Avian malaria, ecological host traits and mosquito abundance in southeastern Amazonia

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SUMMARY

Avian malaria is a vector transmitted disease caused by *Plasmodium* and recent studies suggest that variation in its prevalence across avian hosts is correlated with a variety of ecological traits. Here we examine the relationship between prevalence and diversity of *Plasmodium* lineages in southeastern Amazonia and: (1) host ecological traits (nest location, nest type, flocking behaviour and diet); (2) density and diversity of avian hosts; (3) abundance and diversity of mosquitoes; and (4) season. We used molecular methods to detect *Plasmodium* in blood samples from 675 individual birds of 120 species. Based on cytochrome *b* sequences, we recovered 89 lineages of *Plasmodium* from 136 infected individuals sampled across seven localities. *Plasmodium* prevalence was homogeneous over time (dry season and flooding season) and space, but heterogeneous among 51 avian host species. Variation in prevalence among bird species was not explained by avian ecological traits, density of avian hosts, or mosquito abundance. However, *Plasmodium* lineage diversity was positively correlated with mosquito abundance. Interestingly, our results suggest that avian host traits are less important determinants of *Plasmodium* prevalence and diversity in southeastern Amazonia than in other regions in which they have been investigated.

Key words: Culicidae, Haemosporidian parasites, mosquito diversity, parasite diversity, *Plasmodium*, vectors.

INTRODUCTION

Many factors have been proposed to explain parasite diversity (Poulin, 1997). For example, previous studies have shown that species richness increases towards the equator for some groups of parasitic organisms (Rohde and Heap, 1998; Guernier *et al*. 2004; Nunn *et al*. 2005). However, a recent meta-analysis of 62 studies involving animal, plant and fungal hosts showed that there was no strong evidence for an effect of latitude on parasite species richness (Kamiya *et al*. 2014). Parasite diversity might be determined by characteristics of hosts rather than those of the environment. For example, host body size, population density and geographic range have all been suggested as universal predictors of variation in parasite species richness (Kamiya *et al*. 2014). Nevertheless, the meta-analysis of Kamiya *et al*. (2014) did not include vector-transmitted parasites. Host density can be especially important for parasites, which depend on hematophagous insects for reproduction because the concentration of hosts in a given area potentially affects the prevalence and transmission of vector-borne pathogens by influencing encounter rates between vectors and susceptible hosts (Nunn and Heymann, 2005).

Avian malaria is a worldwide, vector-transmitted disease caused by haemosporidian parasites in the genus *Plasmodium* (Valkiūnas, 2005). These parasites reproduce sexually in female mosquito vectors from the genera *Culex, Aedes, Culiseta, Anopheles, Mansonia, Aedeomyia* and *Coquillettidia* (Diptera: Culicidae) (Valkiūnas, 2005; Njabo *et al*. 2009; Santiago-Alarcon *et al*. 2012). Environmental factors, especially temperature, can play a role in the distribution, prevalence and transmission of these parasites (Gonzalez-Quevedo *et al*. 2014; Oakgrove *et al*. 2014). Temperature constrains not only parasite sporogonic development (LaPointe *et al*. 2010), but also influences the activity and development of the mosquito vectors, which are important determinants of the prevalence and transmission of avian malaria.

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However, temperature, among other environmental variables, did not explain avian malaria prevalence in avian species across several sites in South Africa (Okanga *et al*. 2013). As Okanga *et al*. (2013) pointed out, this suggests that the prevalence of avian malaria parasites may also be determined by factors related to their avian hosts and mosquito vectors (see also Ellis *et al*. 2015).

Several studies have investigated the effect of avian ecological traits on the probability of infection by *Plasmodium* and other related haemosporidians (Ricklefs *et al*. 2005; Fecchio *et al*. 2011, 2013; Svensson-Coelho *et al*. 2013; González *et al*. 2014; Lutz *et al*. 2015; Matthews *et al*. 2016). In these studies, variation in haemosporidian prevalence is thought to be a result of the host's capability to resist and control infection or the result of differential exposure to parasites. However, most studies have not considered the role of vectors in explaining these patterns (but see Medeiros *et al*. 2015).

At a finer spatial scale, infection risk for *Plasmodium* in blue tit populations increased with increasing proximity to a large water source, possibly as result of increased vector abundance (Wood *et al*. 2007). Haemosporidian prevalence was also higher in the wettest of two western Amazonian 100 ha forest plots that were otherwise similar with respect to forest type, altitude, human disturbance and flooding (Svensson-Coelho *et al*. 2013). These studies corroborate the idea that prevalence of blood parasites is higher at sites where vectors are more abundant.

Besides vector abundance, avian host density could also play a role in determining the prevalence of avian malaria parasites. For example, host population density can influence the spread and distribution of parasites by increasing the probability that the vectors and thus the parasites can come into contact with hosts (Anderson and May, 1978; Ellis *et al*. 2017). Furthermore, Drovetski *et al*. (2014) found that haemosporidian lineages infected abundant bird species more frequently than less common host species in four avian communities in Africa, Asia and Europe.

To improve our understanding of avian malaria transmission, we sought to determine which biological factors (mosquito abundance and diversity; host ecological traits and density) explain the prevalence and diversity of *Plasmodium* lineages across seven locations along the Tapajós and Jamanxim rivers in Brazilian Amazonia. Specifically, we predicted that: (1) *Plasmodium* prevalence in a given host species would be positively correlated with the host species' density; (2) *Plasmodium* lineage diversity would be positively correlated with avian host diversity; (3) avian ecological traits would explain some of the variation in *Plasmodium* prevalence; and (4) the prevalence and diversity of avian *Plasmodium* lineages would be positively correlated with the abundance and diversity of mosquitoes. A secondary motivation of our study was to provide information on the diversity and distribution of ornithophilic mosquitoes in a region of Amazonia that has not previously been explored with respect to these insects.

MATERIALS AND METHODS

Sampling sites

The study area was located midway down the Tapajós River, a major south bank tributary of the Amazon River. Sampling was carried out along both banks of the Tapajós River covering both the Rondônia and Tapajós areas of endemism (Silva *et al*. 2005). We also sampled the right bank of its most important tributary, the Jamanxim River (Fig. 1). The area comprises a wide variety of microhabitats, with the *terra firme* and *igapó* forests being the most broadly distributed. Sampling was conducted along six transects of five km in length, beginning in the seasonally flooded forest (*igapó*) and crossing the interior of *terra firme* forest. We also sampled a 250 m transect on a river island. We named these seven sites as follows: the first letter of the label is the first letter of the river name ('T' for Tapajós and 'J' for Jamanxim); the second letter is the margin of the river ('L' for left bank, 'R' for right bank and 'I' for the island); site names also include a number to identify unique sampling sites on the same bank of the same river.

