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Community organization of avian malaria parasites in lowland Amazonia: Prevalence, diversity, and specialization in a local assemblage

Linda Maria Elenor Svensson
University of Missouri-St. Louis, lmsd5d@umsl.edu

COMMUNITY ORGANIZATION OF AVIAN MALARIA PARASITES IN LOWLAND AMAZONIA: PREVALENCE, DIVERSITY, AND SPECIALIZATION IN A LOCAL ASSEMBLAGE

Linda Maria Elenor Svensson Coelho

M.S. in Ecology, Evolution, and Systematics, University of Missouri-St. Louis, 2008

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Advisory Committee

Robert E. Ricklefs, Ph.D.
Chairperson

Patricia G. Parker, Ph.D.

John G. Blake, Ph.D.

Bette A. Loiselle, Ph.D.

Ravinder N.M. Sehgal, Ph.D.

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I dedicate this dissertation to my parents,

Bengt Alrik (in memoriam) and

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GENERAL ABSTRACT

I characterized a lowland Amazonian assemblage of haemosporidian ("malaria") parasites (*Haemoproteus* and *Plasmodium*) of understory birds by analyzing variation in prevalence (proportion of infected host individuals) among years and host species, documenting diversity of haemosporidian evolutionary lineages, and quantifying host specialization. Using standard molecular methods to screen for haemosporidia in 2488 individual birds from 104 species in the Tiputini Biodiversity Station, Ecuador, I found 21.7% to be infected. Prevalence ranged significantly among years and host species. Forty-five putative evolutionary lineages of haemosporidia were identified, by sequencing part of the cytochrome *b* (cyt *b*) gene. Based on a comparative analysis, among host species variation in haemosporidian prevalence related positively to level of sexual dimorphism and negatively to foraging height.

I assigned 385 parasite individuals to cyt *b* lineages. These exhibited a wide range of abundance (one to 91 individuals) and host specialization (one to 23 host species). I quantified host specificity by incorporating both phylogenetic relationships (based on genetic data) and frequency distribution among hosts. Based on null model comparisons, six haemosporidian lineages were more specialized than expected by chance. The hosts of these six haemosporidian lineages were on average more abundant than hosts of generalist lineages, but average body size and survival rate did not differ between hosts of specialists and hosts of generalists. Host specificity was also phylogenetically conserved among haemosporidia. Consequently, I performed a comparative regression

analysis, controlling for the effect of parasite phylogeny, and found no relationships between host specificity and host abundance, body size, or survival rate.

Finally, I applied network analysis in combination with null models to test whether the level of reciprocal specialization (where one parasite lineage associates with only one bird species, which harbors no other parasite lineages) is greater in this tropical assemblage than it is in an equivalent temperate assemblage. Assuming coevolution proceeds towards reciprocal specialization, it should be greater in the tropics, where coevolution has historically been hypothesized to be more important in species diversification. I found no evidence for this hypothesis; instead, reciprocal specialization was greater in the temperate site.

Chapter 1: Diversity and prevalence of *Plasmodium* and *Haemoproteus* in a western Amazonian assemblage

Authors: MARIA SVENSSON-COELHO[1], JOHN G. BLAKE[1,2], BETTE A. LOISELLE[1,2], AMANDA S. PENROSE[1], PATRICIA G. PARKER[1], AND ROBERT E. RICKLEFS[1]

[1]Department of Biology, University of Missouri – St. Louis, One University Blvd., St. Louis, Missouri 63121-4499 USA

[2]Current address: Department of Wildlife Ecology and Conservation and Center for Latin American Studies, 110 Newins-Ziegler Hall, P.O. Box 110430, University of Florida, Gainesville, FL 32611-0430 USA

Corresponding author: Maria Svensson-Coelho. Current address: Departamento Genética e Biologia Evolutiva, Instituto de Biosciências, Universidade de São Paulo, Edifício André Dreyfus, Rua do Matão, 277, São Paulo, Brazil. E-mail: lmsd5d@umsl.edu

ABSTRACT. – PCR and DNA sequencing were used to screen for haemosporidian parasites (*Haemoproteus* and *Plasmodium*) in 2488 individual birds from 104 species and 22 families, primarily understory suboscine passerines, in a lowland Amazonian forest in Ecuador. Putative evolutionary lineages of haemosporidia were identified using the mtDNA gene cytochrome *b* (cyt *b*). Sampling was conducted over nine years and in two 1-ha plots with different levels of moisture, allowing us to assess annual variation in haemosporidian prevalence and whether prevalence is greater in the plot containing more

water, which suggests it offers more suitable vector habitats. Additionally, among-species variation in prevalence was investigated and relationships between traits of hosts and prevalence of haemosporidia were analyzed in a comparative analysis. Finally, host specificity of each recovered parasite lineage was estimated and multiple indices, incorporating varying details of information, were compared. Prevalence of haemosporidia was 21.7% when years and study sites were combined and ranged from 5.6% to 91.2% among well-sampled host species. There was significant variation in prevalence among years, ranging from 14.5% in 2006 to 33.2% in 2009. There was some support for that haemosporidian prevalence increases with the level of sexual dimorphism and decreases with foraging height of a host species. Sixty-five unique cyt *b* haplotypes were identified, some of which were considered variation within the same evolutionary lineage. In total, 45 putative evolutionary lineages were defined based on 363 identified parasites. Fourteen haplotypes were identical to haplotypes found elsewhere, sometimes on different continents. Host specificity varied greatly among parasite lineages, and we found that an index initially developed for community phylogenetics, the standardized effect size of the weighted mean pairwise distance (SES_{MPD}), did not correlate with sample size. This makes it useful in statistical analyses in which host specificity is considered a dependent variable, in that there is no need to control for variable sampling effort.

KEYWORDS: Avian blood parasites, avian malaria, community ecology, compound community, parasite diversity, parasite prevalence

Most wildlife pathogens are capable of infecting multiple host species (Woolhouse et al. 2001; Poulin et al. 2011). Despite this, the compound community (all parasites on all hosts, Holmes and Price 1986; in Esch et al. 1990) of wildlife pathogens is rarely investigated (Esch et al. 1990). Adopting a compound community approach enables us to obtain a more complete understanding of multi-host pathogen community dynamics and is becoming increasingly valuable in light of recent frequent outbreaks of zoonotic diseases (e.g. Cleaveland et al. 2001; Taylor et al. 2001; Wilcox and Gubler 2005).

Birds are infected with a range of pathogens worldwide. The vector-transmitted avian pigmented haemosporidia (*Plasmodium* spp. and *Haemoproteus* spp., Plasmodiidae, hereafter avian haemosporidia), sometimes referred to as avian malaria, constitute one group of common, widespread, and mostly multi-host (restricted to birds) pathogens (Pérez-Tris et al. 2005). Avian haemosporidia are harmful to their hosts (Atkinson and van Riper III 1991; Merino et al. 2000; Cardona et al. 2002; Palinauskas et al. 2011), but the effect of infection, even by the same haemosporidian species, varies among host species (Palinauskas et al. 2008, 2011). Because they can negatively affect their individual hosts, avian haemosporidia can have a detrimental effect on entire avian populations, the most well-known example being the contribution of *Plasmodium relictum* to the decimation of the native Hawaiian avifauna (Warner 1968; van Riper III et al. 1986).

Local studies of avian haemosporidian assemblages are valuable for estimating temporal and among host-species variation in apparent prevalence (proportion of infected hosts; hereafter prevalence), as well as for quantifying host specificity of these parasites, since one avoids the potentially confounding factor of spatial variation in these ecological

properties (Poulin 2007). Prevalence (both community-wide and within host species) has been found to vary both between regions (Greiner et al. 1975; White et al. 1978) and among localities within regions (e.g. Apanius et al. 2000; Bensch and Åkesson 2003; Fallon et al. 2003a; Loiseau et al. 2010; Ricklefs et al. 2011). For instance, based on blood smear data, prevalence of avian haemosporidia is lower in tropical than in temperate regions (Greiner et al. 1975; White et al. 1978). In addition to this spatial variation at various scales, some haemosporidian species have demonstrated significant temporal variation in prevalence; seasonally (Cosgrove et al. 2008), annually (Bensch et al. 2007), and over decades (Fallon et al. 2004). This could be the result of parasite and/or vector sensitivity to climate fluctuations. That is, because dipteran vectors are moisture dependent for their development vector abundance might fluctuate as a response to rainfall patterns or proximity to water sources, which in turn may result in variable parasite prevalence in the bird population (Wood et al. 2007). Furthermore, development time of the infectious stages of *Plasmodium relictum* within its vector, *Culex quinquefasciatus* increases with decreasing ambient temperature and seems to reach a minimum development threshold at 13°C (LaPointe et al. 2009). Because the ambient temperature varies less annually closer towards the equator than it does further away from it (MacArthur 1972), and—more importantly—reaches an average low well above 13°C, one might expect prevalence to vary less in the tropical than in the temperate region from year to year.

Avian haemosporidia are never distributed evenly among host species in an assemblage (Greiner et al. 1975; White et al. 1978; Fallon et al. 2003a; Scheuerlein and Ricklefs 2004; Sehgal et al. 2005; Durrant et al. 2006; Krizanauskiene et al. 2006; Latta

and Ricklefs 2010). Several independent studies have addressed whether among-host species prevalence relates to any ecological and/or life history traits of bird species. For example, prevalence might vary as a result of differential exposure to haemosporidian vectors (Bennett and Fallis 1960; Garvin and Remsen Jr 1997), leading to (1) a positive association between prevalence and foraging or nest height because vectors have been shown to be more abundant towards the canopy (Bennett and Fallis 1960), (2) greater prevalence in open cup nesters compared to cavity and dome nesters because covered nests offer protections against vectors (Fecchio et al. 2011), (3) a positive association between prevalence and body size because larger bodies provide greater surface area for biting (Atkinson and van Riper III 1991), and (4) a positive association between prevalence and host abundance because transmission is greater in denser populations (Anderson and May 1979, 1981; Brown et al. 2001). In addition, some bird species might have impoverished immune systems compared to others, which might relate to measurable host traits. It has, for example, been demonstrated that host survival rate (a proxy for longevity) is positively related to cell-mediated immunity (Tella et al. 2002), suggesting that longer-lived birds have stronger immune systems. Furthermore, the Hamilton-Zuk hypothesis, which applies to chronic parasites like avian haemosporidia, states that "[i]f susceptibility to parasites is important in sexual selection . . . animals that show more strongly developed epigamic characters should be subject to a wider variety of parasites . . ." (Hamilton and Zuk, 1982, p. 385). According to the same authors, this implies that species in which sexual selection is stronger should exhibit greater parasite prevalence.

Studies have, however, found mixed support for whether ecological and life-history traits of hosts relate to blood parasite prevalence. For example, Scheuerlein and Ricklefs (2004) found that male plumage brightness and body size associated with greater prevalence of *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and *Trypanosoma* combined, and that a longer lifespan was associated with higher prevalence of *Plasmodium*. Ricklefs et al. (2005) found a significant upwards concave relationship between haemosporidian prevalence and host abundance (the least and most abundant species exhibited the greatest prevalence), and that body mass was associated with greater prevalence; however, they found no significant relationships between prevalence and nest height, nest type, foraging height, sexual dimorphism, sex, or age. Fecchio et al. (2011) found that social breeding and nest height were associated with higher prevalence of *Haemoproteus*, that birds building open nests exhibited higher prevalence of *Haemoproteus* but lower prevalence of *Plasmodium* compared to birds building closed nests, but no relationship between host body size and prevalence of either parasite genus. The positive association between *Haemoproteus* prevalence and social breeding was verified in Fecchio et al. (2012), but the same study found no significant associations between haemosporidian prevalence and nest type, nest height, weight, incubation time, or migratory behavior. Finally, Read (1991) found prevalence to be greater in monogamous than in polygamous bird species, opposite of what is expected under the Hamilton-Zuk hypothesis of sexual selection. Because of these inconclusive findings, the relationship between host ecology and life-history and parasite prevalence deserves further attention.

