornata* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Formicivora grisea* | 2 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 |
| *Formicivora melanogaster* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Frederickena viridis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Gymnopithys leucaspis* | 7 | 0 | 0.0 | 1 | 14.3 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| *Gymnopithys rufigula* | 7 | 0 | 0.0 | 3 | 42.9 | 0 | 0.0 | 3 | 42.9 | 4 | 57.1 |
| *Gymnopithys salvini* | 62 | 1 | 1.6 | 8 | 12.9 | 0 | 0.0 | 9 | 14.5 | 53 | 85.5 |
| *Hylophylax naevius* | 15 | 0 | 0.0 | 3 | 20.0 | 0 | 0.0 | 3 | 20.0 | 12 | 80.0 |
| *Hylophylax punctulatus* | 5 | 0 | 0.0 | 0 | 0.0 | 1 | 20.0 | 1 | 20.0 | 4 | 80.0 |
| *Hypocnemis cantator* | 6 | 0 | 0.0 | 4 | 66.7 | 0 | 0.0 | 4 | 66.7 | 2 | 33.3 |
| *Hypocnemis flavescens* | 7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 |
| *Hypocnemis hypoxanta* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Hypocnemis ochrogyna* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Hypocnemis peruviana* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Hypocnemis striata* | 32 | 2 | 6.3 | 17 | 53.1 | 2 | 6.3 | 21 | 65.6 | 11 | 34.4 |

| Brazilian Avian Hosts | Samples | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Hypocnemis subflava* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Hypocnemoides maculicauda* | 10 | 1 | 10.0 | 0 | 0.0 | 0 | 0.0 | 1 | 10.0 | 9 | 90.0 |
| *Isleria guttata* | 6 | 0 | 0.0 | 2 | 33.3 | 0 | 0.0 | 2 | 33.3 | 4 | 66.7 |
| *Isleria hauxwelli* | 42 | 1 | 2.4 | 7 | 16.7 | 0 | 0.0 | 7 | 16.7 | 34 | 81.0 |
| *Megastictus margaritatus* | 10 | 0 | 0.0 | 1 | 10.0 | 0 | 0.0 | 1 | 10.0 | 9 | 90.0 |
| *Microrhopias quixensis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Myrmeciza ferruginea* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myrmeciza fortis* | 13 | 0 | 0.0 | 3 | 23.1 | 0 | 0.0 | 3 | 23.1 | 10 | 76.9 |
| *Myrmeciza goeldii* | 5 | 0 | 0.0 | 1 | 20.0 | 0 | 0.0 | 1 | 20.0 | 4 | 80.0 |
| *Myrmeciza hemimelaena* | 25 | 0 | 0.0 | 3 | 12.0 | 0 | 0.0 | 3 | 12.0 | 22 | 88.0 |
| *Myrmoborus leucophrys* | 10 | 0 | 0.0 | 1 | 10.0 | 0 | 0.0 | 1 | 10.0 | 9 | 90.0 |
| *Myrmoborus myotherinus* | 81 | 2 | 2.5 | 16 | 19.8 | 0 | 0.0 | 18 | 22.2 | 63 | 77.8 |
| *Myrmornis torquata* | 9 | 0 | 0.0 | 2 | 22.2 | 0 | 0.0 | 2 | 22.2 | 7 | 77.8 |
| *Myrmotherula axillaris* | 82 | 1 | 1.2 | 21 | 25.6 | 0 | 0.0 | 21 | 25.6 | 60 | 73.2 |
| *Myrmotherula hauxwelli* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Myrmotherula iheringi* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myrmotherula longicauda* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myrmotherula longipennis* | 66 | 0 | 0.0 | 9 | 13.6 | 0 | 0.0 | 9 | 13.6 | 57 | 86.4 |
| *Myrmotherula menetriesii* | 7 | 0 | 0.0 | 1 | 14.3 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| *Myrmotherula multostriata* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Neoctantes niger* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Percnostola minor* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Percnostola rufifrons* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Phlegopsis erythroptera* | 10 | 0 | 0.0 | 1 | 10.0 | 0 | 0.0 | 1 | 10.0 | 9 | 90.0 |
| *Phlegopsis nigromaculata* | 45 | 4 | 8.9 | 14 | 31.1 | 0 | 0.0 | 18 | 40.0 | 27 | 60.0 |
| *Pithys albifrons* | 24 | 0 | 0.0 | 3 | 12.5 | 0 | 0.0 | 3 | 12.5 | 21 | 87.5 |
| *Pygiptila stellaris* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Pyriglena leuconota* | 21 | 0 | 0.0 | 6 | 28.6 | 0 | 0.0 | 6 | 28.6 | 15 | 71.4 |
| *Rhegmatorhina berlepschi* | 12 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 1 | 8.3 | 11 | 91.7 |
| *Rhegmatorhina gymnops* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Rhegmatorhina hoffmannsi* | 11 | 0 | 0.0 | 1 | 9.1 | 0 | 0.0 | 1 | 9.1 | 10 | 90.9 |
| *Rhegmatorhina melanosticta* | 31 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 31 | 100.0 |