Bird sampling

We placed five mist net lines within each transect. These five mist net lines were each separated by 1 km and contained ten nets. The 50 mist nets (12 m length × 3 m height) remained open for three consecutive mornings at each of the seven transect sites. Owing to the small size of the river island, the mist nets were arranged in a single 250 m line and were sampled with the same effort as the others sites. Blood sampling of captured birds took place during two distinct seasons, the dry season for the sites TL1, TL2, TL4, TR1 and JR1 (18 July–3 August 2012) and flooding period for the sites TL1, TL2, TL3 and TI (1–17 October 2012), thus only two sites, TL1 and TL2, were sampled during both the dry and flooding seasons. At each site, to allow sufficient time for transmission of *Plasmodium* to avian hosts, avian blood samples were collected from individual birds shortly after (~2–3 weeks) mosquito vectors were also sampled. Netted birds were bled by brachial venipuncture using heparinized capillary tubes. Blood samples were stored in 95% ethanol until DNA extraction. After blood collection, birds were ringed and released, or euthanized and prepared for museum specimens. All tissue samples and birds were

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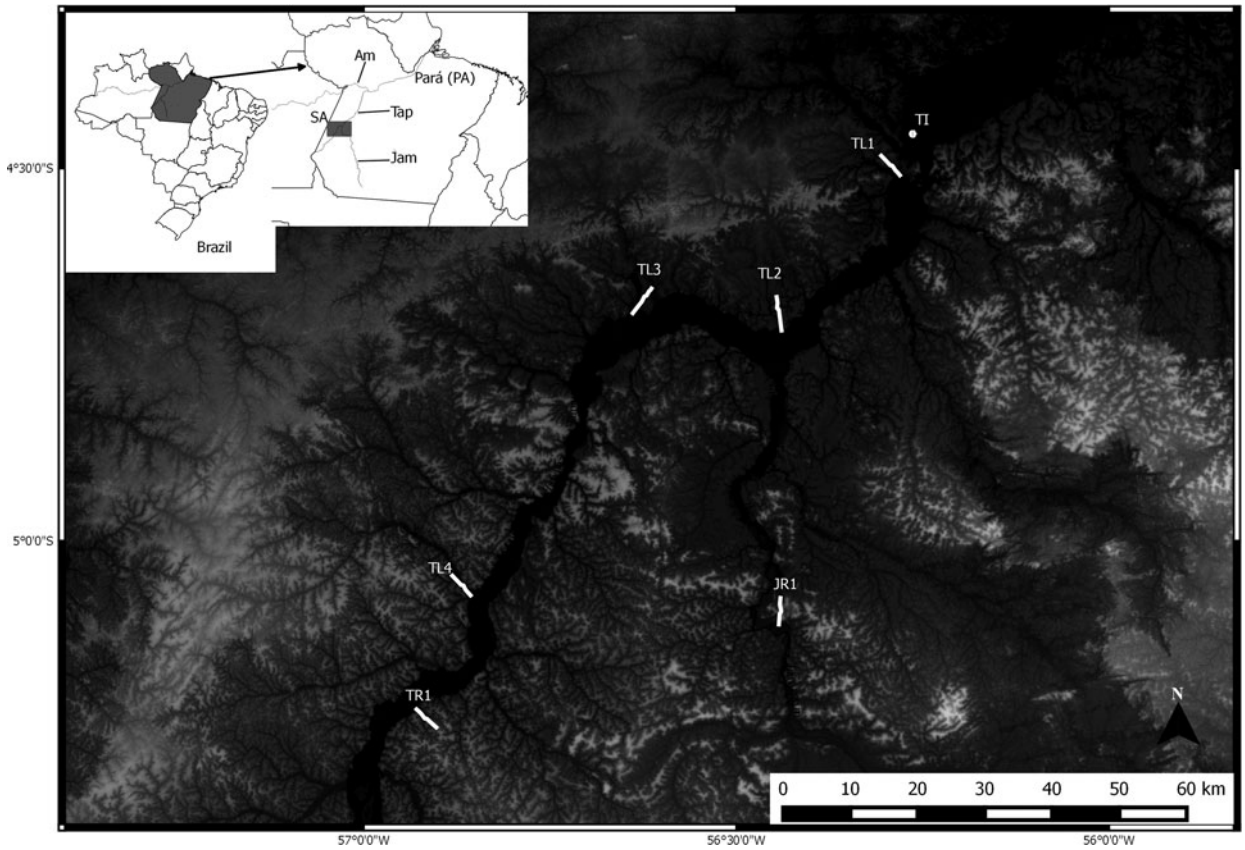

Fig. 1. Map showing the seven sampling sites along the Tapajós and Jamanxim rivers. Background shading corresponds to elevation with lower areas represented by darker shading. The inset shows the state of Pará, Brazil, and the study area (SA) is represented as a dark grey rectangle within the state. Am, Amazon River; Jam, Jamanxim River; and Tap, Tapajós River.

collected or ringed under appropriate permits from Brazil (IBAMA no 22/2012 and ICMBio no 004/2012). Tissue samples and voucher specimens were deposited in the Bird Collection at the Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus, Brazil. Species nomenclature follows Piacentini *et al*. (2015).

Besides mist net sampling, we conducted point count surveys (10 min each), spread along transects at every 500 m, totalling 11 points per transect. Each point was sampled for four consecutive days at each sampling period. We estimated density of individual species in each area using MCDS (Multiple Covariates Distance Sampling) as implemented in the program Distance 6.0 (Thomas *et al*. 2009). The analyses were stratified to obtain density estimates for each area. We truncated 10% of data with larger distances within each species to avoid double counting the same individual, as recommended by Buckland *et al*. (2001). For each species, we compared the following models: (1) half normal and hazard rate set as functions of expansion adjusted by cosine; (2) simple polynomial; and (3) polynomial hermite. We then calculated the Akaike's Information Criterion (AIC) for each model and chose the one with the lowest AIC score.

Mosquito sampling

Mosquitoes were collected using light traps powered by 12 V batteries as designed by Falcão (1981). The traps were installed in the same sampling transects used for mist netting birds, and were distributed in two vertical layers, at ground level (suspended approximately 1 m above the ground) and in the forest canopy (suspended between 10 and 15 m above the ground). A total of 14 traps were installed simultaneously in each transect and these were located at equidistant points starting at the river bank and ending at 4 km from the river bank in the interior of *terra firme* forest. Mosquito collections were made during three consecutive nights, between 18:00 and 21:00 h during both the dry season for the sites TL1, TL2, TL4, TR1 and JR1 (2–13 July 2012) and the flooding season for the sites TL1, TL2, TL3 and TI (12–23 September 2012), thus only two sites, TL1 and TL2, were sampled during both the dry and flooding seasons. Mosquitoes were stored in small plastic vials labelled with the corresponding data for each sample. In the laboratory, the female mosquitoes were separated and identified taxonomically using external morphological characters observed using a stereoscope. We

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only used female mosquitoes for abundance estimation because only females are potential vectors for *Plasmodium*. For species determination, we used keys published by Galindo *et al*. (1954) and Forattini (1962, 1965*a*, *b*, 2002). We used the infrageneric classification scheme of the genus *Anopheles* from McKeon *et al*. (2013), Moreno *et al*. (2013) and Ruiz-Lopez *et al*. (2013), which differentiates among groupings of cryptic but presently unnamed lineages. The abbreviation of genera and subgenera follows the guidelines suggested by Reinert (2001), according to the newly proposed mosquito nomenclature of the Walter Reed Biosystematics Unit, Smithsonian Institution (catalogue available at http://www.mosquitocatalog.org/taxon_table.aspx) and Harbach (2013) in the Mosquito Taxonomic Inventory (www.mosquito-taxonomic-inventory.info/).

Parasite detection

DNA was extracted from avian blood samples using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA), following the Qiagen protocol for blood in 95% ethanol. Total DNA was screened by real-time PCR to detect haemosporidian DNA following the protocol of Bell *et al*. (2015). Positive and negative controls were included in all real-time PCR runs. Samples identified as positive by real-time PCR underwent subsequent nested PCR, as outlined in Bell *et al*. (2015), to amplify a 477 bp fragment of the cytochrome *b* gene.

Positive nested PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA), sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Bio systems, Foster City, CA), and run on an ABI 3100 DNA sequencer (Applied Bio systems, Foster City, CA). For sequencing protocol and primers see Bell *et al*. (2015). 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). 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 specific 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 KU562250–KU562512) and the MalAvi database.

