



Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds

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Identifying the mechanisms driving the distribution and diversity of parasitic organisms and characterizing the structure of parasite assemblages are critical to understanding host–parasite evolution, community dynamics, and disease transmission risk. Haemosporidian parasites of the genera *Plasmodium* and *Haemoproteus* are a diverse and cosmopolitan group of bird pathogens. Despite their global distribution, the ecological and historical factors shaping the diversity and distribution of these protozoan parasites across avian communities and geographic regions remain unclear. Here we used a region of the mitochondrial cytochrome *b* gene to characterize the diversity, biogeographical patterns, and phylogenetic relationships of *Plasmodium* and *Haemoproteus* infecting Amazonian birds. Specifically, we asked whether, and how, host community similarity and geography (latitude and area of endemism) structure parasite assemblages across 15 avian communities in the Amazon Basin. We identified 265 lineages of haemosporidians recovered from 2661 sampled birds from 330 species. Infection prevalence varied widely among host species, avian communities, areas of endemism, and latitude. Composition analysis demonstrated that both malarial parasites and host communities differed across areas of endemism and as a function of latitude. Thus, areas with similar avian community composition were similar in their parasite communities. Our analyses, within a regional biogeographic context, imply that host switching is the main event promoting diversification in malarial parasites. Although dispersal of haemosporidian parasites was constrained across six areas of endemism, these pathogens are not dispersal-limited among communities within the same area of endemism. Our findings indicate that the distribution of malarial parasites in Amazonian birds is largely dependent on local ecological conditions and host evolutionary relationships.

Parasites and pathogens can play an important role in structuring biological communities and maintaining biodiversity (Daszak et al. 2000, Ricklefs 2010). For instance, parasites can influence interactions among competitors and predators (Hatcher et al. 2006), impact the host extinction dynamics (McCallum and Dobson 1995), affect ecosystem functioning and productivity (Hudson et al. 2006), and the success of introduced host species in their non-native range (Torchin et al. 2003). However, the processes structuring these often-hidden communities of parasites and pathogens remain poorly understood, especially when the parasite community is highly diverse and its taxonomic composition is not well established. Therefore, characterizing the structure of parasite associations is the first step in understanding

host–parasite evolution, community dynamics, and disease transmission risk.

Ecological factors may shape parasite assemblage structure because of their potential influence on colonization and extinction of these organisms (Poulin 1997, 2007, Clayton et al. 2016). For example, for free-living organisms, similarity in species composition among communities decreases with increasing geographic distance (Nekola and White 1999), and this ecological pattern has also been demonstrated for parasites (Poulin 2003). This increasing dissimilarity in parasite assemblages with increased geographic distance could be caused by two mechanisms: 1) a decrease in environmental similarity with distance (e.g. gradients in temperature and precipitation, turnover of host species, genetic differences

within a host species) or 2) limits to dispersal (Nekola and White 1999). Historical factors have also been implicated as a determinant of parasite richness. For instance, parasites shifting from co-occurring hosts are more likely to invade and colonize a new host species that is closely related to their current host, therefore similarity and relatedness of hosts within a community may shape its parasite fauna (Poulin 1997, Ricklefs et al. 2014). If evolutionary history of hosts constrains parasite diversity, then one might expect closely related hosts to harbor parasite lineages that are more phylogenetically close than expected by chance.

Avian Haemosporidia from the genera *Plasmodium* and *Haemoproteus* comprise a diverse group of blood parasites that infect birds on all continents, except Antarctica (Valkiūnas 2005). These intracellular parasites reproduce sexually in hematophagous female mosquitos (Diptera: Culicidae), biting midges (Diptera: Ceratopogonidae), and hippoboscids flies (Diptera: Hippoboscidae) (Valkiūnas 2005, Santiago-Alarcon et al. 2012), which act as vectors of *Plasmodium*, *Haemoproteus* (*Parahaemoproteus*), and *Haemoproteus* (*Haemoproteus*), respectively (Valkiūnas 2005, Santiago-Alarcon et al. 2012). The prevalence, distribution, and diversity of these blood parasites are highly variable across biogeographical regions (Valkiūnas 2005, Clark et al. 2014, Drovetski et al. 2014), habitats (Mendes et al. 2005, Loiseau et al. 2012), and host species in the same community (Ricklefs et al. 2005, Fecchio et al. 2013, Svensson-Coelho et al. 2013). Despite this variation, these micro-parasites and their vertebrate hosts are not randomly distributed and can exhibit predictable distributional patterns (Rahbek and Graves 2001, Ishtiaq et al. 2010). For instance, Fallon et al. (2003a) found marked host species effects on haemosporidian distributions on islands of the West Indies, with the central islands of this archipelago sharing similar parasite lineages and the peripheral islands harboring well differentiated parasite communities (Fallon et al. 2005). These results indicate that either ecological traits related to hosts (i.e. host's capability to resist and control infection or differential exposure to parasites), geography (i.e. long periods of isolation), or a combination of the two were the main drivers of distribution and diversity of these parasites. It is therefore important to determine how host ecological traits and geography promote variation in the distribution, diversity, and host specialization of these pathogens across avian communities and biogeographical regions to predict and understand emerging infectious diseases.