Along with prevalence of this group of pathogens, or of single species therein, a relevant parasite trait to consider in multi-host pathogens is host specificity. The quantification of host specificity can include at least two pieces of information: phylogenetic relationships among hosts utilized by a given parasite lineage as well as frequency distribution on the different hosts (Poulin and Mouillot 2003, 2005; Poulin et al. 2011). Because prevalence can vary among localities, a local study allows for incorporation of frequency distribution on alternative host species when estimating host specificity (Poulin 2007). Thus, we use our dataset to compare different host specificity indices to each other in order to determine how much the estimate of host specificity changes when excluding/including host phylogeny and excluding/including prevalence information of several haemosporidian lineages. Additionally, even when one attempts to produce a more biologically realistic measure of host specificity by including phylogenetic and frequency information, one still has to decide what constitutes a specialist and a generalist parasite. We apply an index developed for community phylogenetics (Webb et al. 2002; Kembel et al. 2011) to the avian haemosporidian system to aid in this decision.

Our specific objectives in this study are manifold. First, we describe the diversity of avian haemosporidia and their evolutionary relationships by using molecular techniques in a region that has not previously been explored with respect to these parasites. The lineages recovered are then compared to lineages from other parts of the world to determine whether haemosporidia in our study site are unique to the area. Second, we test whether assemblage-wide and within-host species prevalence of *Plasmodium* and *Haemoproteus* vary annually and/or between our two closely situated

sampling sites. Specifically, we predict that the wetter plot will exhibit elevated haemosporidian prevalence. We make no *a priori* predictions for annual prevalence variation because we do not have climatic data for this period. Instead, we explore whether external factors need to be invoked to explain annual variation or whether it can be attributed to differential sampling effort of primary hosts. Third, we test, using a comparative multiple regression, whether haemosporidian prevalence is related to host species foraging height, nest type, abundance, level of sexual dimorphism, body mass, and apparent survival rate. Here, based on earlier literature described above, we predict prevalence to increase with increasing foraging height, abundance, level of sexual dimorphism, and body mass, to decrease with increasing survival rate, and to be greater in bird species building open nests. Finally, we compare the outcome of several quantitative measures of host specificity and determine which lineages within this site can be considered significantly specialized and generalized. We also investigate whether any of the indices is independent of sample size, which would make it suitable for analyses in which sampling effort is a confounding factor.

METHODS

Sampling. – Sampling took place during the dry season (primarily in January and March) between 2001 and 2010 on two 100-ha plots (Harpia and Puma) in *terra firme* forest, separated by ~ 1.7 km in the Tiputini Biodiversity Station (TBS), Orellana Province, Ecuador (0°38´S, 76°08´W). The area is relatively undisturbed, with the closest indigenous human settlement being ~ 30 km distant. The Harpia plot is located between 201 and 233 m elevation and the Puma plot is located between 209 and 235 m elevation.

The Puma plot contains more streams than the Harpia plot, and while both plots partially flood during the rainy season (April to October), the Puma plot contains more permanent water (Loiselle et al. 2007; Sheth et al. 2009). Consequently, swamp habitats are found only in the Puma plot (Sheth et al. 2009). Over 300 species of bird have been detected in these plots (Blake 2007). The avian assemblage is dominated by relatively sedentary suboscines (Passeriformes, suborder Tyranni), both with respect to species richness and abundance (Blake 2007). The four most species-rich families are the suboscine Thamnophilidae, Tyrannidae, and Furnariidae, and the oscine Thraupidae; the families with the most individuals sampled are the Thamnophilidae and Furnariidae (both suboscines). This study considered primarily understory birds. Bird taxonomy follows the South American Classification Committee (http://www.museum.lsu.edu). Ninety-six nets (12 x 2.6 m, 36-mm mesh) per plot were set at ground level, arranged in eight rectangles (100 x 200 m) of 12 nets, with nets placed ~ 50 m apart. Samples were collected between 0600 and 1300 h ECT and all birds were banded with aluminum leg bands (Loiselle et al. 2007; Blake and Loiselle 2009).

Molecular screening and identification. – Approximately 10 µL of blood was obtained by brachial venipuncture and stored in 1 mL Longmire lysis buffer (Longmire et al. 1997). Work at the Tiputini Biodiversity Station was conducted in accordance with research permit number 13-IC-FAU-DFN (and subsequent renewals), Ministerio del Ambiente, Distrito Forestal Napo, Tena, Ecuador. DNA was extracted by an ammonium acetate/isopropanol protocol (Svensson and Ricklefs 2009) or by phenol-chloroform. DNA samples, along with positive and negative controls, were screened for

haemosporidia by amplifying a segment of mtDNA encoded SSU ribosomal RNA using primers 343F and 496R (Fallon et al. 2003b), followed by gel electrophoresis in a 1% agarose gel for 20 minutes. The presence of a 154-bp band provided evidence of infection. A 552 bp fragment of cytochrome *b* (cyt *b*) was amplified in a nested reaction with outer primers 3932F (inverse of 3932R in Olival et al. 2007) and DW4 (Perkins and Schall 2002), and inner primers 413F and 926R (Ricklefs et al. 2005). PCR reactions for the cyt *b* amplification contained 1X buffer, 200 nM of each dNTP, 2 mM MgCl2, 0.02% BSA, 200 nM of each primer, and 0.5 units of TaKaRa Taq™ (TaKaRa Bio Inc., Shiga, Japan). This method has successfully amplified *Plasmodium* and *Haemoproteus* (both subgenera) previously (Fecchio et al. 2012).

The PCR program for the outer cyt *b* reaction had an initial denaturing period at 94°C for 4 min, 35 cycles of 94°C for 20 s, 49°C for 10 s, 68°C for 45 s, and a final extension at 68°C for 3 min. In the nested PCR reaction, we used 0.5 µL of the outer PCR reaction product and the same concentrations of reagents as in the outer reaction. The PCR program for the nested cyt *b* reaction had an initial denaturing period at 94°C for 1 min, 28 cycles of 94°C for 20 s, 52°C for 10 s, 68°C for 50 s, and a final extension at 68°C for 7 min. The outer reaction was run in 10 µL of reaction mix and the nested reaction was run in 20 µL of the mix. Products were sequenced on an ABI 3100 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA). In some cases, we had strong products from the 702 bp outer reaction and sequenced these instead of the nested product. Unique haplotypes were sequenced in both directions. Cyt *b* sequences were edited in SeqMan II (DNASTAR Inc., Madison, WI, USA) and haplotypes (up to 663 bp long) were aligned in Clustal X v. 2.0.10 (Larkin et al. 2007). Haplotypes found in this

study were matched to known haplotypes in GenBank (http://www.ncbi.nlm.nih.gov), the MalAvi database (Bensch et al. 2009), and our local database as of May 15, 2012. When we detected double peaks in the chromatograms we regarded these as mixed infections. Mixed infections were reconciled manually, by matching the sequence to known haplotypes from the area.

Haemosporidian lineages. – Determining the species or evolutionary lineage of haemosporidia is a challenge because data from multiple genes and morphology are unavailable, and sample sizes of particular haplotypes are usually small. Researchers have used a cyt *b* divergence cutoff of 0.5 – 0.6%, sometimes in combination with host species affiliation, for delineating evolutionary lineages of avian haemosporidia (Ricklefs et al. 2005). Although morphospecies can be distinguished by cyt *b* at similarly low levels (Hellgren et al. 2007), some, for example *P. relictum* (Valkiunas et al. 2009), exhibit much greater cyt *b* divergence.

We combined cyt *b* divergence and host species distribution among closely related haplotypes to group them into putative evolutionary lineages. In an initial neighborjoining tree, we found two shallow clades of at least two haplotypes in *Haemoproteus* and eight such clades in *Plasmodium.* Eight of these 10 clades consisted of three or more haplotypes. Haplotype networks were constructed in order to more accurately view the connections among these shallow clades of parasites. Sequences within the eight clades were extracted into separate files and conserved sites were deleted. Each group was then uploaded in the software Network 4.6 (Fluxus Technology Ltd., Suffolk, England) and median-joining (MJ) haplotype networks (Bandelt *et al.*

1999) were estimated with the highest possible epsilon value (231) and a transition:transversion ratio of 2:1. We then estimated the most parsimonious networks (Polzin and Daneschmand 2003), and show these, including the frequency of each haplotype and their host associations, in Appendices A-H.

Our criteria for either combining two or more haplotypes into the same putative evolutionary lineage or considering a haplotype a unique lineage were as follows. If two haplotypes were separated from each other by four mutations (~0.6%) or less, they were considered the same evolutionary lineage unless (1) both were well-sampled and segregated onto different host species, or (2) they were recovered from different host families (regardless of sample size).

Phylogenetic analysis of parasites. – Six mammalian *Plasmodium* species (*P. vinckei*, *P. cynomolgi*, *P. vivax*, *P. ovale*, *P. berghei*, and *P. chabaudi*; GenBank IDs AB599931, AF069616, AF069619, AF069625, DQ414645, DQ414649) composed the outgroup in a maximum likelihood analysis of 45 ingroup taxa (the most abundant haplotype in each lineage was included), applying the default general time reversible (GTR) + gamma model of evolution and running 100 bootstrap replicates in RAxML BlackBox (Stamatakis et al. 2008). We rooted the tree with mammalian *Plasmodium* spp. because these appear to be the most appropriate outgroup for avian and reptilian haemosporidia based on a Bayesian outgroup-free analysis (Outlaw and Ricklefs 2011). In addition to the ML analysis, we performed a Bayesian analysis in BEAST v. 1.5 (Drummond and Rambaut 2007). For this analysis, we used the HKY + gamma model of evolution and used prior kappa and alpha values estimated in Modeltest v. 3.7 (Posada and Crandall

1998). Starting with a randomly generated tree and the Yule process of speciation, we ran four times a minimum of 10,000,000 generations (sampling every 1000) or until the estimated sample size (ESS) was at least 200 for all parameters.

To determine the degree to which sequences correspond to morphospecies, we downloaded all *Haemoproteus* and *Plasmodium* cyt *b* sequences that had been identified to morphospecies from the MalAvi database (Bensch et al. 2009) as of February 14, 2012. Our sequences overlapped at most by ~ 300 bp with the MalAvi dataset. We performed an ML analysis in RAxML Black Box, described above, again using six mammalian *Plasmodium* spp. as outgroup.

Phylogenetic analysis of birds. – Phylogenetic relationships among bird species with identified parasite infections were estimated by a fragment of the recombination activating gene 1 (RAG-1), which has been used as a part of a phylogenetic reconstruction of suboscines (Moyle et al. 2009). Because we analyzed only 790 of the 4024 characters (20%) used in Moyle et al. (2009), we confirmed that the relationships in our tree matched those in the published paper, for those species shared between both studies. If available, we used RAG-1 data published on GenBank (Appendix J). Otherwise, we sequenced 790 bp of RAG-1 from two individuals per species. RAG-1 was amplified with primers RAG-1F (5'GCA AKA ATA YAC ATC TCA GYA CCA MG 3') and RAG-1R (5' GCT GYA TCA TAT CGR AAT CTC TTY GC 3') developed for this study by searching for conserved regions in an alignment of the RAG-1 sequences in Moyle et al. (2009). PCR reactions contained 1X buffer, 200 nM of each dNTP, 2 mM $MgCl_2$, 0.02% BSA, 200 nM of each primer, and 0.5 units of TaKaRa

Taq™ (TaKaRa Bio Inc., Shiga, Japan). The PCR program had an initial denaturing period at 94°C for 4 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 3 min. Sequences were edited as described for cyt *b* above, and submitted to GenBank (Appendix J). We used Modeltest to find the most appropriate evolutionary model and performed an analysis in BEAST. We selected the model with fewest parameters within the set of models with a delta AIC ≤ 2. Starting with a randomly generated tree and a birth-death process of speciation, we ran four times a minimum of 10,000,000 generations (sampling every 1000) or until the ESS was at least 500 for all parameters. All bird species included in this study were passerine birds, mostly suboscines. As an outgroup, we used several species of Psittaciformes (*Cacatua goffiniana*, GenBankID DQ143355; *Alisterus chloropterus*, GQ505199; *Cyanoramphus novaezelandiae*, GQ505212; *Neophema splendida*, GQ505217; *Coracopsis vasa*, GQ505223; *Psittaculirostris desmarestii*, GQ505242; *Amazona pretrei*, JF807982; *Deroptyus accipitrinus*, JF807984; *Poicephalus meyeri*, JF807989) since this order is most closely related to Passeriformes (Hackett et al. 2008).