Assembled sequences of unique lineages were used to reconstruct a molecular phylogeny using Bayesian inference (BI) as implemented in MrBayes v. 3.2.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and the GTR + I + G model of nucleotide substitution as determined by jModelTest (Darriba *et al*. 2012); *Leucocytozoon fringillarum* (FJ168564) served as the outgroup. The BI analysis was run until the S.D. of split frequencies stabilized below 0·01. Twenty-five percent of resulting trees were discarded as burn in. The resulting consensus tree was visualized in FigTree (Rambaut, 2009).

Statistical analyses and modelling

We were interested in testing whether the prevalence of *Plasmodium* parasites was related to the density of avian hosts within the different sites sampled. To do this we first restricted our analysis to species sampled at least five times within a site; this left us with three sites (TL1, TL2 and TL3) each including more than three species that met our sample size criterion (Table A2). We then calculated prevalence for each species at each site separately as the number of *Plasmodium*-infected individuals divided by the total number of individuals sampled of a particular species at a particular site. Individuals infected with only *Haemoproteus* parasites were considered uninfected for this analysis since we did not sample the arthropod vectors for this parasite genus and because birds can be found with mixed infections of both genera (Valkiūnas, 2005). Individual birds infected with both *Plasmodium* and *Haemoproteus* were considered infected. We then ran three generalized linear models, one for each site, with prevalence as the response variable and host density as the explanatory variable with a quasibinomial error structure to account for overdispersion (Bolker *et al*. 2009). We entered two vectors of infected and uninfected individuals into the model to weight prevalence by sample size as is standard when running such models in R; see Crawley, 2012). Since sampling took place in two seasons for sites TL1 and TL2, we compared prevalence of *Plasmodium* parasites between seasons (within sites; prevalence was calculated for all individuals, i.e. not separated by host species, sampled within each season for each of the two sites) using chi-squared tests to confirm that seasons could be grouped for this analysis.

We tested the hypothesis that *Plasmodium* lineage diversity was related to the diversity of avian hosts across all seven sampling sites. For this we calculated a Simpson's index of diversity for all avian hosts that were sampled (117 species) and one for all parasite lineages within each sampling site using the 'diversity' function in the R package vegan (Oksanen *et al*. 2015). We then used Spearman's rank correlation tests

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(correlation statistic is ρ) to determine whether the variables were correlated across sampling sites.

We were also interested in testing whether the prevalence of *Plasmodium* parasites across avian host species was related to the following host ecological variables: nest location (ground, understory, sub canopy, canopy and cliff/bank), nest type (open cup, closed cup and cavity), flocking behaviour (solitary/family, single species and mixed species), and diet (insectivore, frugivore/granivore and omnivore). We scored these traits for all species sampled using a combination of The Birds of South America Volumes I and II (Ridgely and Tudor, 1989*a*, *b*), 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). We pooled our site data for this analysis because: (1) we did not expect site to influence the relationship of prevalence and ecological host traits (e.g. Matthews *et al*. 2016); and (2) because we did not find any significant differences in the prevalences of individual species sampled at multiple sites in our study (results not shown). We therefore constructed a generalized linear model with *Plasmodium* prevalence (weighted by sample size) as the response variable and each of the ecological variables as explanatory variables. We ran the model with a quasibinomial error structure to account for overdispersion and only included species with at least five individuals sampled ($n = 44$ avian species). Initially we included the taxonomic family of host species as an explanatory variable in the model to account for potential differences in prevalence among families, but sparse sampling led to uninterpretable estimates of family-level prevalences and so we dropped family from our final model. We also ran Wald chi-squared tests on each of the explanatory variables in the model using the function 'wald.test' in the R package aod (Lesnoff and Lancelot, 2012).

Finally, we tested the hypothesis that the prevalence and diversity of *Plasmodium* lineages were positively related to the abundance and diversity of mosquitoes across all seven of our sampling sites using Spearman's rank correlation tests. Here we calculated parasite prevalence for sites rather than for species by dividing the total number of infected individuals by the total number of individuals sampled in a site irrespective of host species. We again calculated a Simpson's index of diversity for *Plasmodium* lineages and one for mosquitoes within each sampling site.

All statistical analyses were performed in R version 3.2.3 (R Core Team, 2015).

RESULTS

Prevalence and diversity of Plasmodium *lineages*

We analysed 675 birds of 120 species sampled in seven communities along the Tapajós and Jamanxim rivers (Fig. 1, Table A1). *Plasmodium* infections were detected in 136 individuals from 51 host species with a prevalence of 20% (Table A1). Infection prevalence varied among well sampled host species (>10 individuals screened), ranging from 0 to 64% (Table A1). *Plasmodium* prevalence was homogeneous across sites, ranging from 12 to 31% ($\chi^2 = 11{\cdot}282$, D.F. $= 6$, $P = 0{\cdot}080$; Table 1). Based on *cytochrome b* divergence, we recovered 89 haemosporidian lineages within the genus *Plasmodium*, of which 81 (91%) were reported for the first time (Fig. 2). Although there are several well resolved and supported clades within the phylogeny, the general pattern is one of many polytomies with low node support (Fig. 2). When mapped onto the phylogeny, host family shows little perceivable pattern within the phylogeny, dominated by the highly sampled family Thamnophilidae. In several cases, individual *Plasmodium* lineages were recovered from more than one host family, with three *Plasmodium* lineages found in individual hosts from four different host families.

Table 1. Prevalence of *Plasmodium* per site along the Tapajós and Jamanxin rivers, southeastern Amazonia, Brazil. Specific site location information can be found in Fig. 1

Location	Sampled individuals	Infected	Prevalence (%)
TL1	151	38	25·2
TL2	137	17	12·4
TL3	142	31	21·8
TL4	60	10	16·7
TR1	61	13	21·3
TI	85	15	17·7
JR1	39	12	30·8
	675	136	20·1

We sampled birds at sites TL1 and TL2 during the dry and flooding periods, allowing us to test for seasonal differences in prevalence. We found no differences in overall prevalence of *Plasmodium* parasites between the two seasons within each site (site TL1, $\chi^2 = 0$, D.F. $= 1$, $P = 1$; site TL2, $\chi^2 = 0{\cdot}703$, D.F. $= 1$, $P = 0{\cdot}402$). For these tests we used ten host species from site TL1 and nine host species from the site TL2 for which we had sampled at least five individuals. We also found no differences in prevalence between seasons for individual host species (results not reported).

Avian host ecology and Plasmodium *prevalence*

Prevalence of *Plasmodium* (calculated for each host species within sites) was positively related to host density at site TL3 (GLM coefficient $= 0{\cdot}186 \pm 0{\cdot}05$ S.E., $P = 0{\cdot}037$, $n = 5$), but not at sites TL1 ($0{\cdot}027 \pm 0{\cdot}09$ S.E., $P = 0{\cdot}769$, $n = 8$) and TL2 ($-0{\cdot}096 \pm 0{\cdot}20$ S.E., $P = 0{\cdot}661$, $n = 6$). However, the