Amazonia is the largest and most diverse tropical forest harboring more than 1300 bird species (Silva et al. 2005). One of the most accepted hypothesis to explain this astonishing diversity is that successive vicariance events, driven by river dynamics, fragmented the biota and promoted allopatric speciation (Ribas et al. 2012). Moreover, many studies have shown that the rivers of the Amazonian basin are major isolation barriers for current plant and animal communities and delineate species distributions in several vertebrate and plant groups (Silva et al. 2005), establishing what is known as areas of endemism. Areas of endemism have been proposed based mainly on terrestrial vertebrate distribution patterns, with eight major areas recognized so far: Napo, Imeri, Guiana, Inambari, Rondônia, Tapajós, Xingu and Belém (Silva et al. 2005). However, the factors that influence the diversity and

distribution of pathogens in their vertebrate hosts, such as avian malaria, either within a single host clade or across areas of endemism within Amazonia are unexplored.

Here we used a region of the mitochondrial cytochrome *b* (cyt *b*) gene to delineate parasite lineages of the genera *Haemoproteus* and *Plasmodium* and examined spatial patterns of prevalence of each genus across six areas of endemism in the Amazon Basin. We then described the phylogenetic relationships of these haemosporidian parasites and tested whether host family had significant phylogenetic signal on the evolutionary history of *Haemoproteus* and *Plasmodium* within Amazonia. Finally, we tested whether the diversity and distribution of avian haemosporidian lineages in 15 avian host communities are structured with respect to: 1) the diversity and distribution of their avian hosts, 2) latitude, and 3) area of endemism in this megadiverse tropical ecosystem. We hypothesize that the dynamics of haemosporidian communities change across areas of endemism. Therefore, we expect to find differences in composition and prevalence of parasites among areas. However, since hosts, and not the environment, are the real habitats for haemosporidians, we predict that their distribution will follow host species' distributions. Lastly, since allopatric speciation is the main mechanism promoting diversity, we do not expect closely related parasites to be sympatrically distributed more than expected by chance.

Methods

Sample collection

We collected blood (88% of all samples) or liver tissue (12%) from 2661 individual birds sampled from 25 localities in six Amazonian areas of endemism (Fig. 1, Supplementary material Appendix 1 Table A1). Due to low sample size in some localities, we considered the avian hosts as a single community if the localities were in the same side of the river in a continuous forest and closer than 50 km. Therefore, our final analyses comprise 15 communities (Supplementary material Appendix 1 Table A1). Netted birds were bled by brachial venipuncture and blood was collected with heparinized capillary tubes. After blood collection, birds were either ringed and released or euthanized and prepared as museum specimens. Birds not likely to be captured by mist nets were collected by firearm in accordance to corresponding permits. Liver samples were taken during specimen preparation. All blood samples except those collected at Gurupi were stored in 95% ethanol until DNA extraction. Blood and liver tissue collected at Gurupi were flash frozen in liquid nitrogen and stored frozen until DNA extraction. All tissue samples and birds were collected or ringed under appropriate permits in Brazil.

Molecular detection of parasites

Samples collected from Chupinguaia, Comodoro, Madeira River, and Porto Trombetas collection sites were screened and amplified following the protocols of Fallon et al. (2003b) and Waldenström et al. (2004). For all other samples blood