| *Schistocichla humaythae* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Schistocichla leucostigma* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Sclateria naevia* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Thamnomanes ardesiacus* | 20 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 20 | 100.0 |
| *Thamnomanes caesius* | 50 | 0 | 0.0 | 7 | 14.0 | 1 | 2.0 | 8 | 16.0 | 42 | 84.0 |
| *Thamnomanes saturninus* | 30 | 1 | 3.3 | 9 | 30.0 | 1 | 3.3 | 11 | 36.7 | 19 | 63.3 |
| *Thamnomanes schistogynus* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |
| *Thamnomanes sp.* | 9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 9 | 100.0 |
| *Thamnophilus torquatus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Thamnophilus aethiops* | 53 | 0 | 0.0 | 7 | 13.2 | 0 | 0.0 | 7 | 13.2 | 46 | 86.8 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Thamnophilus amazonicus* | 2 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 |
| *Thamnophilus capistratus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Thamnophilus doliatus* | 2 | 1 | 50.0 | 0 | 0.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Thamnophilus murinus* | 13 | 0 | 0.0 | 4 | 30.8 | 0 | 0.0 | 4 | 30.8 | 9 | 69.2 |
| *Thamnophilus nigrocinereus* | 11 | 0 | 0.0 | 7 | 63.6 | 0 | 0.0 | 7 | 63.6 | 4 | 36.4 |
| *Thamnophilus palliatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Thamnophilus pelzelni* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Thamnophilus schistaceus* | 14 | 1 | 7.1 | 3 | 21.4 | 0 | 0.0 | 4 | 28.6 | 10 | 71.4 |
| *Thamnophilus stictocephalus* | 7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 |
| *Willisornis poecilinotus* | 120 | 0 | 0.0 | 30 | 25.0 | 0 | 0.0 | 30 | 25.0 | 90 | 75.0 |
| **Thraupidae** | **350** | **45** | **12.9** | **70** | **20.0** | **11** | **3.1** | **126** | **36.0** | **224** | **64.0** |
| *Chlorophanes spiza* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Coereba flaveola* | 7 | 0 | 0.0 | 2 | 28.6 | 0 | 0.0 | 2 | 28.6 | 5 | 71.4 |
| *Conothraupis speculigera* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Coryphospingus cucullatus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Coryphospingus pileatus* | 43 | 2 | 4.7 | 15 | 34.9 | 4 | 9.3 | 21 | 48.8 | 22 | 51.2 |
| *Cyanerpes cyaneus* | 1 | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Cypsnagra hirundinacea* | 22 | 10 | 45.5 | 3 | 13.6 | 2 | 9.1 | 15 | 68.2 | 7 | 31.8 |
| *Dacnis cayana* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Hemithraupis guira* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Lanio fulvus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Lanio versicolor* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Neothraupis fasciata* | 78 | 22 | 28.2 | 10 | 12.8 | 4 | 5.1 | 36 | 46.2 | 44 | 56.4 |
| *Paroaria capitata* | 57 | 1 | 1.8 | 10 | 17.5 | 0 | 0.0 | 11 | 19.3 | 46 | 80.7 |
| *Paroaria coronata* | 13 | 0 | 0.0 | 2 | 15.4 | 0 | 0.0 | 2 | 15.4 | 11 | 84.6 |
| *Ramphocelus carbo* | 40 | 0 | 0.0 | 14 | 35.0 | 0 | 0.0 | 14 | 35.0 | 26 | 65.0 |
| *Saltator atricollis* | 5 | 0 | 0.0 | 2 | 40.0 | 0 | 0.0 | 2 | 40.0 | 3 | 60.0 |
| *Saltator coerulescens* | 13 | 1 | 7.7 | 2 | 15.4 | 0 | 0.0 | 3 | 23.1 | 10 | 76.9 |
| *Saltator grossus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Saltator maximus* | 9 | 0 | 0.0 | 1 | 11.1 | 0 | 0.0 | 1 | 11.1 | 8 | 88.9 |
| *Schistochlamys ruficapillus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Tachyphonus cristatus* | 5 | 2 | 40.0 | 1 | 20.0 | 1 | 20.0 | 4 | 80.0 | 1 | 20.0 |
| *Tachyphonus luctuosus* | 1 | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Tachyphonus phoenicius* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Tachyphonus rufus* | 11 | 4 | 36.4 | 3 | 27.3 | 0 | 0.0 | 7 | 63.6 | 4 | 36.4 |
| *Tachyphonus surinamus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Taeniotriccus andrei* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Tangara cayana* | 7 | 1 | 14.3 | 0 | 0.0 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| *Tangara schrankii* | 5 | 0 | 0.0 | 1 | 20.0 | 0 | 0.0 | 1 | 20.0 | 4 | 80.0 |