Prevalence in hosts. – The estimate of prevalence is strongly affected by small sample size (Jovani and Tella 2006). Jovani and Tella (2006) suggested analyzing prevalence in host species with a minimum sample size of ~15. For 38 species we had a sample size of 15 or more, but because we had 14 captures for one species, we chose this as our minimum criterion when examining host species-specific prevalence. This resulted in our including 39 species in the "well-sampled species" data set. Prior to analysis, prevalence was arcsine square-root transformed and number of individuals sampled was log

transformed to approximate normal distributions. We used the NESTED procedure in SAS v.9.2 (SAS Institute Inc., Cary, NC, USA) to partition the variation among groups (families), subgroups (genera), and within genera[1]. To assess the significance of variation among families, we calculated *F* statistics based on type III sums of squares in the MIXED procedure. Because prevalence varied significantly among families but not among genera within families (see results), we analyzed variation in prevalence among well-sampled host species within each family using the *G*-test adjusted for small sample size (G_{adj}) in Microsoft Excel PopTools v. 3.2 (Hood 2010). For these analyses, prevalence was pooled among years and between plots. Prevalences of *Plasmodium* and *Haemoproteus* were considered both together and separately.

Annual and plot variation in prevalence. – A three-way log-linear model, following Sokal and Rohlf (1995, pp. 743), was used in the package 'MASS' in R v. 2.14 (R Development Core Team 2011) to test for two-way interactions between plot (α), year (β), and infection status (γ). In addition to grouping all infected samples (identified and unidentified), *Plasmodium* and *Haemoproteus* were analyzed separately. This test is a step-wise procedure in which one first tests the null hypothesis that there is no three-way interaction by excluding the last term from the model $\ln f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk}$. If the model without the three-way interaction term does not differ significantly from the full model, one may drop the last term from the model and test for two-way interactions. In cases where the three-way

[1] Nomenclatural changes occurring after this analysis was completed were: *Hylophylax poecilinota* to *Willisornis poecilinotus*, *Myrmotherula erythrura* to *Epinecrophylla erythrura*, and *Myrmotherula fjeldsaai* to *Epinecrophylla fjeldsaai*. These changes did not qualitatively alter our results (not shown).

interaction was significant, we used the G_{adj} test in PopTools within each category (i.e., annual variation was estimated within each plot and plot variation was estimated within each year). Because we were interested in knowing whether prevalence varies among years and/or between plots, we only tested the two-way interaction terms involving infection status, *i.e.*, $\alpha\gamma_{ik}$ and $\beta\gamma_{jk}$. We included six well-sampled ($N > 100$) years in this analysis: 2002, 2003, 2004, 2006, 2009, and 2010.

In addition to analyzing prevalence of *Plasmodium* and *Haemoproteus* together and separately, where hosts and individual parasite lineages were grouped, we split our data to determine annual variation within host families, within host species, and of individual parasite lineages where possible. We used the G_{adj} test described above for all three categories. First, we chose families in which among-species prevalence was homogeneous (Table 1) and thus analyzed annual variation of *Haemoproteus* prevalence within Thamnophilidae and Pipridae and of *Plasmodium* prevalence within Furnariidae. We did not analyze annual variation of *Haemoproteus* within Formicariidae or Tyrannidae because of the very low number of infections. Second, we analyzed annual variation in *Plasmodium* and *Haemoproteus* prevalence within host species with at least six samples per year (*Automolus infuscatus* [Furnariidae], *Glyphorynchus spirurus* [Furnariidae], *Hylophylax naevius* [Thamnophilidae], *Thamnomanes ardesiacus* [Thamnophilidae], and *Thamnomanes caesius* [Thamnophilidae] for *Plasmodium* and *A. infuscatus, G. spirurus, T. caesius*, and *Lepidothrix coronata* [Pipridae] for *Haemoproteus*). Here, we combined plot data. Finally, we analyzed annual variation in prevalence of individual lineages H17L, P4L, P25L, and P41L. In any data set containing cells with zeros, we added one to each cell.

Parasite prevalence and host traits. – We tested whether prevalence was related to host abundance, body size, foraging height, sexual dimorphism, and nest type (Appendix I). Host abundance was estimated by recording the number of individual birds by sight and/or sound along transects in each plot over four years (Blake 2007). Here, we used total records for the study period, which ranged between 9 individuals of *Rhegmatorhina melanosticta* (Thamnophilidae) and 928 individuals of *T. caesius*. Body size of each species was estimated by the average body mass per species, measured in the field by JGB and BAL, and ranged between 8.90 grams in *Myrmotherula axillaris* (Thamnophilidae) and 63.0 g in *Xiphorhynchus guttatus* (Furnariidae). Foraging height, sexual dimorphism, and nest type were obtained from the Handbook of the Birds of the World (Hoyo et al. 2003) and from JGB's personal observations. Foraging height was categorized as 1 = ground, 2 = understory, 3 = midstory, 4 = canopy. Sexual dimorphism was categorized as 1 = no dimorphism, 2 = moderate dimorphism (e.g. different head patterns between male and female), 3 = striking dimorphism (e.g. different body color, sexual ornaments). Nest type was categorized as 1 = closed (domed, cavity) and 2 = open. Body size and abundance were log-transformed prior to analysis, and prevalence was arcsine square-root transformed. We used Grubb's test in GraphPad (<http://graphpad.com/quickcalcs/grubbs2/>) to determine whether our data contained outliers, and we decided to remove the heavily parasitized *Formicarius colma* (Formicariidae) from this analysis ($Z = 3.83$, $P < 0.05$). In addition, *Turdus albicollis* (Turdidae), which forages both on the ground and in the canopy, does not fall within

either of the designated foraging height categories and was excluded from this analysis. Thus, 37 species were included.

Before analyzing the data, we used the test for serial independence (TFSI) (Abouheif 1999) on the RAG-1 phylogeny of birds (above) to determine whether any of the five host traits are phylogenetically independent. We used the permutation method described in Abouheif (1999) to compare our mean *C*-statistic to a null distribution (built from 999 replicates) calculated from the observed data for each trait and considered a one-tailed alpha value of 0.1 to be conservative in rejecting the null hypothesis of independence. We rejected the hypothesis of phylogenetic independence for all variables except abundance and proceeded to analyze our data using the generalized least squares (gls) method (Pagel 1997, 1999), which allows one to incorporate correlated errors (phylogenetic relationships in our case), in the R package 'nlme' (Pinheiro et al. 2011), assuming a Brownian motion of trait evolution (Schluter 2011). The maximum clade credibility tree from the BEAST analysis was used to estimate error correlations. We judged the fit of the model by examining a scatter plot of residuals and fitted values. We included two continuous independent variables (abundance and weight) and three ordered categorical variables (nest type, sexual dimorphism, and foraging height). We judged all possible combinations of models by Akaike's Information Criterion corrected for small sample size (AICc)(Johnson and Omland 2004) and selected those with a delta AICc ≤ 4 (Burnham et al. 2011) for a multi-model inference procedure in the package 'MuMIn' (Barton 2011) in R. Multi-model inference averages the parameter values of each variable (partial beta coefficients in a multiple regression such as ours) after weighting them by the AICc weights (Burnham and Anderson 2002; Burnham et al. 2011). To

determine whether either of these variables relates significantly to haemosporidian prevalence, we tested the null hypothesis that the slope (beta) of the partial regression line equals zero using *Z*-tests.

For a subset of well-sampled species we could also test the relationship between haemosporidian prevalence and apparent survival rate. Because survival rates have not been estimated for all of our well-sampled species, we incorporated this variable in a separate analysis. Apparent annual survival rate, a proxy for longevity, was estimated from 12 years of recapture data (Blake and Loiselle 2008; J. G. Blake unpublished) following methods in Blake and Loiselle (2008) and ranged between 0.43 in *Cyanocompsa cyanoides* (Cardinalidae) and 0.95 in *Myiobius barbatus* (Tyrannidae). Twenty-six host species were included in this set of analyses. Abouheif's TFSI was not significant for apparent survival rate, and we therefore used an ordinary least squares regression of prevalence and survival rate. In all analyses, *Plasmodium* and *Haemoproteus* were analyzed both separately and jointly.

Host specificity. – We estimated host specificity for parasite lineages recovered two times or more and examined several different host specificity indices and how they relate to sample size. Because we had no reason to believe that host specificity should vary between plots, we combined data from the two plots and also included 22 infections from birds found locally outside the Harpia and Puma plots. We removed the single *Baryphthengus martii* (Momotidae, order Coraciiformes) host individual, which was part of *Plasmodium* P24L's host range and the only non-passerine in the data set. This single infection represented 4% of P24L infections and would have a minor influence on the

$MPD_{weighted}$ value. We also removed the two infections found in the migratory *Catharus ustulatus* (Turdidae) because we were interested in estimating host specificity of local parasites only, and these infections could be carried from *C. ustulatus*'s breeding grounds.

We compared traditional indices of host specificity (e.g. Poulin 2007; Poulin et al 2011) to indices developed for community phylogenetics (Webb et al. 2002). The indices range from simple (host breadth; the number of host species utilized) to complex (weighted mean pairwise distance [$MPD_{weighted}$]; incorporating phylogenetic relationships and frequency distribution among hosts). Indices were calculated in the software package *Picante* (Kembel et al. 2010) in R. Five indices were calculated: (1) host breadth, (2) an equivalent to Simpson's *D* (Magurran 2004) (incorporating frequency but not phylogeny and calculated by $D = \sum p_i p_j$, where p_i is the proportion on host *i* and p_j is the proportion on host *j*), (3) mean pairwise distance among hosts (*MPD* calculated by $MPD = 2\sum d_{ij}$, where d_{ij} is the pairwise genetic distance between hosts *i* and *j*) (incorporating phylogeny but not frequency), (4) $MPD_{weighted}$ (incorporating both phylogeny and frequency), and (5) the standardized effect size of the $MPD_{weighted}$ (described in detail below). Pairwise genetic distance between hosts (d_{ij}) was estimated from the RAG-1 sequences obtained as described above.

$MPD_{weighted}$ is equivalent to Rao's quadratic entropy index, *Q* (Rao 1982), which is recommended for use in calculating host specificity of parasites when one has both phylogenetic information about the hosts and abundance data of the parasites (Poulin et al. 2011). This index has previously been used in the avian haemosporidian system

(Fallon et al. 2005; Fecchio et al. 2012; Ventim et al. 2012). $MPD_{weighted}$ is given by the formula

$$MPD_{weighted} = 2 \sum_{i=1}^{S-1} \sum_{j=i+1}^{S-1} d_{ij} p_i p_j ,$$

where S is the number of hosts infected and d_{ij}, p_i , and p_j are as described above. The package *Picante* was designed for community data incorporating phylogenetic relationships among taxa, and *MPD* was implemented to assess within site/sample variation in species diversity, taking into consideration phylogenetic relationships among species (Kembel et al. 2010). Host specificity as assessed by *MPD* is thus a measure of within parasite lineage diversity of hosts.

Because not all parasite lineages are equally well sampled, host specificity values are not directly comparable. Therefore, using null models (Gotelli and Graves 1996), we calculated the standardized effect size of *MPD* (SES_{MPD}) by

$$SES_{MPD} = \frac{MPD_{obs} - mean(MPD_{random})}{SD(MPD_{random})},$$

where MPD_{obs} is the observed $MPD_{weighted}$ described above and MPD_{random} is the *MPD* values calculated from 999 randomly generated host-parasite matrices (Kembel et al. 2011). We used the independent swap algorithm (Gotelli 2000), which retains the number of interactions and parasite host breadth (number of host species from which a parasite lineage has been recovered) for each parasite lineage, to generate our null models, and we performed 1000 iterations of the swaps for each of the 999 randomizations (Kembel et al. 2010). SES_{MPD}, which is in units of standard deviations, is interpreted as the difference between an observed *MPD* value and the mean of an expected (random) distribution of *MPD* values. Positive values indicate that a parasite lineage utilizes very distantly related

hosts (the parasite is "overdispersed" on its hosts, or highly generalized), whereas negative values indicate that a parasite lineage utilizes primarily closely related hosts (the parasite is "clustered" on its hosts, or highly specialized) (Webb et al. 2002). We considered parasite lineages to be significantly generalized or specialized if the *P*-value resulting from comparing the observed and expected *MPD* values was less than 0.05. We cannot perform the randomization procedure on strict host species-specialist. Instead, we determined the minimum sample size necessary in order to reject the hypothesis that a lineage is generalized, based on what we know from our best-sampled lineages (N > 20, 5 lineages). The lineage with the highest skew in frequency on different hosts was H17L, of which 39 of 91 recoveries (43%) were on the host *A. infuscatus*. The distribution of H17L on its hosts thus can be used to determine the most conservative minimum sampling size for detecting generalization. The probability that three random samples of this lineage should be on its preferred host is $0.43^3 = 0.08$ and that four random samples of this lineage should be on its preferred host is $0.43^4 = 0.03$. Thus, in 4 random samples, it is unlikely that all of them will be on the preferred host of this lineage. This indicates that we are likely to identify a generalized lineage sampled 4 times or more.