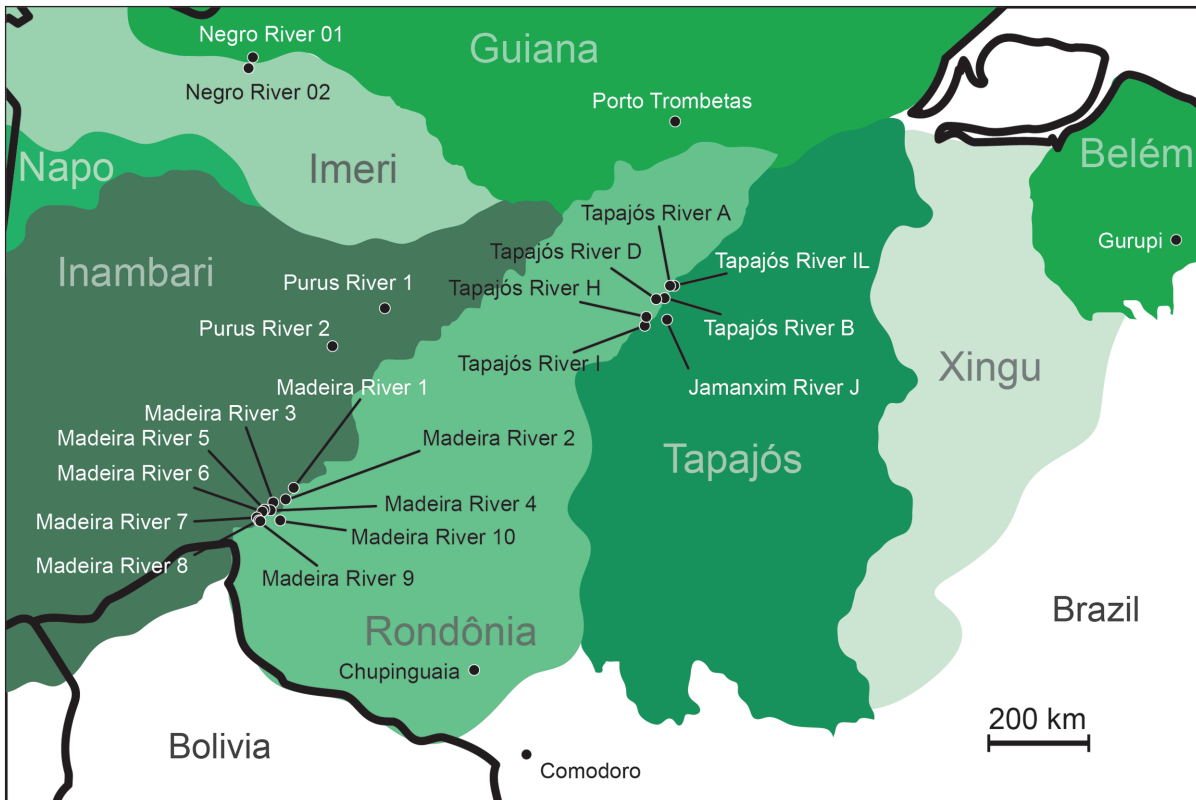


Figure 1. Map of Brazilian Amazonia with 25 sampling locations. Detailed locations (coordinates), number of individuals sampled, number of avian species sampled/infected, and parasite lineage richness per localities can be found in Supplementary material Appendix 1 Table A1.

or liver were processed, screened, and analyzed using the following molecular methods.

DNA was extracted using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA), following the modified Qiagen tissue protocol described by Bell et al. (2015). To check extraction quality and guard against false negatives, a subset of 20 random samples were amplified using the vertebrate cytochrome *b* primers described by Townzen et al. (2008) prior to haemosporidian screening. All samples successfully amplified host DNA. The protocols of Bell et al. (2015) were used to both initially screen samples for haemosporidian DNA with real-time PCR and then to amplify a 477 bp region of the cytochrome *b* gene from positive samples using nested PCR. All real-time PCRs were carried out using iTaq universal SYBR Green Supermix on a CFX96 real-time thermocycler (Bio-Rad, Hercules, CA), whereas nested PCRs were run using OneTaq Quick-Load 2X Master Mix with standard buffer (New England Biolabs, Ipswich, MA). All samples identified as positive by real-time PCR underwent nested PCR amplifications for *Haemoproteus/Plasmodium*. All PCR products were run on 1.25% agarose gels, stained with ethidium bromide, visualized under UV light, and photographed.

Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye terminator ver. 3.1 cycle sequencing kit (Applied Bio systems, Foster City, CA). Cycle sequencing reaction products were purified using ethanol precipitation, re-suspended in 10 μ l of dH₂O, and run on an ABI 3100 DNA sequencer

(Applied Bio systems, Foster City, CA). The primers FIFI and R2 (Ishtiaq et al. 2007) were used for sequencing purified *Haemoproteus/Plasmodium* positive nested PCR products.

Forward and reverse sequences were visualized and assembled using Sequencher ver. 5.0.1 (Gene Codes, 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 analyses.

Assembled sequences were aligned using BioEdit ver. 7.2.0 (Hall 1999) and collapsed to unique haplotypes using the FaBox haplotype collapse and converter tool (Villesen 2007). Sequence identities were verified with a local BLAST against the MalAvi database (Bensch et al. 2009) using BioEdit ver. 7.2.0 (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 specific epithet followed by a number to denote multiple lineages from a single host species. For example, lineage WILPOE01 is the first lineage obtained from *Willisornis poecilinotus*. All sequences were deposited in GenBank (Accession no. KU562119-KU562842) and in the MalAvi database.

Phylogenetic reconstruction

Assembled sequences of unique haplotypes were used to reconstruct molecular phylogenies. The GTR + I + G model of nucleotide substitution was implemented for all phylogeny reconstruction as determined by jModelTest (Guindon and Gascuel 2003, Darriba et al. 2012). For clarity of visualizing phylogenetic patterns, lineages from the Brazilian Amazon were split into two separate alignments based on parasite genus, *Haemoproteus* or *Plasmodium*. For both genera, *Leucocytozoon fringillarum* (Accession no. FJ168564) served as the outgroup. Bayesian inference and maximum likelihood phylogenies were reconstructed for each genus using the programs MrBayes (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) and RAxML (Stamatakis 2014) respectively. In MrBayes 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 ver. 1.40 (Rambaut 2009).

To determine whether host family had a significant phylogenetic signal on the evolutionary history of *Haemoproteus* and *Plasmodium* within Amazonia, a Maddison and Slatkin (1991) randomization test was conducted for each haemosporidian genus in R (ver. 3.3.2; R Development Core Team) as described by Bush et al. (2016). A significant result indicates that the host family distribution within the tree topology is more conserved than expected by chance, demonstrating a significant host family phylogenetic constraint.