| *Thraupis episcopus* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| **Tityridae** | **89** | **3** | **3.4** | **4** | **4.5** | **0** | **0.0** | **7** | **7.9** | **82** | **92.1** |
| *Laniocera hypopyrra* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Myiobius atricaudus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myiobius barbatus* | 12 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 1 | 8.3 | 11 | 91.7 |
| *Onychorhynchus coronatus* | 22 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 22 | 100.0 |
| *Pachyramphus marginatus* | 7 | 1 | 14.3 | 0 | 0.0 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| *Pachyramphus minor* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Pachyramphus polychopterus* | 1 | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Pachyramphus rufus* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Pachyramphus validus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Schiffornis amazona* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Schiffornis turdina* | 32 | 1 | 3.1 | 1 | 3.1 | 0 | 0.0 | 2 | 6.3 | 30 | 93.8 |
| **Troglodytidae** | **86** | **2** | **2.3** | **11** | **12.8** | **0** | **0.0** | **13** | **15.1** | **73** | **84.9** |
| *Campylorhynchus turdinus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Cantorchilus leucotis* | 15 | 1 | 6.7 | 4 | 26.7 | 0 | 0.0 | 5 | 33.3 | 9 | 60.0 |
| *Cantorchilus longirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Cyphorhinus arada* | 15 | 0 | 0.0 | 3 | 20.0 | 0 | 0.0 | 3 | 20.0 | 12 | 80.0 |
| *Microcerculus marginatus* | 21 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 21 | 100.0 |
| *Pheugopedius coraya* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Pheugopedius genibarbis* | 23 | 0 | 0.0 | 3 | 13.0 | 0 | 0.0 | 3 | 13.0 | 20 | 87.0 |
| *Troglodytes musculus* | 9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 9 | 100.0 |
| **Turdidae** | **85** | **0** | **0.0** | **17** | **18.5** | **0** | **0.0** | **17** | **18.5** | **68** | **80.0** |
| *Turdus albicollis* | 23 | 0 | 0.0 | 1 | 4.3 | 0 | 0.0 | 1 | 4.3 | 22 | 95.7 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Turdus amaurochalinus* | 35 | 0 | 0.0 | 9 | 25.7 | 0 | 0.0 | 9 | 25.7 | 26 | 74.3 |
| *Turdus fumigatus* | 8 | 0 | 0.0 | 2 | 25.0 | 0 | 0.0 | 2 | 25.0 | 6 | 75.0 |
| *Turdus hauxwelli* | 9 | 0 | 0.0 | 4 | 44.4 | 0 | 0.0 | 4 | 44.4 | 5 | 55.6 |
| *Turdus ignobilis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Turdus leucomelas* | 5 | 0 | 0.0 | 1 | 20.0 | 0 | 0.0 | 1 | 20.0 | 4 | 80.0 |
| *Turdus ruviventris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Turdus subalaris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Tyrannidae** | **733** | **27** | **3.7** | **37** | **5.1** | **0** | **0.0** | **64** | **8.8** | **669** | **91.3** |
| *Attila cinnamoneus* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Attila spadiceus* | 13 | 0 | 0.0 | 1 | 7.7 | 0 | 0.0 | 1 | 7.7 | 12 | 92.3 |
| *Camptostoma obsoletum* | 26 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 26 | 100.0 |
| *Casiornis fuscus* | 6 | 0 | 0.0 | 1 | 16.7 | 0 | 0.0 | 1 | 16.7 | 5 | 83.3 |
| *Casiornis rufus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Cnemotriccus fuscatus* | 20 | 0 | 0.0 | 1 | 5.0 | 0 | 0.0 | 1 | 5.0 | 19 | 95.0 |
| *Cnipodectes subbrunneus* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Corythopis torquatus* | 15 | 0 | 0.0 | 1 | 6.7 | 0 | 0.0 | 1 | 6.7 | 14 | 93.3 |
| *Culicivora caudacuta* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Elaenia chilensis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Elaenia chiriquensis* | 138 | 5 | 3.6 | 2 | 1.4 | 0 | 0.0 | 7 | 5.1 | 131 | 94.9 |
| *Elaenia cristata* | 96 | 0 | 0.0 | 5 | 5.2 | 0 | 0.0 | 5 | 5.2 | 91 | 94.8 |
| *Elaenia flavogaster* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Elaenia parvirostris* | 10 | 2 | 20.0 | 0 | 0.0 | 0 | 0.0 | 2 | 20.0 | 8 | 80.0 |