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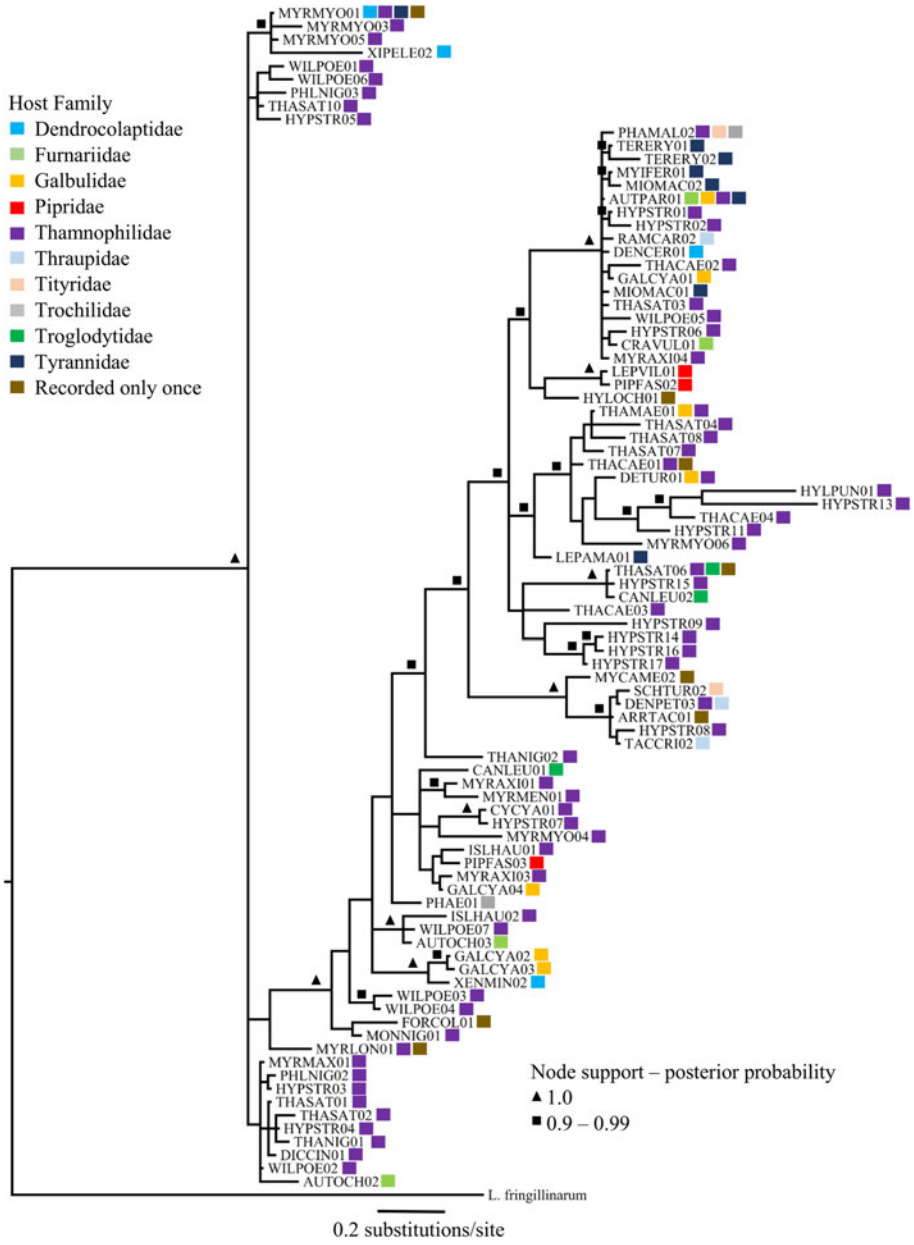

Fig. 2. BI phylogenetic reconstruction of *Plasmodium* lineages recovered from sites along the Tapajós and Jamanxim rivers. Posterior probability support above 0·9 is noted at the base of nodes and host family is noted next to terminal taxon labels.

relationship at site TL3 was based on only five host species, and would not be considered significant after a Bonferroni correction (after three tests, alpha = 0·05/3 or 0·017; Table A2). The diversity of *Plasmodium* parasites was not related to the diversity of avian hosts ($\rho = 0{\cdot}49$, $P = 0{\cdot}268$, $n = 7$).

We found no relationship between ecological traits of the hosts and the prevalence of *Plasmodium* parasites. We report coefficients and significance of each category of the explanatory ecological variables in Table 2 (results of each host trait modelled separately can be found in Table A3). We also ran Wald chi-squared tests for each ecological variable across all coefficients and none were significant (nest location: $\chi^2 = 1{\cdot}4$, D.F. = 4, $P = 0{\cdot}85$; nest type: $\chi^2 = 4{\cdot}6$, D.F. = 2, $P = 0{\cdot}10$; flocking behaviour: $\chi^2 = 1{\cdot}6$, D.F. = 2, $P = 0{\cdot}45$; diet: $\chi^2 = 2{\cdot}1$, D.F. = 2, $P = 0{\cdot}35$). For this analysis, we used 504 birds of 44 species with a minimum of five individuals sampled per species. *Plasmodium* infections were detected in 101 individuals from 23 host species with a prevalence of 20% (Table A4).

Mosquitos, prevalence and diversity of Plasmodium *lineages*

We collected 511 female mosquitoes from 56 species and morpho-species belonging to 11 genera (Tables A5 and A6). We ran paired Mann–Whitney *U* tests

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Table 2. The results of a generalized linear model relating four avian ecological traits to the prevalence of *Plasmodium* parasites in avian hosts

	Estimate of coefficient	S.E.	*t* value	*P*
Intercept	−0·262	0·704	−0·372	0·712
Understory	−0·022	0·801	−0·028	0·978
Sub canopy	−0·622	0·891	−0·699	0·490
Canopy	0·157	1·545	0·101	0·920
Cliff or Bank	0·252	1·857	0·136	0·893
Closed cup	−1·233	1·023	−1·206	0·237
Cavity	−1·212	0·677	−1·790	0·083
Single species	−1·800	1·509	−1·193	0·242
Mixed species	0·123	0·559	0·220	0·827
Frugivore/ Granivore	−18·759	2562·700	−0·007	0·994
Omnivore	−1·536	1·061	−1·447	0·157

All of the explanatory ecological variables were categorical. We therefore report the estimate of the coefficient of each of the levels of those variables in relation to a base level. The base levels are as follows: nest location is ground, nest type is open cup, flocking is solitary/family, diet is insectivore. We report the estimate of the coefficient for each variable, its S.E., *t* value and *P* value; the null deviance of the model is 198·01 on 43 D.F. and the residual deviance is 128·35 on 33 D.F.

to compare mosquito abundance between seasons in sites TL1 and TL2. In site TL1, mosquito abundance was higher in the flooding period than in the dry season ($U = 21{\cdot}5$, $P < 0{\cdot}001$), but in site TL2, there was no difference in mosquito abundance between seasons ($U = 169$, $P = 0{\cdot}441$). In the other five sites, mosquito collection took place in only one season, preventing further seasonal comparisons (Tables A5 and A6).

The prevalence of *Plasmodium* parasites in avian hosts (calculated for an entire site and not by host species) was not related to the diversity ($\rho = -0{\cdot}46$, $P = 0{\cdot}302$) or the abundance ($\rho = -0{\cdot}22$, $P = 0{\cdot}641$) of mosquitoes across the seven sampling sites. The diversity of *Plasmodium* parasites was also not related to the diversity of mosquitoes ($\rho = 0{\cdot}67$, $P = 0{\cdot}102$), but was positively related to the abundance of mosquitoes ($\rho = 0{\cdot}79$, $P = 0{\cdot}034$) across sampling sites (Fig. 3). Since each of these tests represents a separate hypothesis, we did not apply a Bonferroni correction to the resulting *P* values.

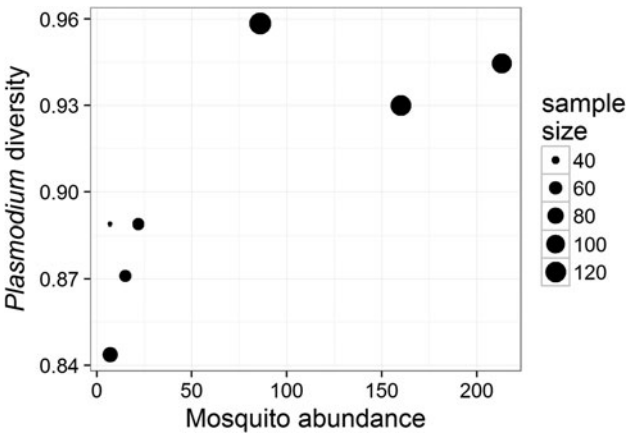

Fig. 3. Relationship between the diversity of *Plasmodium* parasites (calculated using Simpson's index of diversity) and mosquito abundance at each of the sampled sites; point size is scaled to the number of individual birds sampled at each site. The two variables are positively correlated ($\rho = 0{\cdot}79$, $P = 0{\cdot}034$).