Composition analysis

To determine the effect of area of endemism on host and parasite communities within Amazonia, data were organized into two binary (presence-absence) matrices: one for the distribution of parasite lineages in areas of endemism, and another for the distribution of bird species.

Permutational multivariate analyses of variance (PERMANOVA) was used to determine whether parasite and host assemblages differed across areas of endemism. The Jaccard index was used as a dissimilarity measure and 10 000 permutations for each model were performed to determine statistical significance. Latitude was also included as an explanatory variable to test its effect on the composition of parasite lineages and host species. Analyses were conducted in R (ver. 3.2.2; R Development Core Team) using the package vegan (Oksanen et al. 2013).

To test for an association between the compositions of host communities and parasite assemblages across localities, 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 determine statistical significance.

To test whether the parasite assemblage in each area of endemism was composed of lineages that were phylogenetically closer than expected by chance, occurrence data were

separated by area of endemism to build two binary matrices, one for *Plasmodium* and one for *Haemoproteus*. The Jaccard index was calculated to measure the pairwise dissimilarities between parasite lineages from different areas of endemism, and a matrix for each haemosporidian 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 for each test.

Results

A total of 2661 samples were collected from Amazonia (Supplementary material Appendix 1 Table A1 and A2). Haemosporidian infections were detected in 463 individuals (17.4% prevalence), with *Plasmodium* accounting for 407 (87.9%) of all infections (Supplementary material Appendix 1 Table A3). *Plasmodium* prevalence (15.3%) was significantly higher than *Haemoproteus* prevalence (2.4%) ($\chi^2 = 271.92$, $df = 1$, $p < 0.001$) (Supplementary material Appendix 1 Table A3). There were 56 coinfections identified in Amazonian birds, with dual *Plasmodium* infection being most common (Supplementary material Appendix 1 Table A4).

Haemosporidian infection varied among 15 avian communities, with overall infection prevalence varying from 7.3 to 33.3% (Supplementary material Appendix 1 Table A1). Infection prevalence significantly differed across the six areas of endemism: overall prevalence ($\chi^2 = 40.0$, $df = 5$, $p < 0.001$), *Haemoproteus* prevalence ($\chi^2 = 28.11$, $df = 5$, $p < 0.001$), and *Plasmodium* prevalence ($\chi^2 = 23.504$, $df = 5$, $p < 0.001$) (Fig. 2, Supplementary material Appendix 1 Table A2). Across areas of endemism, overall infection prevalence ranged from 11.9% in Guiana to 31.0% in Tapajós (Fig. 2, Supplementary material Appendix 1 Table A2). The areas of Belém, Rondônia, and Tapajós showed significantly higher prevalence for *Haemoproteus*, *Plasmodium*, and both genera combined (Supplementary material Appendix 1 Table A2).

Samples were obtained from 17 avian orders, 45 families, and 330 species (Supplementary material Appendix 1 Table A5 and A6). Infection prevalence varied across host families and orders, with no infections found in eight avian orders, although these host orders were poorly sampled with only 40 samples collected from these orders combined (Supplementary material Appendix 1 Table A5). Passeriformes were the most sampled host order with 2412 (90.6%) samples collected, although higher haemosporidian prevalence was seen in Columbiformes, Falconiformes, Gruiformes, Piciformes, and Psittaciformes (Supplementary material Appendix 1 Table A5). *Plasmodium* prevalence was higher than *Haemoproteus* prevalence in all avian orders, except Columbiformes, where *Haemoproteus* was more prevalent (Supplementary material Appendix 1 Table A5).

Among Passeriformes, samples were collected from 22 host families and 251 host species. Thamnophilidae