| *Elaenia ruficeps* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Empidonomus varius* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Euscarthmus rufomarginatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Fluvicola nengeta* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Griseotyrannus aurantioatrocristatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hemitriccus flammulatus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Hemitriccus griseipectus* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Hemitriccus inornatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hemitriccus iohannis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Hemitriccus margaritaceiventer* | 15 | 0 | 0.0 | 1 | 6.7 | 0 | 0.0 | 1 | 6.7 | 14 | 93.3 |
| *Hemitriccus minor* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |
| *Hemitriccus minor pallens* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hemitriccus striaticollis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Hemitriccus zosterops* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Knipolegus poecilocercus* | 6 | 1 | 16.7 | 0 | 0.0 | 0 | 0.0 | 1 | 16.7 | 5 | 83.3 |
| *Lathrotriccus euleri* | 4 | 1 | 25.0 | 0 | 0.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Leptopogon amaurocephalus* | 18 | 0 | 0.0 | 2 | 18.2 | 0 | 0.0 | 2 | 18.2 | 16 | 88.9 |
| *Lophotriccus eulophotes* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Lophotriccus galeatus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Mionectes macconnelli* | 20 | 0 | 0.0 | 2 | 10.0 | 0 | 0.0 | 2 | 10.0 | 18 | 90.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Mionectes oleagineus* | 34 | 0 | 0.0 | 1 | 2.9 | 0 | 0.0 | 1 | 2.9 | 33 | 97.1 |
| *Myiarchus swainsoni* | 40 | 3 | 7.5 | 3 | 7.5 | 0 | 0.0 | 6 | 15.0 | 34 | 85.0 |
| *Myiarchus ferox* | 8 | 0 | 0.0 | 1 | 12.5 | 0 | 0.0 | 1 | 12.5 | 7 | 87.5 |
| *Myiarchus sp.* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myiarchus swainsoni* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Myiarchus tyrannulus* | 15 | 0 | 0.0 | 4 | 26.7 | 0 | 0.0 | 4 | 26.7 | 11 | 73.3 |
| *Myiodynastes maculatus* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Myiopagis caniceps* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Myiopagis gaimardii* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Myiopagis viridicata* | 8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 100.0 |
| *Myiophobus fasciatus* | 7 | 1 | 14.3 | 0 | 0.0 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| *Myiornis sp.* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Myiozetetes cayanensis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Myiozetetes similis* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Ornithion inerme* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Phaeomyias murina* | 13 | 1 | 7.7 | 0 | 0.0 | 0 | 0.0 | 1 | 7.7 | 12 | 92.3 |
| *Phylloscartes virescens* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Pitangus sulphuratus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Platyrinchus coronatus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Platyrinchus platyrhynchos* | 15 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 15 | 100.0 |
| *Platyrinchus saturatus* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Poecilotriccus fumifrons* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Poecilotriccus latirostris* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Poecilotriccus sylvia* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Ramphotrigon fuscicauda* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Ramphotrigon megacephalum* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Ramphotrigon ruficauda* | 12 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 1 | 8.3 | 11 | 91.7 |
| *Rhynchocyclus olivaceus* | 4 | 0 | 0.0 | 1 | 25.0 | 0 | 0.0 | 1 | 25.0 | 3 | 75.0 |