We estimated pairwise correlations between all indices of lineages infecting more than one species (i.e., those that have SES_{MPD} values). For all indices, increasing values represent decreasing specificity. *D* ranges between zero and one, *MPD* and $MPD_{weighted}$ between zero and the maximum possible pairwise distance (or less than this, after incorporating prevalence for $MPD_{weighted}$), which varies, depending on the phylogeny, between one and *N*, where *N* is the number of host species utilized by a parasite species. We also determined whether any indices correlated significantly with sample size.

Sample size and host breadth were log transformed prior to analysis. We used the software package *corrgram* (Wright 2006) for R to calculate pairwise Pearson's correlations of specificity indices and sample size.

RESULTS

Prevalence variation. – In total, 2488 individual birds from 104 species were screened for avian haemosporidia. Of these, 539 individuals (21.7%) of 73 species (70.2%) were infected (Appendix M).

Due to cyt *b* sequencing failure, 176 haemosporidian infections were not identified to genus. *Plasmodium* was found in 223 of 2312 birds (9.6%) and *Haemoproteus* in 149 of 2312 birds (6.4%). Prevalence varied greatly among species, from 0% to 100%. All of our well-sampled species (N = 39) were infected with avian haemosporidia; assemblage-wide prevalence was lowest in *Pipra filicauda* (Pipridae; 5.6%, N = 107) and highest in *F. colma* (91.2%, N = 34) (Fig. 1). Considering only those well-sampled species exhibiting some level (i.e., in which at least some infections were identified to genus) of *Plasmodium* or *Haemoproteus* infection, *Plasmodium* prevalence varied from 1.9% in *P. filicauda* to 89.7% in *F. colma*, and *Haemoproteus* prevalence varied from 1.0% in *Pipra pipra* (Pipridae) to 50% in *A. infuscatus*. Host species with high prevalence of *Haemoproteus* showed low prevalence of *Plasmodium* and vice versa (Fig.1). From the nested ANOVA analysis, prevalence varied significantly among families but not among genera for *Plasmodium* (among families F = 6.3, df = 6,11, P = 0.0045; among genera F = 1.0, df = 21,11, P = 0.50) but prevalence did not vary significantly at any level for *Haemoproteus* (among families F = 3.0, df = 6,11, P =

0.055; among genera $F = 1.0$, df = 21,11, $P = 0.52$). Within families, *Plasmodium* varied among species in four of five families and *Haemoproteus* prevalence varied among species in one of five families (Table 1).

Prevalence in well-sampled years varied annually between 9.8% in Harpia in 2006 and 40.9% in Puma in 2009 (Fig. 2). Both *Plasmodium* and *Haemoproteus* prevalence were lowest in 2006 and highest in 2009, but the peak of *Haemoproteus* in the Puma plot in 2009 was particularly noticeable (Fig. 2). The log-linear model for the three-way table indicated that assemblage-wide combined prevalence varied significantly among years and between plots (Table 2). Prevalence of *Haemoproteus* varied significantly among years but not between plots. The three-way interaction was significant for *Plasmodium* ($G = 11.2$, df = 5, $P = 0.048$), preventing us to proceed with testing the significance of two-way interactions. Thus, we applied the G-test within each plot to test for annual variation and within each year to test for plot variation in prevalence. Only within the Harpia plot did *Plasmodium* prevalence vary annually ($G_{adj} = 28.6$, df= 5, $P < 0.001$) and only in 2004 was *Plasmodium* prevalence significantly higher in the Puma plot than in the Harpia plot ($G_{adj} = 9.44$, df = 1, $P = 0.002$). In all years combined, 259 of 1225 (21.1%) birds were infected in the Puma plot and 197 of 1222 (16.1%) birds were infected in the Harpia plot. The sample sizes per host species correlated significantly between the two plots (Pearson's $r = 0.86$, df = 102, $P < 0.001$), suggesting that differential sampling effort of host species cannot account for the higher prevalence in the Puma plot.

We analyzed annual variation in *Plasmodium* and *Haemoproteus* prevalence within host species with at least six samples per year, and three years of data (Tables 3,

4). Only one of seven species exhibited significant annual variation in *Plasmodium* prevalence (Table 3), whereas two of four species exhibited significant annual variation in *Haemoproteus* prevalence (Table 4). Three species (*A. infuscatus*, *G. spirurus*, and *T. caesius*) were sampled sufficiently to assess annual variation in prevalence of both *Plasmodium* and *Haemoproteus*, and whereas *T. caesius* did not exhibit variation in either parasite genus, both *A. infuscatus* and *G. spirurus* showed consistent patterns: *Haemoproteus* but not *Plasmodium* prevalence varied annually.

Within host families, annual variation in prevalence was observed only in Thamnophilidae (of *Haemoproteus*) (Table 5). No variation was found within Furnariidae (of *Plasmodium* prevalence) or Pipridae (of *Haemoproteus* prevalence). No families exhibited plot variation in prevalence.

The dominant haemosporidian lineage, H17L, exhibited significant among-year variation in prevalence, whereas P4L, P25L, and P41L did not (Table 6). This led us to question how the prevalence of individual parasite lineages depends on variation in host sample sizes among years. P4L is a strict host species specialist and H17L and P41L are generalists but primarily infect one or two host species. H17L was recovered from 23 host species, but 44% of positives were found in *A. infuscatus* and 20% in *G. spirurus*, with the remaining 36% being roughly equally divided among the 21 remaining hosts. Likewise, 52% of P41L were recovered from *G. spirurus*, with the remainder distributed evenly among 7 other host species. P25L was found in 16 species of bird, primarily Thamnophilidae but found on occasion in other families. To determine whether the more specialized lineages vary accordingly to their preferred hosts' abundance, we plotted the abundance of parasite and primary host(s) (Fig. 3).

This close association of individual parasite lineage prevalence and the abundance of their preferred host would also indicate that within their preferred hosts, individual parasite lineages do not exhibit annual prevalence variation among years even if they do when data from all hosts are combined. We confirmed this for H17L, the only well sampled lineage that exhibited annual variation in prevalence, and its two primary hosts (within *A. infuscatus* G_{adj} = 3.10, df = 4, P = 0.541; within *G. spirurus* G_{adj} = 2.51, df = 4, P = 0.642). Neither of these two species were sampled in 2006, hence only years 2002, 2003, 2004, 2009, and 2010 were included.

Prevalence and host traits. – Abouheif's test for serial independence (Abouheif 1999) led us to reject the null hypothesis of independence among host species for all traits but abundance (and survival rate in the reduced data set). That is, foraging height, nest type, body weight, and sexual dimorphism exhibit significant phylogenetic signal and among-species comparisons should therefore take into consideration the statistical non-independence of these data (Table 7). Combined genera and *Haemoproteus* prevalence exhibited host phylogenetic signal, but *Plasmodium* prevalence did not.

For some data sets several sub-models had high AICc weights with delta AICc ≤ 4 (Table 8), and we used multi-model inference to determine the beta coefficients (Table 9). Only two of the six host traits related significantly with haemosporidian prevalence. Prevalence increased with the level of sexual dimorphism (combined data and *Plasmodium*), and decreased with foraging height (*Plasmodium* only).

Survival rate was not significantly related to either *Plasmodium* (b = 0.06, df = 24, P = 0.639), *Haemoproteus* (b = -0.13, df = 24, P = 0.382), or combined (b = -0.10, df = 24, P = 0.434) prevalence.

Recaptures. – Recaptures and multiple infections composed a small fraction of our sample. We were able to analyze repeated blood samples for 91 individuals (90 had two samples separated among years, while one bird had three samples). Of these, 28 were infected with haemosporidia at least at one point, where 11 went from being uninfected to infected, nine went from being infected to uninfected, and eight were infected at both sampling occasions. In none of these eight cases did we manage to identify (by cyt *b* sequencing) the parasite lineages from both capture dates. Recaptured individuals were included in other analyses only the first year they were sampled.

Mixed infections. – At least 34 host individuals (9.4% of those from which we obtained cyt *b* chromatograms) were infected with more than one parasite haplotype, as evident from chromatograms exhibiting multiple peaks. In eight host individuals exhibiting mixed infections, both parasite haplotypes were identified by matching the sequence to previously identified haplotypes from single infections. In 20 host individuals exhibiting mixed infections, one of the parasite haplotypes could be identified, and we could identify the 20 remaining unknown haplotypes to genus, by subtracting the known haplotype from the sequence. In six host individuals, neither parasite infection could be identified. Nineteen individuals harbored two parasite lineages of the same genus, whereas only 9 individuals harbored both a *Plasmodium* sp. and a *Haemoproteus* sp.

lineage. *Plasmodium* (N = 27) and *Haemoproteus* (N = 29) were found nearly equally often in mixed infections. Because we matched up mixed infections to already identified haplotypes, abundant haplotypes are likely to be overrepresented. We do not perform any analyses here comparing rare and common haplotypes; thus, this has no bearing on our results. Instead, our manual reconciliation of mixed infections increased our sample size for several common haplotypes and allowed us to perform more robust analyses of annual abundance variation of well-sampled individual haemosporidian lineages.

Phylogenetic analysis. – We obtained cyt *b* sequence data for 361 individuals (67% of infected) and found 65 haplotypes (40 of which were recovered at least twice from the host assemblage, and 25 of which were recovered from only one host individual; GenBank ID XXXXX - XXXXX). Forty-five haplotypes were *Plasmodium* (P1-P45) and 20 haplotypes were *Haemoproteus* subgenus *Parahaemoproteus* (H1-H20). Including those reconciled from mixed infections, 363 parasites were identified by cyt *b* to haplotype and 383 infections were identified to genus. Abundance of non-unique haplotypes varied between two and 82 cases. *Plasmodium* was more abundant (217 individuals; 60%) than *Haemoproteus* (146 individuals; 40%)(χ^2 = 13.9, df = 1, P < 0.01), despite the most abundant haplotype being *Haemoproteus* sp. H17 (N = 82). The second most abundant haplotype was *Plasmodium* sp. P25 (N = 24). Although *Haemoproteus* of the subgenus *Parahaemoproteus* was abundant within this assemblage, we found no *Haemoproteus* of the subgenus *Haemoproteus*, normally associated with dove (Columbiformes) hosts (Santiago-Alarcon et al. 2009), probably because we found no infected doves in Tiputini.

Of the 10 shallow clades of parasite cyt *b* haplotypes examined for host species sharing, two were not visualized in haplotype networks because they contained only 2 haplotypes each. One consisted of P3 and P4 (0.2% divergent), found in 27 *F. colma* individuals. The closest relative to this group (~ 3% divergent) was P2, found exclusively in 8 *Formicarius analis* (Formicariidae) individuals (Fig. 4). The other group consisted of P5 and P6 (0.2% divergent), both recovered only once each but from the same host species (*Chamaeza nobilis*, Formicariidae). *C. nobilis* was poorly sampled (n=3; 2 of which were infected). The closest relative to this group of haplotypes was P7 (~ 6.6% divergent) found in only one *Hypocnemis hypoxantha* (Thamnophilidae) individual. In both of these cases, it is clear that the haplotypes can be combined into two putative evolutionary lineages; P4L (including P3 and P4) and P5L (including P5 and P6). After examining the remaining eight shallow clades in haplotype networks (Appendix A-H), we delineated a total of 45 putative evolutionary lineages, 15 *Haemoproteus* subgenus *Parahaemoproteus* and 30 *Plasmodium* (Fig. 4). Each lineage consisting of more than one haplotype is designated by an "L" following the ID number (Fig. 4). In most cases, grouping of haplotypes into evolutionary lineages by our method was straightforward; less straightforward cases represent a small proportion of identified infections (17 of 363) and are unlikely to have a large impact on our analyses. In two cases (H16, Appendix A and P30, Appendix F), we considered poorly sampled haplotypes unique lineages because they were found in different host families from their well-sampled close (i.e., within 0.5% divergence in cyt *b*) relatives. In two cases, we grouped haplotypes that were more than 0.5% divergent (P8 and P25, Appendix C, and P40 and P41, Appendix G) because they were intersected by a poorly sampled haplotype. In the final case, we kept

P22 separate from P24L because all three recoveries of P22 were from the genus *Automolus*. Here, we used the G_{adj} test (after adding a value of 1 to each cell) to determine whether P24L and P22 significantly segregate onto *Automolus* and non-*Automolus* hosts (G_{adj} = 10.4, df = 3, P = 0.02).