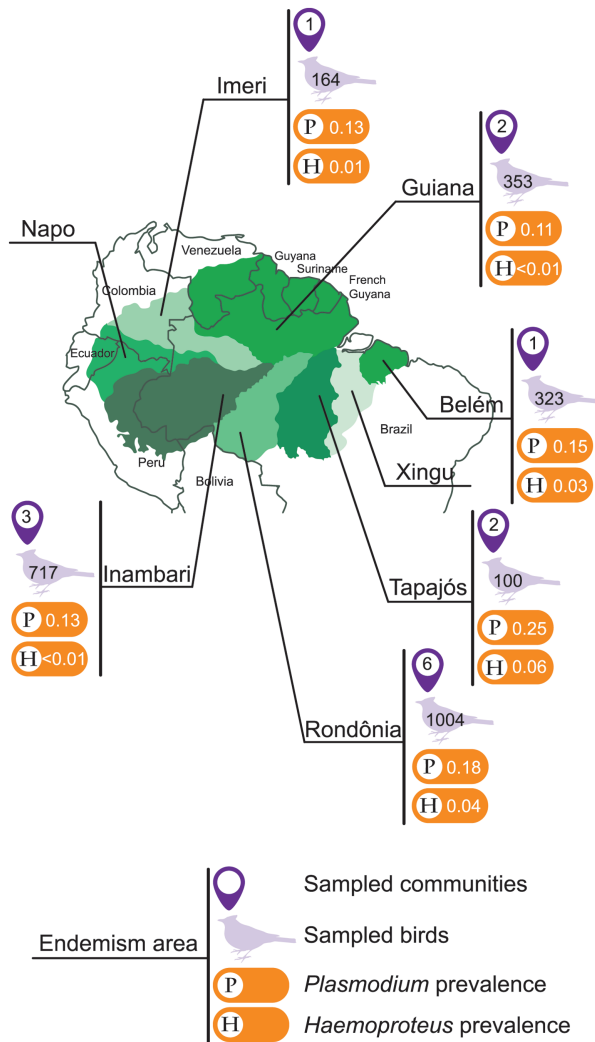


Figure 2. Map of Amazon Basin with six areas of endemism sampled. The proportions of infected birds are presented by area of endemism. Detailed prevalence by avian community or host taxon can be found in Supplementary material Appendix 1 Table A1 and A6.

(935 samples), Dendrocolaptidae (415 samples), Pipridae (345 samples), and Tyrannidae (223 samples) were the most frequently sampled avian families. Infection prevalence varied among host families, with Formicariidae (37.9%), Thraupidae (30.4%), and Thamnophilidae (23.7%) showing the highest prevalence. All infected passerine families had higher prevalence of *Plasmodium* than *Haemoproteus* (Supplementary material Appendix 1 Table A5).

Parasite and host phylogeny

We recovered 265 lineages of haemosporidians from Amazonian birds, of which 91.4% were novel lineages (Supplementary material Appendix 1 Table A3). *Plasmodium* lineages significantly outnumbered *Haemoproteus* lineages ($\chi^2 = 200.52$, $df = 1$, $p < 0.001$), although the percentages of newly identified lineages did not differ between the two parasite genera ($\chi^2 = 0.40$, $df = 1$, $p = 0.526$) (Supplementary material Appendix 1 Table A3).

Haemoproteus and *Plasmodium* lineages from 2661 Amazonian host samples were used for phylogenetic reconstruction, with each parasite genus analyzed separately because their reciprocal monophyly is well documented. A total of 51 *Haemoproteus* lineages (Fig. 3) and 214 *Plasmodium* lineages (Fig. 4) were included in each respective phylogenetic tree. Although nodal support was general high for both parasite genera (Fig. 3 and 4), both phylogenetic trees included several large polytomies, especially for *Plasmodium* (Fig. 4). For both parasite genera, host families were generally spread throughout the phylogeny partly biased by the fact that Thamnophilidae comprised a third of all samples studied. (Fig. 3 and 4). However, a clade of *Haemoproteus* (*Haemoproteus*) from Columbidae were sister to all other *Haemoproteus* (*Parahaemoproteus*) (Fig. 3) and a clade of *Plasmodium* lineages parasitizing Tyrannidae clustered together (Fig. 4). Conservation of host family, when mapped onto the phylogenies differed between the two haemosporidian genera, with a significant host family phylogenetic signal for *Haemoproteus* ($p < 0.01$), but not for *Plasmodium* ($p = 0.102$).

Compositional analysis

Composition analysis demonstrated that both parasite and host communities differed significantly across areas of endemism and also as a function of latitude (Table 1). Areas of endemism that were more similar in avian community composition were also more similar in their parasite communities (Mantel statistic $r = 0.33$, $p = 0.005$). Haemosporidian prevalence varied widely among areas of endemism (Fig. 2, Supplementary material Appendix 1 Table A2), with areas of endemism north of the Amazon River (Guiana and Imeri) having significantly lower haemosporidian prevalence than areas of endemism south of the Amazon River (Belém, Tapajós, Inambari and Rondônia) ($\chi^2 = 34.37$, $df = 1$, $p < 0.001$). No correlation was found between the phylogenetic distance of parasite lineages and their occurrence in areas of endemism, for either *Haemoproteus* lineages (Mantel statistic $r = 0.08$, $p = 0.11$), or *Plasmodium* lineages (Mantel statistic $r = -0.05$, $p = 0.99$).

Discussion

The Brazilian Amazon harbors a high diversity of haemosporidian parasites with 265 lineages within the genera *Plasmodium* and *Haemoproteus* identified in samples from 330 bird species. The biogeography of the six Amazonian areas of endemism sampled in this study defined haemosporidian diversity and distribution. Areas of endemism contained unique parasite communities that differed not only in parasite prevalence, but also in community composition. Parasite communities in each area of endemism differed, in part, due to differences in host communities, with areas containing more similar host communities harboring more similar parasite communities. Avian community structure in Amazonia closely matches areas of endemism (Cracraft 1985, Silva et al. 2005, Wesselingh et al. 2009), and this study provides the first example of vector borne parasite