DISCUSSION

We investigated biotic factors that may determine the prevalence and diversity of *Plasmodium* parasites in Amazonian birds. We found that the diversity of *Plasmodium* lineages was positively correlated with mosquito abundance across the seven bird communities we sampled. Neither bird density nor bird diversity explained prevalence or lineage diversity of *Plasmodium* parasites among avian hosts. None of the four ecological traits of avian hosts explained *Plasmodium* prevalence. The lack of seasonal differences in prevalence of *Plasmodium* found along the Tapajós River needs to be considered with caution since we tested this with samples collected from only two communities.

Our finding that *Plasmodium* lineage diversity is correlated with mosquito abundance and not with any avian host traits suggests that diversity and distribution of these parasites might be constrained by the final host (Culicidae) in our bird–parasite–mosquito system. Ishtiaq *et al*. (2008) demonstrated that the movement of *Plasmodium* lineages among southwest Pacific Islands might be restricted by the lack of overlap in the distributions of competent vector species. One possibility is that the mosquito community in our study region only has a few competent mosquito vectors and their signal was washed out by the huge mosquito diversity found in southeastern Amazonia. Alternatively, regional processes at the level of host populations, such as immunity to particular parasite lineages or differential exposure to certain parasite lineages, might mask any density-dependent influence of avian hosts and mosquitoes vectors on prevalence. For instance, the distribution and diversity of many parasites among host populations are known to be highly variable and one of the main reasons behind this is the inequality of individual host immune responses in defending themselves against particular parasites (Poulin, 2007).

Our results demonstrate that *Plasmodium* lineage diversity and prevalence in Amazonian birds does not vary with host ecological traits and avian host density. We expected that host population density would be positively correlated to avian malaria prevalence since it affects vector–host-encounter rates (Dobson, 2004). Although several studies

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have found evidence for higher haemosporidian prevalence in denser host populations (Matthews *et al*. 2016; Ricklefs *et al*. 2016; Ellis *et al*. 2017), many others failed to find such an association (Svensson-Coelho *et al*. 2013; Gonzalez-Quevedo *et al*. 2014). Kilpatrick *et al*. (2006) showed that West Nile virus transmission within a local host community was influenced by extreme heterogeneity in mosquito feeding patterns. At least in some sites, transmission of multi-host pathogens such as avian malaria, may be influenced by heterogeneity in host–vector compatibility more than by bird density (Kilpatrick *et al*. 2006; Medeiros *et al*. 2013). Specifically, for avian malaria, higher abundance of vectors does not lead to a higher host–vector-encounter rate. For example, Medeiros *et al*. (2015) showed that vectors overutilized some bird species regardless of their abundance and *Plasmodium* prevalence may be associated with vector utilization rather than vector abundance.

Nest characteristics among many other ecological traits of host individuals might be associated with variation in haemosporidian prevalence (Ricklefs *et al*. 2005; Fecchio *et al*. 2011, 2013; Svensson-Coelho *et al*. 2013; González *et al*. 2014; Lutz *et al*. 2015; Matthews *et al*. 2016). However, based on simple correlations, these studies relied on the premise that nestlings or adults are more exposed to the vectors in the nest according to its architecture. Moreover, mixed results found in these studies suggest that the relationship between nest type and risk of infection by haemosporidian parasites might be location dependent. An analysis of the identity of host blood found in engorged female mosquitoes could provide a general test for this pattern and confirm whether host nest type can predict haemosporidian prevalence in birds.

Lack of seasonal variation in *Plasmodium* prevalence found in two communities sampled along the Tapajós River confirms the temporal stability of these parasites in tropical birds. There are three main possible explanations for this: (1) the abundance of mosquito vectors might differ between seasons (i.e. site TL1), but even the lower number of actively feeding mosquitoes is sufficient to ensure transmission especially if these few mosquitoes are the most competent vector for more prevalent *Plasmodium* lineages; (2) infections in birds last long enough to span more than a single season and thus mask the changes in mosquito abundance between seasons; and (3) vector abundance might be stable over seasons (i.e. site TL2), allowing *Plasmodium* transmission throughout the year. If *Plasmodium* parasites have a dynamic aspect in tropical bird communities, it may vary with years or decades and not between seasons within the same year.

Several species of mosquito belonging to the genera *Aedeomyia, Anopheles, Coquillettidia, Culex, Culiseta, Mansonia, Aedes* (*Ochlerotatus* and *Stegomyia*) have been implicated in the transmission of *Plasmodium* spp. in birds (Valkiūnas, 2005; Njabo *et al*. 2009). Among the species of mosquito inhabiting our study area in Tapajos, *Ad*. (*Ady*.) *squamipennis* is known as a natural vector of avian malaria in Venezuela (Gabaldon *et al*. 1981). Another Neotropical mosquito *Culex* (*Melanoconion*) *ocossa*, together with *Ad*. (*Ady*.) *squamipennis* could be responsible for the transmission of avian malaria in some regions of Panama (Loaiza and Miller, 2013). Unfortunately, we were not able to analyse the engorged females of these mosquitoes to study vectorial capacity or host specificity and thus future research on this is necessary as a first step to fully understand the transmission risk and high diversity of avian malaria parasites in the Neotropical region.

The absence of any perceivable pattern of host family within the phylogeny can be attributed to both the high level of sampling from the host family Thamnophilidae and the low level of host specificity known for *Plasmodium* (Beadell *et al*. 2004, 2009; Valkiūnas, 2005; Dimitrov *et al*. 2010; Ishtiaq *et al*. 2010). This low level of host specificity is shown in those lineages recovered from three and four different host families, spanning different host orders. Host switching is an important evolutionary mechanism in avian haemosporidian parasites with closely related haemosporidian lineages conserved within higher host taxa (Waldenström *et al*. 2002; Križanauskienė *et al*. 2006; Ricklefs *et al*. 2014). Due to high levels of host switching and subsequent dispersal, cospeciation is not thought to have played a large role in the evolutionary history of *Plasmodium* (Ricklefs *et al*. 2014; Lauron *et al*. 2015). However, the high host diversity in Amazonia would be an ideal system to explore the existence of coevolutionary links between *Plasmodium* parasites and avian hosts in future studies.

The heterogeneous prevalence of *Plasmodium* across bird species in southeastern Amazonia, regardless of their ecological traits, suggests that constraints on the distribution of these parasites are related to vectors within these assemblages. This highlights the importance of exposure to vectors in explaining avian malaria prevalence (Medeiros *et al*. 2015). Nonetheless, the results have to be interpreted cautiously, because of low sample sizes within sites and the low number of sites in general. Therefore, the next step in understanding the factors promoting the high diversity and heterogeneity of *Plasmodium* lineages in this region of Amazonia, as well as the mechanisms that produce variation in the prevalence of these vector-borne parasites across avian hosts, must include studies that integrate rates of vector exposure, feeding preference and vectorial capacity of the mosquitoes in the same area.