Fourteen of 65 (21.5%) haplotypes recovered from birds in Ecuador were identical to haplotypes from elsewhere from a variety of host species and geographic locations (Appendix N). The only lineages that appear restricted to South America of these are P24L, H3, and H4, which have been found only in Guyana (Durrant et al. 2006), and H8, H9L, and H10 which have been found only in Brazil (Fecchio et al. 2012) prior to this study. Interestingly, three well-sampled lineages at our site (P4L, P41L, and H17L) have not been found in any other locality to date. These three were most often recovered from host species not extensively sampled elsewhere.

In our phylogenetic analysis composed of sequences of identified morphospecies and the haplotypes recovered in our study, only four of our lineages either matched exactly or were closely related to and grouped (with strong support) with sequences from known morphospecies. These are *Haemoproteus coatneyi* (H5 exactly matched OZ21 identified morphologically in Svensson and Ricklefs 2009), *Haemoproteus enucleator* (H18 grouped with ALCLEU01 identified morphologically in Beadell et al. 2006), *Haemoproteus paruli* (H1 grouped with TABI02 identified morphologically in Ricklefs and Fallon 2002), and *Plasmodium elongatum* (P37 exactly matched GRW06 identified morphologically in Valkiunas et al. 2008). *H. enucleator* has not previously been found in South America (Valkiunas 2005), and although the *H. enucleator* sequence in MalAvi groups with strong bootstrap support with our P18, they are ~ 2.5% divergent and likely

represent different but closely related species. *H. paruli* and *H. coatneyi* cannot readily be distinguished morphologically (Valkiūnas 2005), however, two independent researchers identified TABI02 to *H. paruli* and OZ21 to *H. coatneyi*, and at Tiputini these are distinguished both genetically and by host species association. Interestingly, none of our sequences were closely related to either *Plasmodium relictum* or *Plasmodium juxtanucleare*, both of which have been found in South America previously (Valkiunas 2005). We collected blood smears for a fraction of birds during the last two sampling years, but in a preliminary assessment we only detected trophozoites of known positives, rendering morphological identification impossible.

Two of the lineages at Tiputini match lineages recovered from mosquito vectors in Gager et al's (2008) study (although each exhibits one bp difference) in Panama, in which *Plasmodium* exhibited high vector specificity. Our P1 (rare in our study), found in *Turdus lawrencii* (Turdidae), was found in *Turdus grayi* (Turdidae) and the vector *Aedeomyia squamipennis* (Culicidae) (in that study called PAN6), and our P24L, found in a variety of host species and families matched that of PAN2 found in *Culex (Melanoconion) ocossa* (Culicidae) in Panama (Gager et al. 2008). Accordingly, one would expect *A. squamipennis* and *C. (M.) ocossa* to be competent *Plasmodium* vectors also at Tiputini. We have no information about vectors from our study site.

Host specificity. – Parasite lineages that were recovered more than twice were obtained from between one and 23 (H17L) host species, and hosts harbored between one and nine (*G. spirurus* and *H. naevius*) parasite lineages (Fig. 4, Table 10). Parasite lineages were distributed heterogeneously both among species and among host families (Fig. 4). Half of

the parasite haplotypes were found in the family Thamnophilidae, which was also the most abundantly sampled family. Of non-unique lineages (N = 32), 17 were family specific, nine of which were also species specific (Fig. 4). Host breadth (number of host species utilized by a parasite lineage) and parasite richness (number of parasite lineages recovered from a host species) both increased with increased sampling (Figs. 5-6).

Two parasite lineages were considered significantly specialized according to our SES_{MPD}, and we consider an additional four significantly specialized because they were found in at least four individuals and in only one host species (Table 10). H17L and P25L infected multiple species but occurred primarily on only a few close relatives (Fig. 4). No lineages exhibited significantly greater host generalization than expected under the random distribution.

$MPD_{weighted}$, *MPD*, *D*, and host breadth correlated significantly with each other (Fig. 7), and SES_{MPD} correlated with both *MPD* indices. In addition, all indices except SES_{MPD} correlated significantly with sample size (Fig. 7).

DISCUSSION

Annual and plot variation in prevalence. – We found significant among-year and between-plot variation in assemblage-wide prevalence in our study. Some of this variation might be attributed to moisture availability. Wood et al. (2007), for example, demonstrated an increased incidence of *Plasmodium* infection and Lachish et al. (2011) showed that *Plasmodium circumflexum* infection rates are consistently higher closer to a large water source (the River Thames in both studies), presumably as a consequence of proximity to suitable vector habitats. We found evidence for this in the between-plot

variation in prevalence: the wetter Puma plot exhibited significantly greater combined prevalence than did the Harpia plot, corroborating the earlier studies on haemosporidian prevalence and moisture associations. However, the plot variation in prevalence was not ubiquitously upheld when considering only *Haemoproteus* or *Plasmodium* prevalence (only in 2004 did *Plasmodium* exhibit significantly greater prevalence in the Puma plot).

Annual variation in prevalence, which is much more pronounced than the plot variation in prevalence, might also be attributed to climatic factors. However, annual variation in prevalence could also be caused by fluctuations in abundance of primary hosts, abundance of individual parasite lineages, abundance of vectors, or a combination of any of the above. We have neither climatic nor vector data from this site to directly address all these possibilities. Regardless, if external factors such as moisture or temperature were primarily responsible for affecting parasite prevalence, one would expect (1) prevalence within host species to vary concordantly with assemblage-wide prevalence and (2) relative abundance of individual parasite lineages to vary independently of the abundance of their primary host species.

Sample size is drastically reduced when we analyze prevalence variation within host species or abundance variation of individual parasite lineages. Nonetheless, our results do not provide convincing support for either of these two predictions. First, although two of three host species exhibit significant annual variation in *Haemoproteus* prevalence, in only one of seven host species does *Plasmodium* prevalence vary annually, indicating that in most cases prevalence remains homogeneous over years within host species. It is curious that within *G. spirurus* and *A. infuscatus*, *Haemoproteus* but not *Plasmodium* prevalence varies annually, perhaps an indication that fluctuations in vector

abundance is responsible for annual variation in prevalence since the two genera utilize different vectors (Atkinson and van Riper III 1991, Valkiūnas 2005). Second, the abundance of individual parasite lineages appears strongly associated with the sample size of their primary hosts, and the significant annual variation in prevalence of *Haemoproteus* H17L analyzed at the assemblage level (i.e., incorporating data from all potential host species) disappears when only data from its primary hosts are analyzed. This suggests that external factors have little influence on the abundance of individual parasite lineages.

Previous studies addressing climatic influences on avian haemosporidia are in the temperate region (e.g. Wood et al. 2007; Lachish et al. 2011). Our study, on the other hand, is set on the equator in the Amazonian rainforest. Temperatures in our site are unlikely to ever drop below the critical 13°C for parasite development, and precipitation is likely to be sufficient on a yearly basis to provide ample breeding habitats for haemosporidian vectors.

Prevalence heterogeneity among host species. Few ecological and life history traits of the resident hosts in this Amazonian study site seem to influence prevalence of avian haemosporidia. No significant relationships were found when considering *Haemoproteus* on their own, but we found support for our prediction that greater levels of sexual dimorphism is associated with greater combined and *Plasmodium* prevalence. We also found that, contrary to our prediction, prevalence of *Plasmodium* decreases with increasing foraging height.

The positive association between parasite prevalence and level of sexual dimorphism supports the Hamilton-Zuk hypothesis, according to which species under strong sexual selection are more burdened by chronic parasites than species in which sexual selection is less important (Hamilton and Zuk 1982; Andersson 1994). At the population level this should result in greater parasite prevalence in dimorphic than in monomorphic species (Poulin and Forbes 2011). Scheuerlein and Ricklefs (2004) also found haemosporidian prevalence on blood smears to associate positively with male plumage brightness, but Ricklefs et al. (2005) failed to find such a relationship in Missouri forest birds for which prevalence was assessed by PCR. The positive association between haemosporidian prevalence and level of sexual dimorphism was obtained even though we included manakins in our analysis. All six manakin species sampled here, which engage in elaborate lek displays to attract mates, provide a glaring contradiction to the Hamilton-Zuk hypothesis because they exhibit significantly lower average prevalence than the average of the remaining bird assemblage (11.9% in Pipridae [$N = 6$] versus 24.9% in other species [$N = 32$, excluding the outlier *F. colma*], $t = 2.64$, $P = 0.012$).

Read (1991) suggested that alleles that simultaneously confer resistance to rare and common parasites may spread throughout a population via female choice of resistant males, leading to reduced prevalence in species under strong sexual selection. Read (1991) also argues that because such alleles might not be present in all species, one can find both positive and negative associations between parasite prevalence and strength of sexual selection, making the Hamilton-Zuk hypothesis as it traditionally stands difficult to falsify (Read 1991). One might therefore speculate that when traits and behaviors

evolved to attract mates come at an exceptionally high cost to the individual, and when reproductive skew is high as is the case in manakins (Ryder et al. 2009), only those males that are resistant to the great majority of parasites, common and rare, gain access to females.

The negative relationship between foraging height and *Plasmodium* prevalence indicates that infection rate might vary vertically. Garvin and Remsen Jr. (1997) found that prevalence of haemosporidia increased with increasing nest height, and for the same reasons they provide (greater vector exposure towards the canopy) we predicted increased prevalence in canopy foragers. Instead, we found that *Plasmodium* prevalence is higher in ground foragers. There are several possible explanations for this. First, studies have shown that vertical stratification in abundance, sex ratio, and age structure varies among blood-sucking dipteran species (Snow and Wilkes 1977; Veras and Castellon 1998; Derraik et al. 2005) and it is possible that in this particular site, haemosporidian vectors tend to be more abundant towards the ground. A survey of vectors in Tiputini would help answer this. Second, the relationship between prevalence and foraging height could also exist because foraging height differs among taxa. For example, Tyrannidae forage in the midstory and canopy whereas Formicariidae forage on the ground, although variation in foraging height over at least three of our categories are observed within Pipridae, Thamnophilidae, and Furnariidae. In Pipridae, the species foraging at lower heights is *L. coronata*, which also exhibits the highest prevalence. In Thamnophilidae, *Schistocichla leucostigma* is the only ground forager, and it has the highest prevalence. Third, if individual parasite lineages show preference for hosts based on their foraging height, the more abundant parasite lineages might drive this pattern.

However, the three better sampled *Plasmodium* lineages, P25L, P24L, and P41L, were recovered from birds foraging from the ground to the midstory; thus, it is unlikely that individual parasite lineages show preference for birds foraging at particular heights.

One caveat with the investigation of host trait and parasite prevalence relationships is that, even when partitioning our data into *Plasmodium* and *Haemoproteus*, we overlook the relative prevalence of individual parasite lineages. Some parasite lineages might impact some hosts more than others (Palinauskas et al. 2008, 2011). Such among-host species variation in susceptibility to the same pathogen is a potential confounding factor in this study. Some measures of immunity vary more among than within host species (Tella et al. 2002). Tella et al. (2002) demonstrated positive relationships between cell-mediated immunity, longevity, and incubation period in a sample of 50 species of bird. However, immunity can also be acquired throughout a bird's life as a response to primary infection of a particular parasite. Indeed, Cellier-Holzem et al. (2010) demonstrated that secondary infection of *Plasmodium relictum* had a much lower effect on the health of domestic canary (*Serinus canaria*) individuals, indicating that a primary infection improves immunity to the same pathogen later in life. What we can conclude here is that factors other than immunity of birds (both innate and acquired), such as traits that might alter the probability of vector encounter, do not seem the most important determinants of prevalence within host species. Instead, we found support for the Hamilton-Zuk hypothesis, which is based on the interaction between host immunity and parasite infectivity. This implies that individual host compatibility might hold the key to understanding the pattern of population level parasite prevalence.