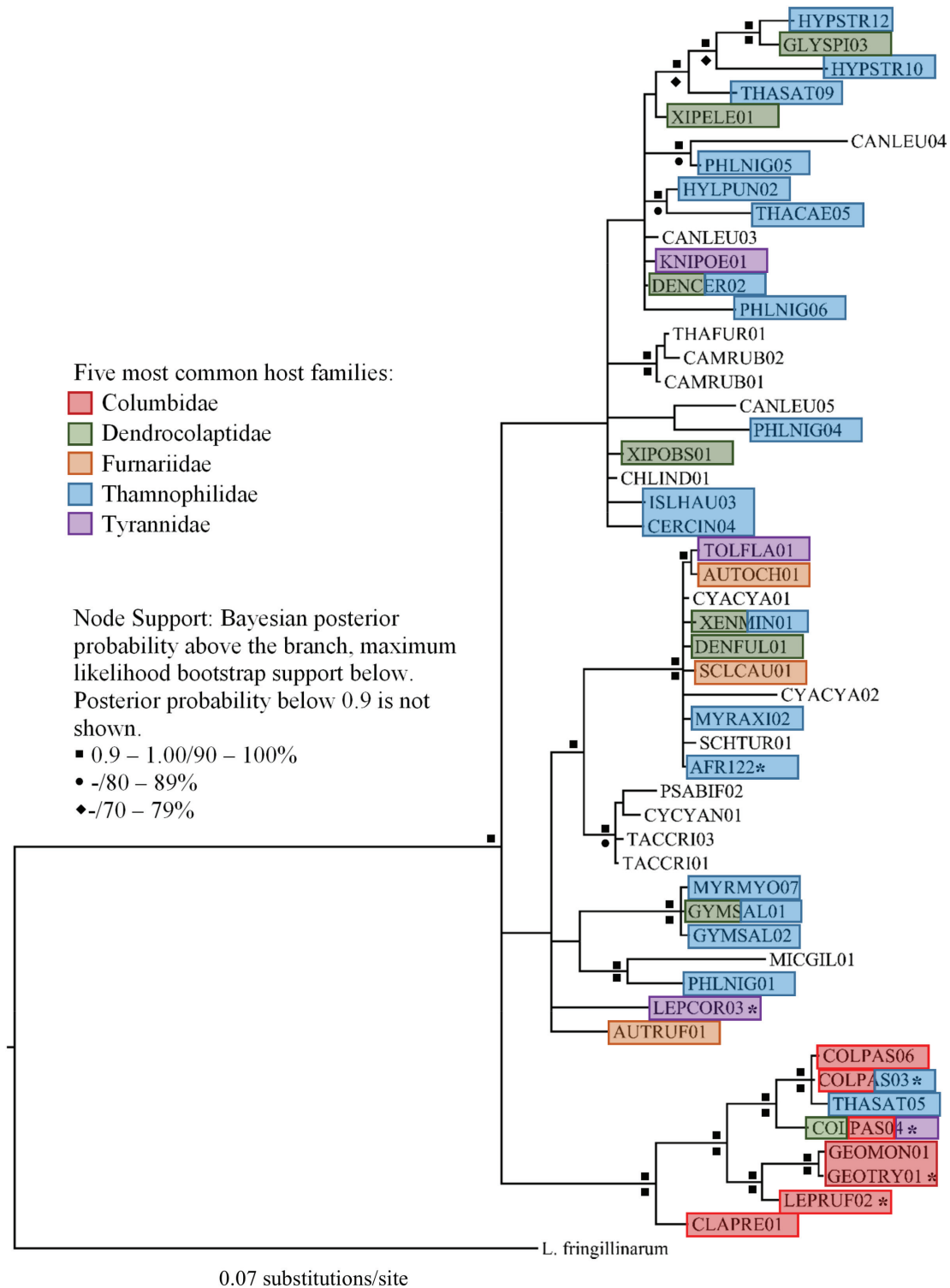


Figure 3. Phylogenetic reconstruction of Amazonian *Haemoproteus* lineages. Previously reported lineages (MalAvi) are indicated with an asterisk. The five most common host families are indicated by colored blocks.

communities also conforming to these areas. Avian haemosporidian distribution was affected by host distribution, which in turn is structured by the unique biogeography of Amazonia.

Several studies have shown that avian *Plasmodium* are generalist parasites and that generalism occurs due a high frequency of host shifting events, presumably mediated

by the more generalist feeding preferences of their vectors, allowing *Plasmodium* spp. to exploit a broad range of vertebrate hosts (Santiago Alarcon et al. 2012, Medeiros et al. 2013). Despite low host specificity of *Plasmodium* (with some lineages infecting a variety of different host families) and absence of a significant host family phylogenetic constraint on the haemosporidian phylogenies, we found