| *Rhytipterna immunda* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Rhytipterna simplex* | 10 | 0 | 0.0 | 3 | 30.0 | 0 | 0.0 | 3 | 30.0 | 7 | 70.0 |
| *Sublegatus modestus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Suiriri islerorum* | 16 | 0 | 0.0 | 2 | 12.5 | 0 | 0.0 | 2 | 12.5 | 14 | 87.5 |
| *Suiriri suiriri* | 31 | 12 | 38.7 | 0 | 0.0 | 0 | 0.0 | 12 | 38.7 | 19 | 61.3 |
| *Terenotriccus erythrurus* | 23 | 0 | 0.0 | 1 | 4.3 | 0 | 0.0 | 1 | 4.3 | 22 | 95.7 |
| *Todirostrum cinereum* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Tolmomyias flaviventris* | 8 | 1 | 12.5 | 1 | 12.5 | 0 | 0.0 | 2 | 25.0 | 6 | 75.0 |
| *Tolmomyias sulphurescens* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Tyrannus melancholichus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Tyrannus savana* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| **Vireonidae** | **41** | **3** | **7.3** | **1** | **2.4** | **0** | **0.0** | **4** | **9.8** | **37** | **90.2** |
| *Cyclarhis gujanensis* | 21 | 3 | 14.3 | 0 | 0.0 | 0 | 0.0 | 3 | 14.3 | 18 | 85.7 |
| *Hylophilus amaurochalinus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Hylophilus brunneiceps* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hylophilus ochraceiceps* | 16 | 0 | 0.0 | 1 | 6.3 | 0 | 0.0 | 1 | 6.3 | 15 | 93.8 |
| *Hylophilus semicinereus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Hylophilus sp.* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Piciformes** | **158** | **22** | **13.9** | **15** | **9.5** | **1** | **0.6** | **38** | **24.1** | **120** | **75.9** |
| **Bucconidae** | **62** | **19** | **30.6** | **2** | **3.2** | **1** | **1.6** | **22** | **35.5** | **40** | **64.5** |
| *Bucco capensis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Bucco tamatia* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Malacoptila fusca* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Malacoptila rufa* | 13 | 1 | 7.7 | 1 | 7.7 | 0 | 0.0 | 2 | 15.4 | 11 | 84.6 |
| *Malacoptila semicincta* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |
| *Monasa morphoeus* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |
| *Monasa nigrifrons* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Nonnula ruficapilla* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Notharchus tectus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Nystalus chacuru* | 18 | 14 | 77.8 | 0 | 0.0 | 1 | 5.6 | 15 | 83.3 | 3 | 16.7 |
| *Nystalus maculatus* | 8 | 4 | 50.0 | 0 | 0.0 | 0 | 0.0 | 4 | 50.0 | 4 | 50.0 |
| **Capitonidae** | **1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **1** | **100.0** |
| *Capito auratus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Galbulidae** | **38** | **2** | **5.3** | **9** | **23.7** | **0** | **0.0** | **11** | **28.9** | **27** | **71.1** |
| *Brachygalba lugubris* | 4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 100.0 |

| | | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Brazilian Avian Hosts | Samples | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Galbula albirostris* | 5 | 0 | 0.0 | 1 | 20.0 | 0 | 0.0 | 1 | 20.0 | 4 | 80.0 |
| *Galbula cyanescens* | 6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 |
| *Galbula cyanicollis* | 15 | 2 | 13.3 | 8 | 53.3 | 0 | 0.0 | 10 | 66.7 | 5 | 33.3 |
| *Galbula ruficauda* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Jacamerops aureus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| **Picidae** | **49** | **0** | **0.0** | **3** | **6.1** | **0** | **0.0** | **3** | **6.1** | **45** | **91.8** |
| *Campephilus rubricollis* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 66.7 |
| *Celeus elegans* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Celeus grammicus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Celeus undatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Colaptes campestris* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Melanerpes cruentatus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Piculus flavigula* | 3 | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 1 | 33.3 | 2 | 66.7 |