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APPENDIX

Table A1. *Plasmodium* prevalence from seven bird communities in southeastern Amazonia, Brazil. Species of birds are organized taxonomically

Order	Family	Scientific name	Sampled individuals	Infected	Prevalence (%)
Accipitriformes	Accipitridae	*Leucopternis melanops*	1	0	0
Columbiformes	Columbidae	*Leptotila rufaxilla*	5	0	0
Columbiformes	Columbidae	*Geotrygon montana*	4	0	0
Apodiformes	Trochilidae	*Glaucis hirsutus*	3	0	0
Apodiformes	Trochilidae	*Threnetes leucurus*	1	0	0
Apodiformes	Trochilidae	*Phaethornis superciliosus insignis*	4	1	25
Apodiformes	Trochilidae	*Phaethornis* sp.	1	1	100
Apodiformes	Trochilidae	*Campylopterus largipennis*	1	0	0
Trogoniformes	Trogonidae	*Trogon collaris*	1	0	0
Coraciiformes	Alcedinidae	*Chloroceryle aenea*	4	0	0
Coraciiformes	Momotidae	*Baryphthengus martii*	1	0	0
Galbuliformes	Galbulidae	*Galbula cyanicollis*	9	5	55·6
Galbuliformes	Galbulidae	*Jacamerops aureus*	1	0	0
Galbuliformes	Bucconidae	*Malacoptila rufa*	5	0	0
Galbuliformes	Bucconidae	*Monasa nigrifrons*	3	1	33·3
Piciformes	Ramphastidae	*Ramphastos vitellinus*	1	0	0
Piciformes	Picidae	*Veniliornis affinis*	1	0	0
Piciformes	Picidae	*Celeus elegans*	5	0	0
Falconiformes	Falconidae	*Micrastur ruficollis*	1	0	0
Falconiformes	Falconidae	*Micrastur semitorquatus*	1	1	100
Passeriformes	Thamnophilidae	*Myrmornis torquata*	5	2	40
Passeriformes	Thamnophilidae	*Epinecrophylla leucophthalma*	15	0	0
Passeriformes	Thamnophilidae	*Epinecrophylla* sp.	1	1	100
Passeriformes	Thamnophilidae	*Myrmotherula axillaris*	19	6	31·6
Passeriformes	Thamnophilidae	*Myrmotherula longipennis*	17	5	29·4
Passeriformes	Thamnophilidae	*Myrmotherula menetriesii*	1	1	100
Passeriformes	Thamnophilidae	*Isleria hauxwelli*	19	4	21·1
Passeriformes	Thamnophilidae	*Thamnomanes saturninus*	25	10	40
Passeriformes	Thamnophilidae	*Thamnomanes caesius*	9	3	33·3
Passeriformes	Thamnophilidae	*Dichrozona cincta*	5	2	40
Passeriformes	Thamnophilidae	*Thamnophilus schistaceus*	4	2	50
Passeriformes	Thamnophilidae	*Thamnophilus nigrocinereus*	11	7	63·6
Passeriformes	Thamnophilidae	*Thamnophilus aethiops*	4	1	25
Passeriformes	Thamnophilidae	*Thamnophilus amazonicus*	1	1	100
Passeriformes	Thamnophilidae	*Myrmoderus ferrugineus*	1	0	0
Passeriformes	Thamnophilidae	*Hypocnemoides maculicauda*	10	0	0
Passeriformes	Thamnophilidae	*Hylophylax naevius*	6	1	16·7
Passeriformes	Thamnophilidae	*Hylophylax punctulatus*	5	1	20
Passeriformes	Thamnophilidae	*Myrmelastes leucostigma*	2	1	50
Passeriformes	Thamnophilidae	*Myrmoborus myotherinus*	22	9	40·9
Passeriformes	Thamnophilidae	*Cercomacroides nigrescens*	2	0	0
Passeriformes	Thamnophilidae	*Hypocnemis striata*	32	17	53·1
Passeriformes	Thamnophilidae	*Willisornis poecilinotus*	14	7	50
Passeriformes	Thamnophilidae	*Phlegopsis nigromaculata*	28	11	39·3
Passeriformes	Thamnophilidae	*Rhegmatorhina gymnops*	4	1	25
Passeriformes	Thamnophilidae	*Rhegmatorhina berlepschi*	12	1	8·3
Passeriformes	Conopophagidae	*Conopophaga aurita*	4	1	25
Passeriformes	Formicariidae	*Formicarius colma*	5	1	20
Passeriformes	Formicariidae	*Formicarius analis*	1	0	0
Passeriformes	Scleruridae	*Sclerurus macconnelli*	2	0	0
Passeriformes	Scleruridae	*Sclerurus caudacutus*	2	0	0
Passeriformes	Dendrocolaptidae	*Dendrocincla fuliginosa*	5	0	0
Passeriformes	Dendrocolaptidae	*Dendrocincla merula*	14	0	0
Passeriformes	Dendrocolaptidae	*Deconychura longicauda*	5	0	0
Passeriformes	Dendrocolaptidae	*Sittasomus griseicapillus*	1	0	0
Passeriformes	Dendrocolaptidae	*Certhiasomus stictolaemus*	3	0	0
Passeriformes	Dendrocolaptidae	*Glyphorynchus spirurus*	26	0	0
Passeriformes	Dendrocolaptidae	*Xiphorhynchus ocellatus*	1	0	0
Passeriformes	Dendrocolaptidae	*Xiphorhynchus elegans*	18	0	0
Passeriformes	Dendrocolaptidae	*Xiphorhynchus obsoletus*	4	0	0

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Table A1. (*Cont.*)