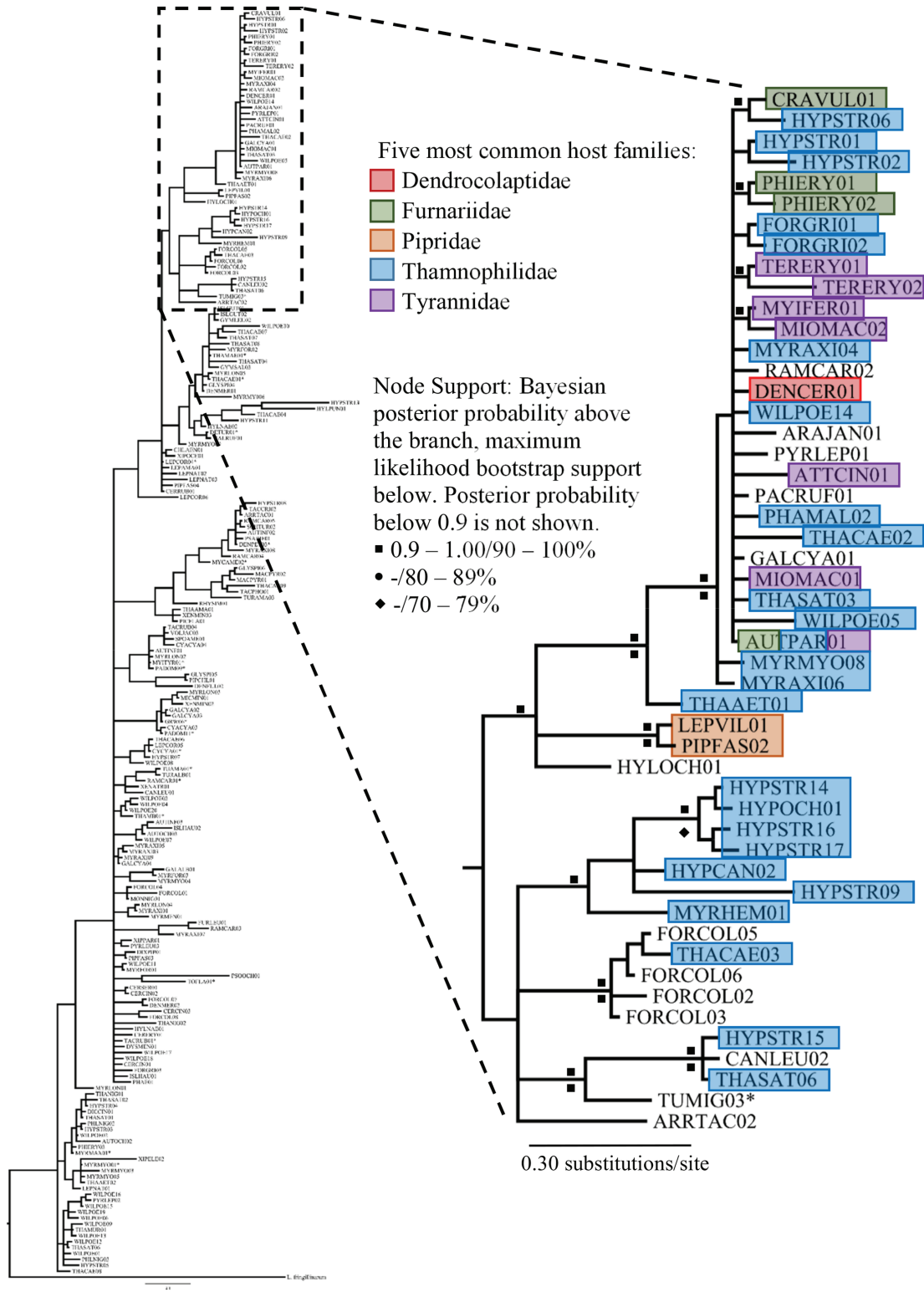


Figure 4. Phylogenetic reconstruction of Amazonian *Plasmodium* lineages. Enclosed subtree shown in detail. Previously reported lineages (MalAvi) are indicated with an asterisk. The five most common host families are indicated by colored blocks.

evidence that the distribution and community structure of *Plasmodium* that infected Amazonian birds was influenced by host distribution and geography. Across sampling locations, host community similarity predicted parasite similarity, and area of endemism constrained the distributions

of parasites. This pattern of host distribution determining haemosporidian distribution matches what is known for haemosporidian parasites within North America (Ellis et al. 2015). The higher lineage diversity recorded for *Plasmodium* as compared to *Haemoproteus* across Amazonia confirms the

Table 1. PERMANOVA results. Model 1 tests for changes in parasite assemblage composition among areas of endemism and in different latitudes, whereas 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			1.00	
Model 2: composition of host assemblage						
Explanatory variables	df	SS	MS	F	R ²	p-value
Areas of endemism	5	2.1870	0.43739	1.5161	0.44212	<0.001
Latitude	1	0.4516	0.45160	1.5653	0.09130	<0.001
Residuals	8	2.3080	0.28850		0.46658	
Total	14	4.9465			1.00	

pattern suggested by Clark et al. (2014) for South America. These authors hypothesized that *Plasmodium* spp. may have experienced an exceptional radiation within South America potentially due to the higher diversity of avian hosts and habitats compared to other biogeographical regions.