| *Picumnus fulvescens* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Picumnus pygmaeus* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Picumnus rufiventris* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Veniliornis affinis* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Veniliornis mixtus* | 15 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 15 | 100.0 |
| *Veniliornis passerinus* | 7 | 0 | 0.0 | 1 | 14.3 | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 |
| **Ramphastidae** | **8** | **0** | **0.0** | **1** | **12.5** | **0** | **0.0** | **1** | **12.5** | **7** | **87.5** |
| *Pteroglossus aracari* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |

| Brazilian Avian Hosts | Samples | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Pteroglossus viridis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Ramphastos tucanus* | 1 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| *Ramphastos vitellinus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Selenidera gouldii* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Selenidera reinwardtii* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Psittaciformes** | **20** | **2** | **10.0** | **3** | **15.0** | **0** | **0.0** | **5** | **25.0** | **15** | **75.0** |
| **Psittacidae** | **20** | **2** | **10.0** | **3** | **15.0** | **0** | **0.0** | **5** | **25.0** | **15** | **75.0** |
| *Aratinga aurea* | 2 | 2 | 100.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 |
| *Aratinga cactorum* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Aratinga jandaya* | 2 | 0 | 0.0 | 1 | 50.0 | 0 | 0.0 | 1 | 50.0 | 1 | 50.0 |
| *Aratinga nenday* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |
| *Aratinga pertinax* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Brotogeris chiriri* | 5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 |
| *Myiopsitta monachus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Pionites leucogaster* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Pyrrhura lepida* | 3 | 0 | 0.0 | 2 | 66.7 | 0 | 0.0 | 2 | 66.7 | 1 | 33.3 |
| **Strigiformes** | **9** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **9** | **100.0** |
| **Strigidae** | **9** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **9** | **100.0** |
| *Ciccaba hulula* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Ciccaba virgata* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Glaucidium brasilianum* | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 |

| Brazilian Avian Hosts | Samples | *Haemoproteus* | | *Plasmodium* | | *Haem/Plas* | | Total Infected | | Uninfected | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| *Megascops usta* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Megascops watsonii* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Pulsatrix perspicillata* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Tinamiformes** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| **Tinamidae** | **2** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **2** | **100.0** |
| *Crypturellus parvirostris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Crypturellus soui* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| **Trogoniformes** | **6** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **6** | **100.0** |
| **Trogonidae** | **6** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** | **6** | **100.0** |
| *Trogon collaris* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Trogon rufus* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| *Trogon rufus chryochlorus* | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| *Trogon viridis* | 2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 100.0 |
| **Grand Total** | **4521** | **156** | **3.5** | **552** | **12.2** | **22** | **0.5** | **730** | **16.1** | **3791** | **83.9** |

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