Host specificity. – Most specialized haemosporidia at our study site belonged to the genus *Plasmodium*, contradicting the traditional consensus that *Haemoproteus* is the most specialized genus (see Atkinson and van Riper III 1991). Here, in fact, the three most generalized lineages were *Haemoproteus*, and all of the strict host species specialists were *Plasmodium*. Although counterintuitive, the parasite lineage with the greatest host breadth, *Haemoproteus* sp. H17L, which was recovered from 23 species, was also significantly specialized. This is because two species in the same family hosted 64% of the H17L population. The second best sampled lineage, *Plasmodium* sp. P25L ($N = 34$), was also significantly specialized despite being recovered from 16 host species. The specialization of P25L could not be attributed to the preference of any one or two host species, but 90% of the recoveries were from the family Thamnophilidae. Thus, it appears sufficient that a parasite lineage is restricted to family of bird in order to be deemed significantly specialized by the SES_{MPD}. However, the identical haplotype to P8, here grouped within P25L, was recovered frequently in the West Indies and the Missouri Ozarks (Ricklefs et al. 2005; there named OZ 06). In these other sites, this lineage infected multiple host species and families, although it was most often recovered from Parulidae. P25L, thus, might be an example of a parasite with high alpha specificity but low beta specificity (Krasnov et al. 2011); that is, locally it might be restricted to, for example, a host family, but the identity of the host family on which P25L specializes varies geographically.

Host specificity indices were correlated with sample size, but this association weakened remarkably as more information was included in the index and disappeared

entirely when we used the standardized effect sizes of the weighted *MPD* index in place of the observed values. Thus, SES_{MPD} might be a promising host specificity index to use as a parasite trait in studies where sample size varies among parasite species.

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TABLES

Table 1. Prevalence range (in percent) of avian haemosporidia (*Plasmodium* [*Pla*], *Haemoproteus* [*Hae*], and combined genera) among well-sampled bird species ($N \geq 14$) within families at the Tiputini Biodiversity Station, Ecuador.

Family	Combined	G_{adj}	df [a]	*P*	*Pla*	G_{adj}	df	*P*	*Hae*	G_{adj}	df	*P*
Furnariidae	7.7 – 58.7	66.9	8	<0.001	0.0 – 11.5	13.8	8	0.086	3.1 – 50.0	76.7	8	<0.001
Thamnophilidae	6.1 – 44.4	37.8	17	0.003	0.0 – 26.7	29.2	17	0.033	0.0 – 10.5	13.8	17	0.685
Formicariidae	54.5 – 91.2	9.65	1	0.002	45.0 – 86.7	9.62	1	0.002	3.3 – 5.0	0.07	1	0.795
Tyrannidae	14.8 – 25.0	1.19	1	0.276	2.0 – 22.2	7.73	1	0.005	0.0 – 4.1	0.214	1	0.644
Pipridae	5.6 – 19.4	9.64	5	0.086	0.0 – 11.3	18.4	5	0.002	0.0 – 4.2	13.8	5	0.685

[a] Degrees of freedom are *N*-1, where *N* is the number of species included in the analysis.

Table 2. Log-likelihood test for three-way tables of year (YR), plot, and infection status (I) of both haemosporidian genera (Combined) and *Haemoproteus*.[a]

	Combined			*Haemoproteus*		
Interaction	*G*	Df[b]	*P*	*G*	df	*P*
YR x I	57.8	10	<0.001	33.0	10	<0.001
Plot x I	21.7	6	0.001	12.2	6	0.058

[a] Because the three-way interaction was significant for the *Plasmodium* dataset, it was analyzed differently (see methods).

[b] Degrees of freedom in the two-way interaction tests are (*a*-1)(*b*-1)*c*, where *a* and *b* represent the number of categories in each of the two variables tested and *c* represents the number of categories in the third variable.

Table 3. Species level variation in the number of hosts infected (I) and uninfected (U) with *Plasmodium* among years. AUTINF = *Automolus infuscatus*, GLYSPI = G*lyphorynchus spirurus*, HYLNAE = *Hylophylax naevius*, MYRAXI = *Myrmotherula axillaris*, THAARD = *Thamnomanes ardesciacus*, THACAE = *Thamnomanes caesius*, LEPCOR = *Lepidothrix coronata*.

	I	U	G_{adj}	df[a]	*P*
AUTINF	4	60	0.179	3	0.981
GLYSPI	17	287	2.34	3	0.673
HYLNAE	7	60	0.180	2	0.914
MYRAXI	7	18	2.61	2	0.271
THAARD	11	60	4.03	3	0.258
THACAE	5	51	3.15	3	0.369
LEPCOR	16	121	14.5	4	0.006

[a] Degrees of freedom are *n*-1, where *n* is the number of years.

Table 4. Species level variation in the number of hosts infected (I) and uninfected (U) with *Haemoproteus* among years. AUTINF = *Automolus infuscatus*, GLYSPI = G*lyphorynchus spirurus*, THACAE = *Thamnomanes caesius*, LEPCOR = *Lepidothrix coronata*.

	I	U	G_{adj}	df[a]	*P*
AUTINF	31	33	12.1	3	0.007
GLYSPI	24	280	9.92	4	0.042
THACAE	5	51	1.55	3	0.671
LEPCOR	6	131	4.45	4	0.348

[a] Degrees of freedom are *N*-1, where *N* is the number of years.

Table 5. Log-likelihood test of a three-way table of year (YR), plot, and infection status (I) within well-sampled families that did not exhibit among-species variation in prevalence (Table 1). Prevalence of *Plasmodium* (*Pla*) and *Haemoproteus* (*Hae*) was analyzed separately. No three-way interactions were significant.

Family	Interaction	*G*	df [a]	*P*
Furnariidae	YR x Plot	15.7	10	0.109
Plasmodium	YR x I	14.6	10	0.147
	Plot x I	10.6	6	0.100
Thamnophilidae	YR x Plot	18.1	10	0.053
Haemoproteus	YR x I	22.5	10	0.013
	Plot x I	5.28	6	0.509
Pipridae	YR x Plot	10.2	14	0.746
Haemoproteus	Year x I	18.3	14	0.196
	Plot x I	2.06	8	0.979

[a] Degrees of freedom in the two-way interaction tests are (*a*-1)(*b*-1)*c*, where *a* and *b* represent the number of categories in each of the two variables tested and *c* represents the number of categories in the third variable.

Table 6. Annual variation in prevalence of individual haemosporidian lineages.

Lineage	*N*	G_{adj}	df [a]	*P*
H17L	81	11.9	5	0.036
P25L	32	8.61	5	0.126
P4L	25	2.50	5	0.776
P41L	25	11.0	5	0.051

[a] Degrees of freedom are *N*-1, where *N* is the number of years.

Table 7. Observed *C*-statistic values from 1000 permutations of Abouheif's test for serial independence (TFSI), standard deviations (STD), and one-tailed *P*-values of host phylogeny and host traits (average body weight, foraging height, nest type, sexual dimorphism, abundance, and apparent survival rate) and haemosporidian prevalence (combined, *Plasmodium* only [*Pla*], and *Haemoproteus* only [*Hae*]). Survival is based on the reduced (N = 26) dataset.

Variable	*C*	STD	*P*
Weight	0.370	3.92	0.002
Foraging	0.182	2.10	0.030
Nest type	0.487	4.96	0.002
Dimorphism	0.585	5.95	0.001
Abundance	0.066	0.935	0.173
Survival	0.038	0.673	0.242
Prevalence	0.210	2.46	0.017
Pla	0.038	1.02	0.158
Hae	0.398	4.72	0.001

Table 8. Model summary for each of the 3 data sets on which the generalized least squares analysis of haemosporidian prevalence and host traits was performed. Only models with delta AICc ≤ 4 are shown. FH = Foraging height, W = Weight, SD = Sexual dimorphism. *N* = 37 host species.

Data set	Variable	AICc	Delta AICc	AICc weights
	SD	-30.22	0	0.44
Combined	Intercept	-29.77	0.45	0.35
	W	-27.65	2.57	0.12
	W + SD	-26.87	3.35	0.08
Plasmodium	FH+SD	-15.83	0	0.86
	FH	-12.24	3.58	0.14
Haemoproteus	Intercept	-26.27	0	0.69

Table 9. Beta coefficients, 95% confidence intervals, *Z*-values, and *P*-values of the multiple generalized least squares regression including 37 host species with haemosporidian prevalence as the dependent variable, after averaging models with a delta AICc ≤ 4. Only significant beta coefficients are shown.

Data set	Variable	Beta	95% CI	*Z*	*P*
Combined	Dimorphism	0.12	0.03 : 0.20	2.68	0.007
Plasmodium	Foraging	-0.16	-0.22 : -0.09	4.88	<0.001
	Dimorphism	0.16	0.07 : 0.26	3.32	<0.001

Table 10. Host specificity of lineages recovered twice or more at Tiputini measured as the number of host species utilized, Simpson's *D*, *MPD*, $MPD_{weighted}$, SES_{MPD}. Sample size (*N*) and significance based on the two-tailed *Z*-value are also shown. Lineages are sorted by phylogenetic placement in Fig. 4. An asterisk indicates significant specialization. A question mark indicates that sample size is too small to determine whether the lineage is significantly specialized.

Lineage	*N*	Host species	*D*	*MPD*	$MPD_{weighted}$	SES_{MPD}	*P*
H1	4	4	0.750	0.016	0.012	-1.60	0.110
H9L	12	9	0.861	0.067	0.060	1.68	0.093
H8	5	5	0.800	0.072	0.058	1.52	0.129
H10	11	7	0.810	0.059	0.054	1.18	0.238
H12	4	3	0.625	0.005	0.003	-1.95	0.051
H13	19	8	0.814	0.047	0.029	-1.12	0.263
H14	2	2	0.500	0.011	0.005	-1.19	0.234
H17L	91	23	0.774	0.054	0.031	-2.26	0.024*
P2	8	1	0	0	0		*
P4L	27	1	0	0	0		*
P5L	2	1	0	0	0		?
P17	2	1	0	0	0		?
P9	7	1	0	0	0		*
P12L	5	3	0.640	0.015	0.010	-1.46	0.144
P14	3	3	0.667	0.009	0.006	-1.64	0.101

P25L	34	16	0.908	0.033	0.023	-2.62	0.009*
P16	2	1	0	0	0		?
P10	2	1	0	0	0		?
P20	10	4	0.480	0.053	0.020	-1.05	0.294
P22	3	2	0.444	0.012	0.005	-1.18	0.238
P24L	25	18	0.931	0.061	0.054	0.88	0.379
P26	2	1	0	0	0		?
P27	3	1	0	0	0		?
P28	5	3	0.560	0.036	0.018	-0.85	0.395
P29	9	1	0	0	0		*
P30	2	2	0.500	0.009	0.005	-1.29	0.197
P32L	9	4	0.691	0.014	0.010	-1.81	0.070
P33L	12	6	0.806	0.024	0.015	-1.93	0.054
P36	7	3	0.612	0.034	0.015	-1.06	0.289
P37L	3	3	0.667	0.039	0.026	-0.36	0.719
P41L	28	8	0.676	0.051	0.026	-1.34	0.180
P43L	6	5	0.778	0.049	0.035	-0.17	0.865