Order	Family	Scientific name	Sampled individuals	Infected	Prevalence (%)
Passeriformes	Dendrocolaptidae	*Xiphorhynchus guttatus*	2	0	0
Passeriformes	Dendrocolaptidae	*Campylorhamphus procurvoides*	1	0	0
Passeriformes	Dendrocolaptidae	*Dendroplex picus*	4	0	0
Passeriformes	Dendrocolaptidae	*Dendrocolaptes certhia*	3	1	33·3
Passeriformes	Xenopidae	*Xenops minutus*	8	1	12·5
Passeriformes	Furnariidae	*Automolus rufipileatus*	2	0	0
Passeriformes	Furnariidae	*Automolus subulatus*	1	0	0
Passeriformes	Furnariidae	*Automolus ochrolaemus*	11	2	18·2
Passeriformes	Furnariidae	*Automolus paraensis*	3	1	33·3
Passeriformes	Furnariidae	*Philydor erythrocercum*	2	0	0
Passeriformes	Furnariidae	*Philydor pyrrhodes*	5	0	0
Passeriformes	Furnariidae	*Synallaxis rutilans*	3	0	0
Passeriformes	Furnariidae	*Cranioleuca vulpina*	2	1	50
Passeriformes	Pipridae	*Pipra fasciicauda*	2	2	100
Passeriformes	Pipridae	*Ceratopipra rubrocapilla*	18	0	0
Passeriformes	Pipridae	*Lepidothrix nattereri*	23	0	0
Passeriformes	Pipridae	*Lepidothrix vilasboasi*	3	1	33·3
Passeriformes	Pipridae	*Lepidothrix iris*	1	0	0
Passeriformes	Pipridae	*Heterocercus linteatus*	13	0	0
Passeriformes	Pipridae	*Dixiphia pipra*	2	0	0
Passeriformes	Pipridae	*Chiroxiphia pareola*	1	0	0
Passeriformes	Onychorhynchidae	*Onychorhynchus coronatus*	8	0	0
Passeriformes	Onychorhynchidae	*Terenotriccus erythrurus*	2	1	50
Passeriformes	Onychorhynchidae	*Myiobius barbatus*	4	1	25
Passeriformes	Tityridae	*Schiffornis turdina*	8	1	12·5
Passeriformes	Cotingidae	*Lipaugus vociferans*	1	0	0
Passeriformes	Platyrinchidae	*Platyrinchus saturatus*	1	0	0
Passeriformes	Platyrinchidae	*Platyrinchus platyrhynchos*	6	0	0
Passeriformes	Rhynchocyclidae	*Mionectes oleagineus*	6	1	16·7
Passeriformes	Rhynchocyclidae	*Mionectes macconnelli*	4	2	50
Passeriformes	Rhynchocyclidae	*Leptopogon amaurocephalus*	1	1	100
Passeriformes	Rhynchocyclidae	*Corythopis torquatus*	1	0	0
Passeriformes	Rhynchocyclidae	*Rhynchocyclus olivaceus*	2	0	0
Passeriformes	Rhynchocyclidae	*Tolmomyias sulphurescens*	2	0	0
Passeriformes	Rhynchocyclidae	*Tolmomyias flaviventris*	1	0	0
Passeriformes	Tyrannidae	*Attila spadiceus*	5	1	20
Passeriformes	Tyrannidae	*Ramphotrigon ruficauda*	2	0	0
Passeriformes	Tyrannidae	*Myiarchus ferox*	1	1	100
Passeriformes	Tyrannidae	*Myiarchus* sp.	1	0	0
Passeriformes	Tyrannidae	*Rhytipterna simplex*	5	0	0
Passeriformes	Tyrannidae	*Cnemotriccus fuscatus*	5	0	0
Passeriformes	Tyrannidae	*Knipolegus poecilocercus*	6	0	0
Passeriformes	Vireonidae	*Hylophilus semicinereus*	1	0	0
Passeriformes	Vireonidae	*Tunchiornis ochraceiceps*	6	1	16·7
Passeriformes	Troglodytidae	*Microcerculus marginatus*	2	0	0
Passeriformes	Troglodytidae	*Pheugopedius genibarbis*	3	1	33·3
Passeriformes	Troglodytidae	*Cantorchilus leucotis*	11	4	36·4
Passeriformes	Polioptilidae	*Ramphocaenus melanurus*	1	0	0
Passeriformes	Turdidae	*Turdus fumigatus*	7	2	28·6
Passeriformes	Turdidae	*Turdus albicollis*	3	0	0
Passeriformes	Passerellidae	*Arremon taciturnus*	4	2	50
Passeriformes	Thraupidae	*Coryphospingus cucullatus*	1	0	0
Passeriformes	Thraupidae	*Lanio surinamus*	3	0	0
Passeriformes	Thraupidae	*Lanio cristatus*	2	1	50
Passeriformes	Thraupidae	*Ramphocelus carbo*	11	2	18·2
Passeriformes	Thraupidae	*Sporophila angolensis*	5	0	0
Passeriformes	Thraupidae	*Saltator maximus*	2	0	0
Passeriformes	Thraupidae	*Saltator coerulescens*	5	0	0
Passeriformes	Thraupidae	*Saltator grossus*	2	0	0
Passeriformes	Cardinalidae	*Cyanoloxia rothschildii*	6	0	0
			675	136	20·1

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Table A2. The 11 bird species used to test the effect of host density (individuals per 100 ha) on prevalence of *Plasmodium* from three transects along the Tapajós River. The geographic locations of sites can be found in Fig. 1

Species	Location	Sampled individuals	Infected	Density
Ceratopipra rubrocapilla	TL2	5	0	17·29
Galbula cyanicollis	TL1	6	4	1·99
Glyphorynchus spirurus	TL1	5	0	6·95
	TL2	8	0	11·12
	TL3	5	0	8·34
Hypocnemis striata	TL1	9	6	12·46
	TL3	10	8	38·53
Lepidothrix nattereri	TL1	7	0	5·75
	TL2	9	0	6·79
	TL3	6	0	6·27
Myrmoborus myotherinus	TL1	6	3	18·91
	TL3	5	4	29·32
Myrmotherula axillaris	TL1	5	3	11·05
Myrmotherula longipennis	TL1	5	1	1·65
Phlegopsis nigromaculata	TL1	6	5	2·23
	TL2	5	2	11·18
Willisornis poecilinotus	TL2	5	1	0·00
Xiphorhynchus elegans	TL2	7	0	12·13
	TL3	6	0	5·51
		120	37	

Table A3. In the main text of our paper we report the results of a multiple regression (GLM) modelling prevalence as a function of four explanatory variables (nest location, nest type, flocking and diet). Here we report the results of four separate models of each explanatory variable by itself, for comparison. Each model is a GLM with a 'quasibinomial' error structure (to account for overdispersion as in the model presented in the main text); coefficients are reported as in Table 2 of the main text

	Estimate of coefficient	S.E.	*t* value	*P*
Model 1 – nest location				
Intercept	−0·571	0·732	−0·78	0·44
Understory	−0·413	0·808	−0·511	0·613
Sub canopy	−1·025	0·849	−1·208	0·234
Canopy	−1·221	1·354	−0·902	0·373
Cliff or Bank	−0·528	1·871	−0·282	0·779
Model 2 – nest type				
Intercept	−0·952	0·269	−3·538	0·001
Closed cup	−0·735	1·019	−0·722	0·475
Cavity	−0·879	0·592	−1·485	0·145
Model 3 – flocking				
Intercept	−1·225	0·335	−3·661	0·001
Single species	−1·26	1·522	−0·828	0·412
Mixed species	0·14	0·468	0·298	0·767
Model 4 – diet				
Intercept	−0·931	0·221	−4·217	0·0001
Frugivore/Granivore	−18·061	2417·171	−0·007	0·994
Omnivore	−1·503	0·878	−1·711	0·095

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Table A4. The 44 species used in the analysis of an effect of avian ecological traits on *Plasmodium* prevalence in southeastern Amazonia