Parasites from the genus *Haemoproteus* are known to be more host specific (Beadell et al. 2004, 2009, Valkiūnas 2005, Dimitrov et al. 2010, Ishtiaq et al. 2010) than *Plasmodium*, which we have also found in this study, with *Haemoproteus* exhibiting a significant host family phylogenetic signal. This higher host specificity of *Haemoproteus* may explain why they are less diverse than *Plasmodium* in Amazonia, since their higher specificity allows fewer opportunities for these parasites to invade and colonize a new host and then speciate (Poulin 1997, Ricklefs et al. 2014). The extreme host diversity of Amazonia may benefit *Plasmodium* with its ability to jump between phylogenetically distant hosts, but equally disadvantage *Haemoproteus* owing to its inability to do so. This differing effect of host specificity between the two genera may generate the disparity between *Plasmodium* and *Haemoproteus* diversity seen in this study.

Habitat is also known to affect host specificity (Loiseau et al. 2012, Moens and Pérez-Tris 2016) with geographical barriers limiting the movement of specialist lineages (Mata et al. 2015). The high host diversity found in Amazonia 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 lack of phylogenetic signal in avian host family for Amazonian *Plasmodium* lineages, where avian host diversity is exceptionally high, supports this hypothesis. Our findings suggest that the diversity and distribution of these two genera of parasites are shaped by factors related to geography and host associations. Further studies including denser sampling in non-passerine hosts will help to better understand host specificity within the Amazon Basin, especially since haemosporidian prevalence varied across host families. This will shed additional light on host specificity of these parasites and confirm whether host specificity is a specific parasite lineage trait or whether it varies geographically.

Related avian hosts might share phylogenetically similar lineages of malaria parasites because they have similar

ecological and life-history traits and immunological responses (Ricklefs and Fallon 2002). Therefore, infecting hosts with similar immune responses could promote parasite range expansion among closely related host species. However, intraspecific geographic variation in host responses to parasites (Ardia 2005) and local host–parasite interactions selecting for greater host resistance (Best et al. 2011) might lead to the geographic differentiation of parasite lineages and increase the influence of geographic factors in structuring haemosporidian assemblages. Transmission of multi-host pathogens, such as avian malaria, is driven by heterogeneity in host–parasite compatibility (Medeiros et al. 2013), in other words, some traits of avian hosts make them more susceptible to certain lineages of parasites and vectors as well. This suggests that host–parasite compatibility is evolutionarily labile and that the relationship between a particular host and its parasites changes geographically. The role of geography in structuring avian malaria parasite assemblages was shown in insular West Indian (Fallon et al. 2005) and Melanesian birds (Olsson-Pons et al. 2015) and also in this study of Amazonian birds.

Our results clearly demonstrate that the diversity and distribution of haemosporidian lineages in Amazonian bird hosts vary with latitude. Previous studies have shown that species richness increases towards the equator in free-living (Gaston and Blackburn 2000) and some parasitic and pathogenic organisms (Rohde and Heap 1998, Guernier et al. 2004, Nunn et al. 2005). However, studies of latitudinal gradients in parasites are less common and often conflicting in their results. For example, Poulin (1995) did not find any relationships between latitude and richness of gastrointestinal parasites of several bird and mammal species. And while Nunn et al. (2005) showed no increased risk of helminths and viruses in primates close to the equator, they did find an increased risk of blood protozoan parasites. In fact, Nunn et al. (2005) argued that the higher diversity of protozoan parasites found in primates near the tropics could be caused by greater abundance or diversity of biting insects closer to the equator or by climatic effects on vector behavior and parasite development. In the present study we were unable to sample vectors, but our analysis showed that similarity of haemosporidian lineages was determined by bird community similarity and latitude. Future studies should include

the vector community to examine the degree to which haemosporidian parasite assemblages are influenced by intermediate vertebrate host communities and definitive insect vector assemblages, and whether latitude is associated with differences in parasite prevalence, diversity, and specialization in this bird-parasite-vector system.

The structure of avian haemosporidian parasites in Amazonia is determined by host community similarity and geography. Lineage sharing between closely related hosts might be the primary mode of dispersal of these parasites between avian communities in Amazonia and then host shifting across more distantly related host species occurred within areas of endemism to create geographical signatures. The known lack of host specificity for *Plasmodium* (Beadell et al. 2004, 2009, Valkiūnas 2005, Dimitrov et al. 2010, Ishtiaq et al. 2010) and at the same time a strong biogeographical structure reinforces that host switching or ongoing dispersal of these parasites is the main mechanism promoting diversification in *Plasmodium* in the Amazon Basin (see Weckstein 2004 for ectoparasite-bird system). The differences in host family phylogenetic constraint found for *Plasmodium* and *Haemoproteus* suggest that *Plasmodium* infection and distribution patterns were mostly shaped by geography, whereas *Haemoproteus* patterns of diversity and distribution were shaped primarily by host associations. Because vectors are important in distributing haemosporidian parasites widely throughout an avian community, these invertebrate hosts should be incorporated into future analyses to explain patterns of malaria distribution and diversity.

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Supplementary material (Appendix ECOG-03058 at <www.ecography.org/appendix/ecog-03058>). Appendix 1.