FIGURES

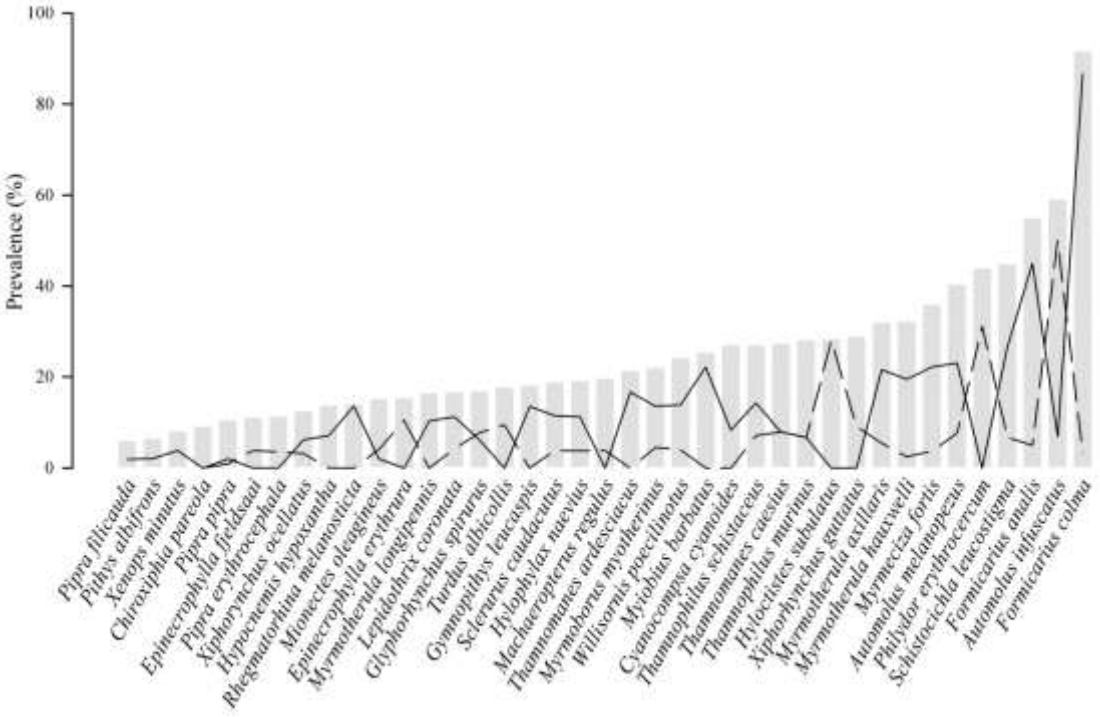


Figure 1. Haemosporidian prevalence in well-sampled ($N \geq 14$) bird species (grey bars). Prevalence of *Plasmodium* (black line) and *Haemoproteus* (dashed line) is also shown.

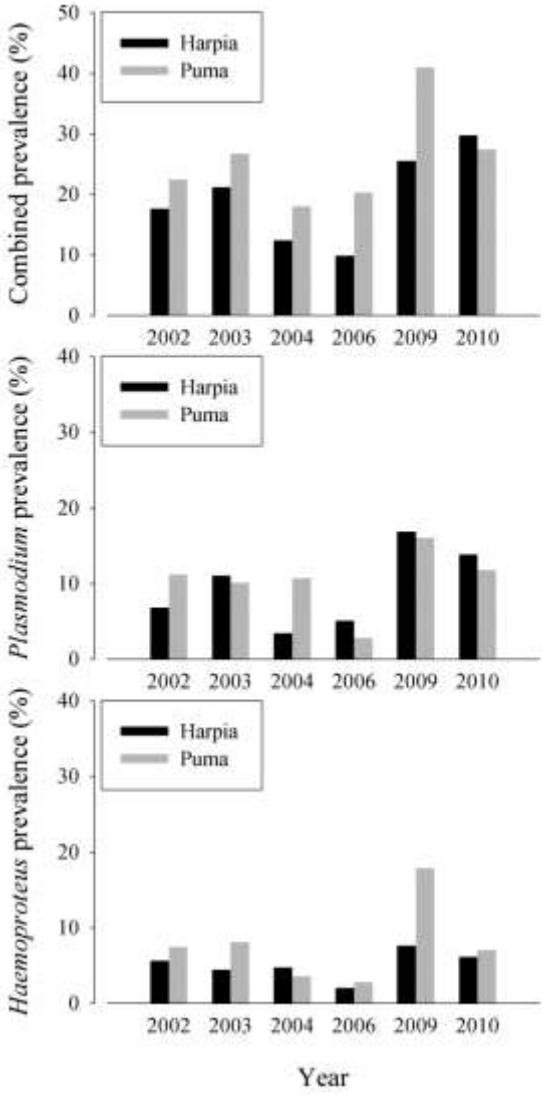


Figure 2. Annual variation in assemblage-wide prevalence of any haemosporidian infection (top), *Plasmodium* infection (middle) and *Haemoproteus* infection (bottom) in 6 well-sampled years in two 100-ha plots (Harpia and Puma).

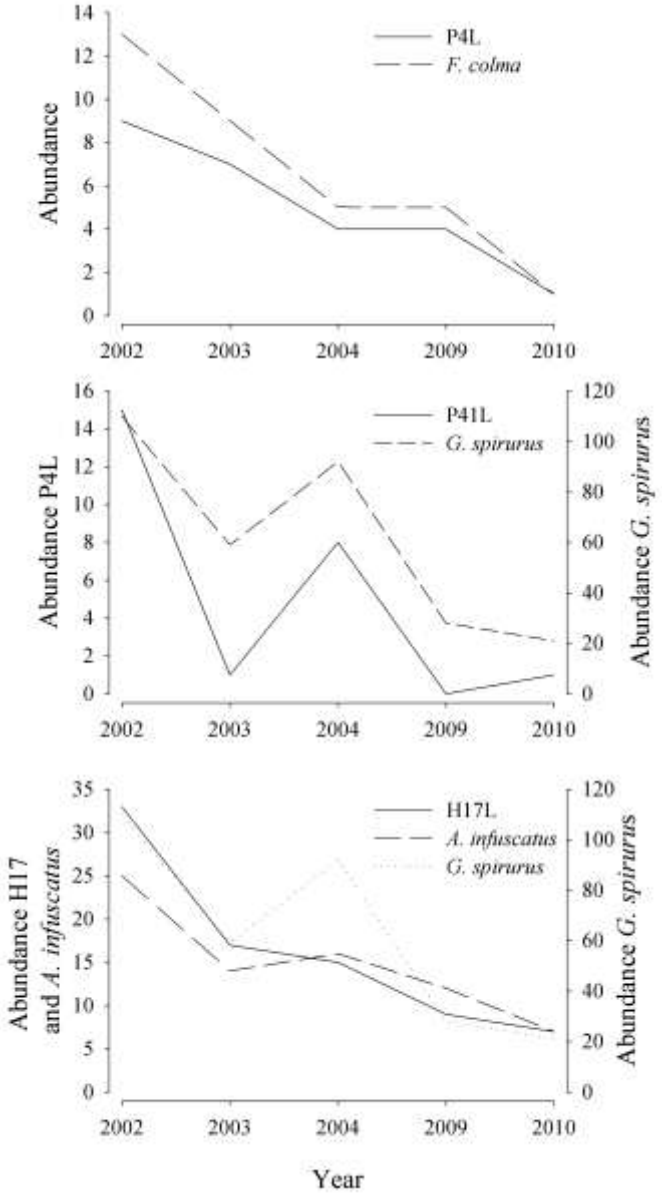
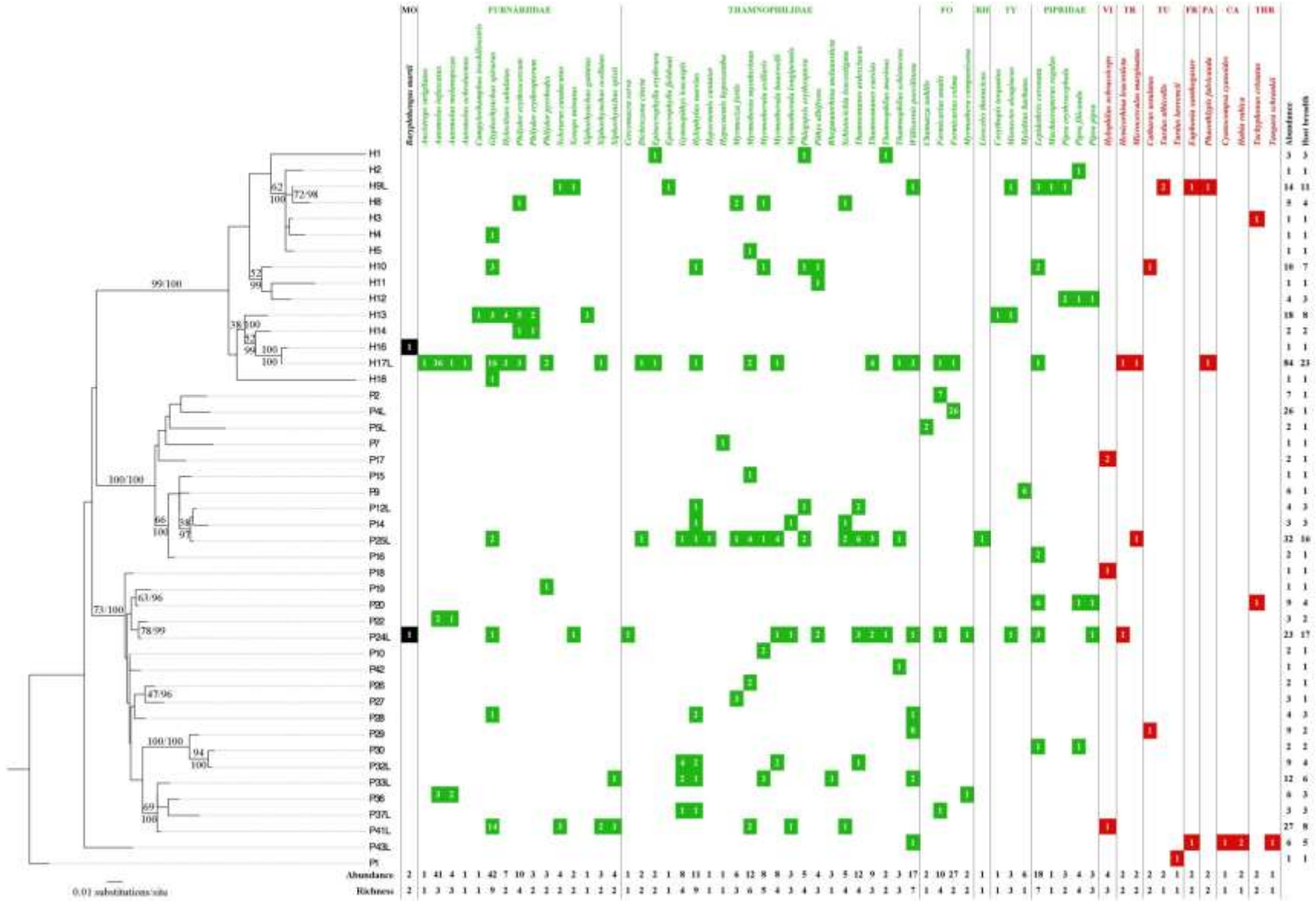


Figure 3. Annual variation in the abundance of the most frequently recovered specialized parasite lineages and their primary host(s).


MO
FURNARIIDAE
THAMNOPHILIDAE
FO
RH
TY
PIPRIDAE
VI
TR
TU
FR
PA
CA
THR
Abundance
Host breadth
H1
H2
H9L
H8
H3
H4
H5
H10
H11
H12
H13
H14
H16
H17L
H18
P2
P4L
P5L
P7
P17
P15
P9
P12L
P14
P25L
P16
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P22
P24L
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P42
P26
P27
P28
P29
P30
P32L
P33L
P36
P37L
P41L
P43L
P1
99/100
72/98
100/100
73/100
63/96
78/99
47/96
100/100
94
69
0.01 substitutions/site

Figure 4. The maximum likelihood tree of haemosporidian lineages recovered in Ecuador, rooted with mammalian *Plasmodium. Haemoproteus* begin with an "H" and *Plasmodium* with a "P". Lineages composed of multiple haplotypes are indicated by "L." Bootstrap values from the ML analysis (left of slash or top of branch) and posterior probabilities from the Bayesian analysis (right or bottom) are show on branches for relationships that were supported by at least one method (i.e., bootstrap ≥ 70, PP ≥ 0.95). The table shows the number of recoveries of each lineage (abundance), partitioned by host species. Only *Baryphthengus martii* (Momotidae) is a non-passerine bird (black). Suboscine passerines (Tyranni) are shown in green and oscine passerines (Passeri) are shown in red. Some families are abbreviated: MO = Momotidae, FO = Formicariidae, RH = Rhinocryptidae, TY = Tyrannidae, VI = Vireonidae, TR = Troglodytidae, TU = Turdidae, FR = Fringillidae, PA = Parulidae, CA = Cardinalidae, THR = Thraupidae.