	Number of individuals		Ecological traits analysed			
Specie name	Sampled	Infected	Nest location	Nest type	Flocking	Diet
Attila spadiceus	5	1	Understory	Open cup	Solitary/family	Insectivore
Automolus ochrolaemus	11	2	Cliff or Bank	Cavity	Mixed species	Insectivore
Cantorchilus leucotis	11	4	Understory	Closed cup	Solitary/family	Insectivore
Celeus elegans	5	0	Sub canopy	Cavity	Solitary/family	Omnivore
Ceratopipra rubrocapilla	18	0	Understory	Open cup	Solitary/family	Frugivore/ Granivore
Cyanoloxia rothschildii	6	0	Understory	Open cup	Solitary/family	Omnivore
Deconychura longicauda	5	0	Sub canopy	Cavity	Mixed species	Insectivore
Dendrocincla fuliginosa	5	0	Sub canopy	Cavity	Mixed species	Insectivore
Dendrocincla merula	14	0	Sub canopy	Cavity	Mixed species	Insectivore
Epinecrophylla leucophthalma	15	0	Understory	Open cup	Single species	Insectivore
Formicarius colma	5	1	Understory	Cavity	Mixed species	Insectivore
Galbula cyanicollis	9	5	Sub canopy	Cavity	Mixed species	Insectivore
Glyphorynchus spirurus	26	0	Sub canopy	Cavity	Mixed species	Insectivore
Heterocercus linteatus	13	0	Understory	Open cup	Solitary/family	Frugivore/ Granivore
Hylophylax naevius	6	1	Understory	Open cup	Solitary/family	Insectivore
Hylophylax punctulatus	5	1	Understory	Open cup	Solitary/family	Insectivore
Hypocnemis striata	32	17	Understory	Open cup	Mixed species	Insectivore
Hypocnemoides maculicauda	10	0	Understory	Closed cup	Solitary/Family	Insectivore
Isleria hauxwelli	19	4	Understory	Open cup	Mixed species	Insectivore
Knipolegus poecilocercus	6	0	Ground	Closed cup	Solitary/family	Insectivore
Lepidothrix nattereri	23	0	Sub canopy	Open cup	Solitary/family	Omnivore
Leptotila rufaxilla	5	0	Understory	Open cup	Solitary/family	Frugivore/ Granivore
Malacoptila rufa	5	0	Ground	Cavity	Mixed species	Insectivore
Mionectes oleagineus	6	1	Understory	Closed cup	Mixed species	Omnivore
Myrmoborus myotherinus	22	9	Understory	Open cup	Solitary/family	Insectivore
Myrmornis torquata	5	2	Ground	Open cup	Solitary/family	Insectivore
Myrmotherula axillaris	19	6	Understory	Open cup	Mixed species	Insectivore
Myrmotherula longipennis	17	5	Sub canopy	Open cup	Mixed species	Insectivore
Onychorhynchus coronatus	8	0	Sub canopy	Open cup	Solitary/family	Insectivore
Philydor pyrrhodes	5	0	Sub canopy	Open cup	Solitary/family	Insectivore
Phlegopsis nigromaculata	28	11	Ground	Open cup	Solitary/family	Insectivore
Platyrinchus platyrhynchos	6	0	Sub canopy	Open cup	Solitary/family	Insectivore
Ramphocelus carbo	11	2	Canopy	Open cup	Single species	Omnivore
Rhegmatorhina berlepschi	12	1	Understory	Open cup	Solitary/family	Insectivore
Rhytipterna simplex	5	0	Canopy	Open cup	Mixed species	Insectivore
Saltator coerulescens	5	0	Canopy	Open cup	Mixed species	Frugivore/ Granivore
Schiffornis turdina	8	1	Sub canopy	Cavity	Solitary/family	Insectivore
Sporophila angolensis	5	0	Sub canopy	Open cup	Mixed species	Omnivore
Thamnomanes saturninus	25	10	Sub canopy	Open cup	Mixed species	Insectivore
Thamnophilus nigrocinereus	11	7	Sub canopy	Open cup	Solitary/family	Insectivore
Turdus fumigatus	7	2	Canopy	Open cup	Solitary/family	Omnivore
Willisornis poecilinotus	14	7	Understory	Cavity	Solitary/family	Insectivore
Xenops minutus	8	1	Sub canopy	Cavity	Mixed species	Insectivore
Xiphorhynchus elegans	18	0	Sub canopy	Cavity	Mixed species	Insectivore
	504	101				

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Table A5. Mosquito species caught across five sites during the dry season. The geographic locations of sites can be found in Fig. 1

Subfamily	Tribe	Species/morph-species	Sites				
			TL1	TL2	TL4	TR1	JR1
Anophelinae		*Anopheles* (*Nyssorhynchus*) *nuneztovari* s.l.	3	0	3	0	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) *oswaldoi* s.l.	0	26	0	0	0
Culicinae	Aedini	*Psorophora* (*Janthinosoma*) *amazonica cf.*	0	0	2	0	0
Culicinae	Culicini	*Culex* (*Culex*) *chidesteri*	0	9	0	0	0
Culicinae	Culicini	*Culex* (*Culex*) sp. F-3	0	0	0	0	1
Culicinae	Culicini	*Culex* (*Melanoconion*) *ocossa*	0	0	2	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *ribeirensis*	0	2	0	4	2
Culicinae	Culicini	*Culex* (*Melanoconion*) *ribeirensis* F-1	0	0	0	4	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Group Intrincatus	0	6	7	0	3
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Section Melanoconion	0	0	0	2	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Subgroup Penai	0	6	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-2 Section Spissipes	0	3	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-2 Group Atratus	0	5	0	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *albicosta*	0	3	0	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *hermanoi*	0	3	0	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *juxtamansonia*	6	2	0	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *lynchi*	0	6	2	0	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *calosomata*	0	0	6	0	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) spp.	0	5	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) spp. Section Melanoconion	0	0	0	5	1
			9	76	22	15	7

Table A6. Mosquito species caught across four sites during the flooding season. The geographic locations of sites can be found in Fig. 1

Subfamily	Tribe	Species/morph-species	Site			
			TL1	TL2	TL3	TI
Anophelinae		*Anopheles* (*Nyssorhynchus*) *benarrochi*	1	0	0	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) *nuneztovari* s.l.	3	2	1	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) *oswaldoi* s.l.	2	18	19	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) *rangeli*	0	0	1	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) *trianmulatus* s.l.	2	0	5	0
Anophelinae		*Chagasia bonneae*	0	5	0	0
Culicinae	Aedeomyiini	*Aedeomyia* (*Aedeomyia*) *squamipennis*	0	0	0	2
Culicinae	Culicini	*Culex* (*Anoedioporpa*) *quasioriginator*	1	0	0	0
Culicinae	Culicini	*Culex* (*Culex*) *chidesteri*	1	1	1	0
Culicinae	Culicini	*Culex* (*Culex*) sp. F-1	1	1	0	0
Culicinae	Culicini	*Culex* (*Culex*) sp. F-2	0	1	2	0
Culicinae	Culicini	*Culex* (*Culex*) sp. F-3	0	0	1	0
Culicinae	Culicini	*Culex* (*Culex*) spp. Group coronator	4	1	1	0
Culicinae	Culicini	*Culex* (*Culex*) *surinamensis cf.*	1	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *inadmirabilis*	0	0	0	1
Culicinae	Culicini	*Culex* (*Melanoconion*) *pedroi*	1	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *pilosus*	0	3	42	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *ribeirensis*	0	9	6	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *ribeirensis* F-1	1	1	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *saramaccensis cf.*	0	0	3	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Group Intrincatus	2	10	55	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Section Spissipes	0	0	5	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Section Melanoconion	0	0	2	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Subgroup Penai	0	4	2	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-1 Group Atratus	0	1	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-2 Group Atratus	2	0	6	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-3 Section Spissipes	0	4	15	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-2 Section Melanoconion	1	0	0	0

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Table A6. (*Cont.*)

Subfamily	Tribe	Species/morph-species	Site			
			TL1	TL2	TL3	TI
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-3 Section Melanoconion	0	0	1	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-4 Section Melanoconion	1	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) sp. F-5 Section Melanoconion	0	0	1	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *spissipes*	0	1	1	0
Culicinae	Culicini	*Culex* (*Melanoconion*) *theobaldi*	0	2	3	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *albicosta*	5	1	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *hermanoi*	5	2	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *juxtamansonia*	25	2	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *lynchi*	1	4	0	0
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) *venezuelensis*	0	0	4	0
Culicinae	Mansoniini	*Mansonia* (*Mansonia*) *titillans*	10	0	0	2
Culicinae	Sabethini	*Sabethes* (*Sabethes*) sp. F-1	0	1	0	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *apicalis*	1	0	2	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *calosomata*	1	0	1	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *geometrica*	0	0	6	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) *geometrica* F-1	1	0	19	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) sp. F-1	0	2	0	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) sp. F-2	1	1	1	0
Culicinae	Uranotaeniini	*Uranotaenia* (*Uranotaenia*) sp. F-3	0	0	1	0
Culicinae	Incerta	Culicidae morphotype 1	2	0	0	0
Anophelinae		*Anopheles* (*Nyssorhynchus*) sp.	0	1	0	0
Culicinae	Aedini	*Aedes* (*Ochlerotatus*) sp.	1	0	0	0
Culicinae	Culicini	*Culex* (*Melanoconion*) spp. Section Melanoconion	0	5	6	3
Culicinae	Mansoniini	*Coquillettidia* (*Rhynchotaenia*) sp.	0	1	0	0
			77	84	213	8

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