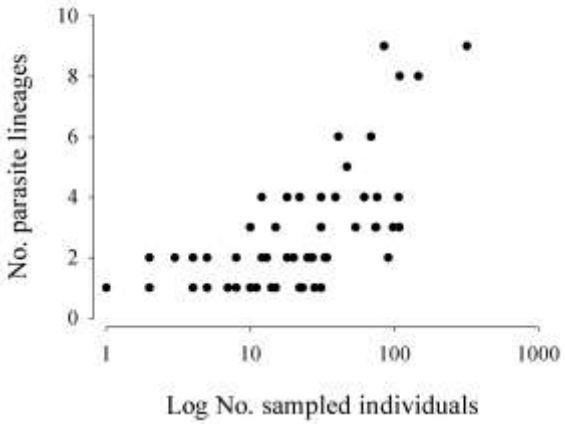


Figure 5. Number of parasite lineages (parasite richness) per host species as a function of host sample size. The relationship is significant (b = 22.8, t = 9.62, $P < 0.01$, R^2 = 0.60).

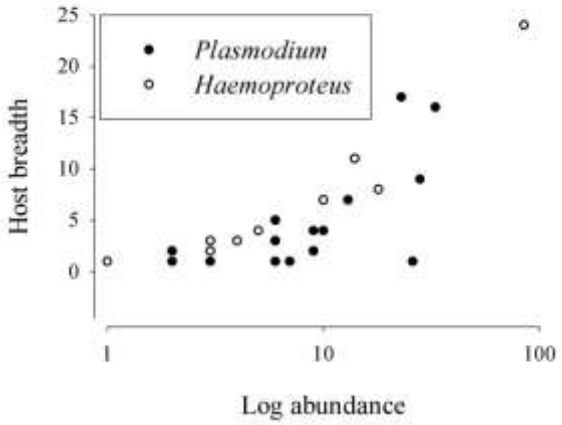


Figure 6. Host breadth (number of host species) of individual parasite lineages as a function of parasite abundance. The relationships between abundance and (1) *Plasmodium* and (2) *Haemoproteus* are significant ($b = 2.0$, $t = 6.78$, $P < 0.01$, $R^2 = 0.68$ and $b = 2.9$, $t = 5.71$, $P < 0.01$, $R^2 = 0.80$, respectively).

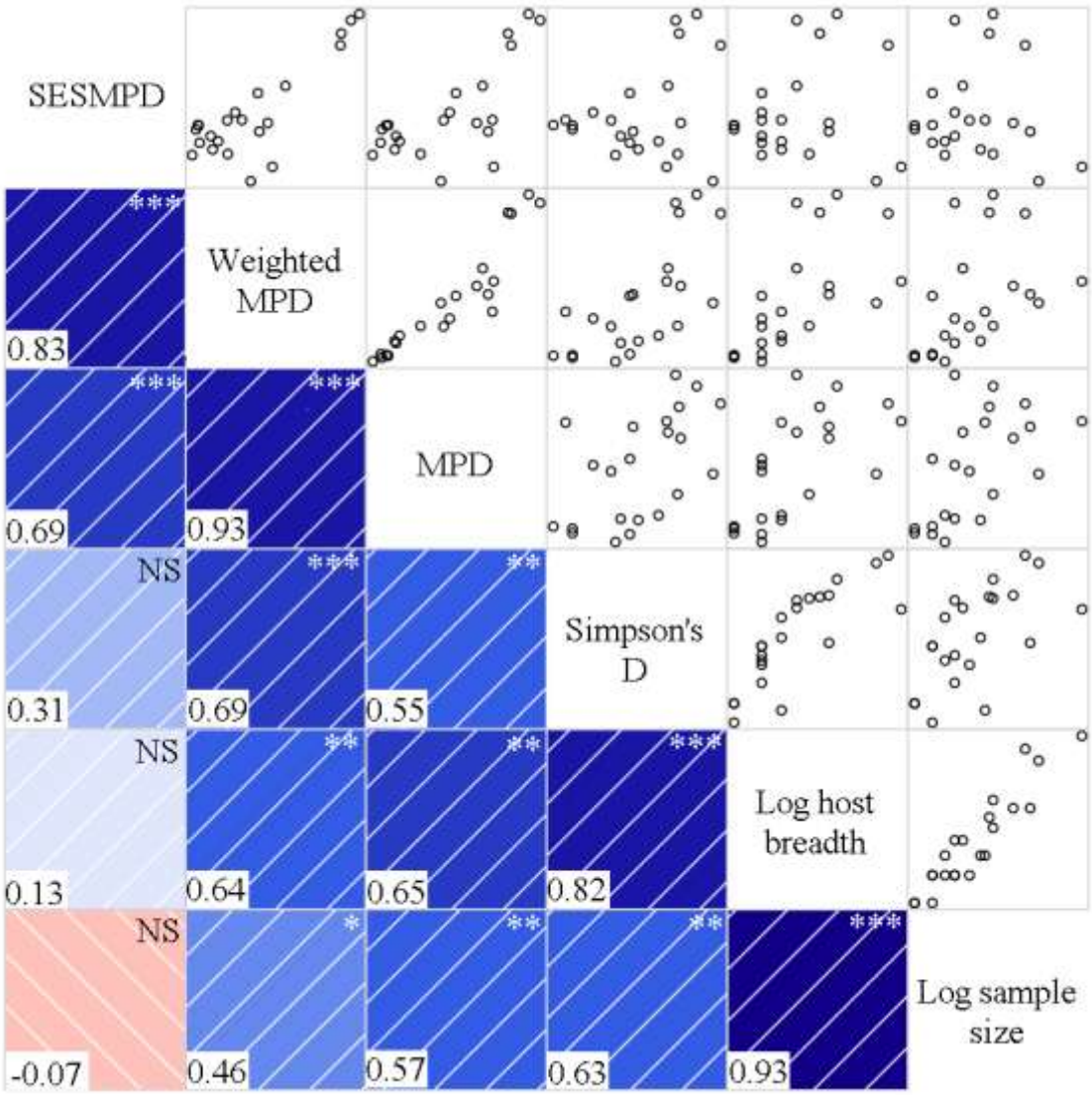
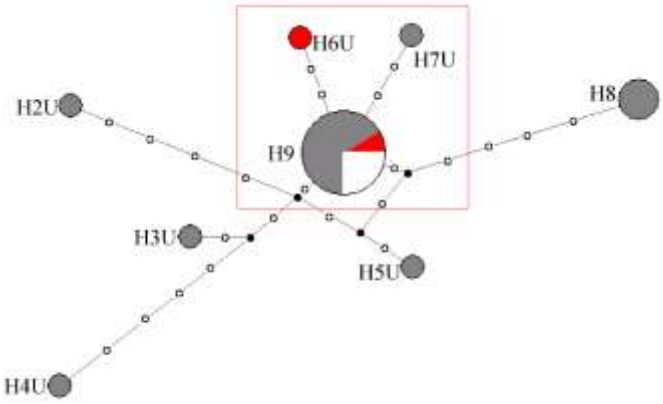


Figure 7. Correlogram of four indices of host specificity (SES_{MPD}, $MPD_{weighted}$, MPD, Simpson's D), host breadth (number of host species), and sample size of parasite lineages recovered at Tiputini. Pearson's correlation coefficient is shown in the bottom left corner of the lower panel. Significance is indicated by asterisks in the top left corner of the lower panel (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant).

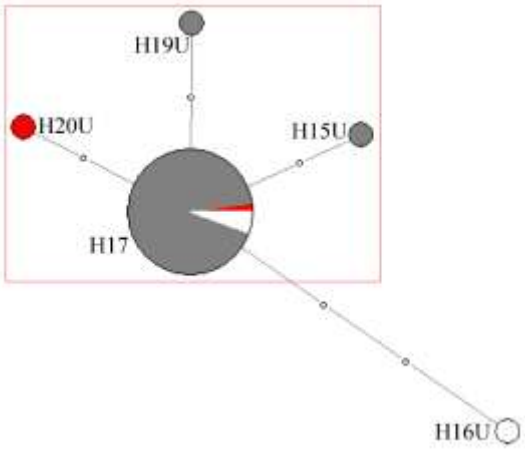
Appendix A. Haplotype network and table listing host species of clade Haem1. Color-code: white = non-shared families; grey = non-shared species within families that are shared; color = shared species. Empty circles in the networks are mutations, filled circles are median vectors. Haplotypes that are considered variation within one evolutionary lineage are boxed in red.



Family	Species	H2U	H3U	H4U	H5U	H6U	H7U	H8	H9
Furnariidae	GLYSPI			1					
	PHIERY							1	
	SCLCAU								1
	XENMIN						1		
Parulidae	PHAFUL								1
Pipridae	MACREG								1
	LEPCOR								3
	PIPERY								1
	PIPFIL	1							
Thamnophilidae	HYLPOE								1

	MYRAXI	1
	EPIFJE	1
	MYRMYO	1
	SCHLEU	1
Fringillidae	EUPXAN	1
Thraupidae	TACCRI	1
Turdidae	TURALB	1 1
Tyrannidae	MIOOLE	1

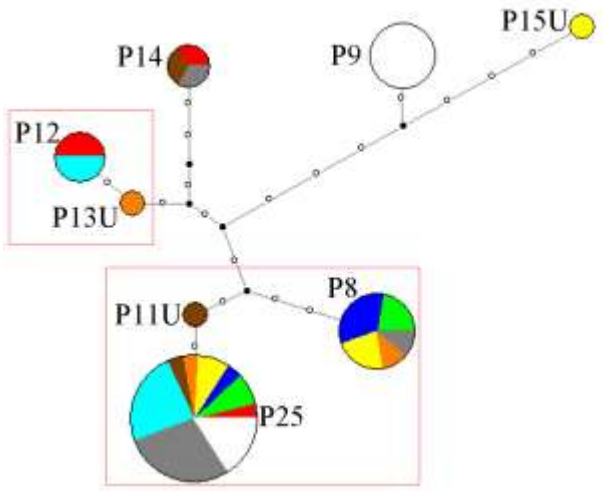
Appendix B. Haplotype network and table listing host species of clade Haem2. Color-code as in Appendix A



Family	Species	H15U	H16U	H17	H19U	H20U
Formicariidae	FORANA			1		
	FORCOL				1	
Furnariidae	GLYSPI			16		
	XIPOCE	1				
	ANCSTR			1		
	AUTINF			39		
	AUTMEL			1		
	AUTOCH			2		
	HYLSUB			6		
	PHIERT			1		
	PHIERY			3		
	PHIPYR			2		
Momodidae	BARMAR		1			

Parulidae	PHAFUL	1		
Pipridae	LEPCOR	1		
Thamnophilidae	DICCIN	1		
	EPIERY	1		
	HYLNAE	1		
	MYRHAU	1		
	MYRMYO	2		
	THACAE	4		
	THASCH	1		
	WILPOE	2		1
Troglodytidae	HENLEU	1		
	MICMAR	1		

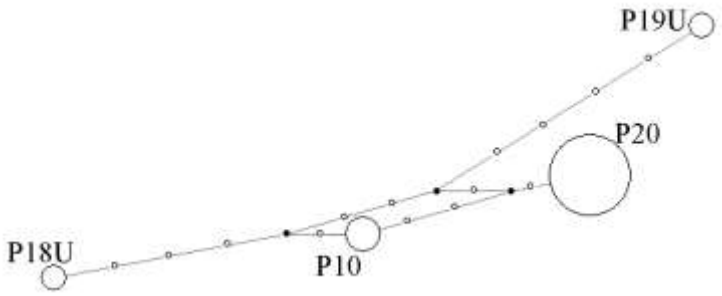
Appendix C. Haplotype network and table listing host species of clade Plas1. P11, P8, and P25 are within 6 mutations (~ 0.9%) of each other and share several species, thus, they are considered one lineage (P25L). P12 and P14 share host species, but are more than 1% divergent. P12 and P13 do not share host species but are only 0.2% divergent, and poorly sampled. Thus, P12 and P13 are one lineage (P12L), but P14 is separate. P9 is host species specialized, and P15 is distant from the generalized haplotypes and is considered unique. Color-code as in Appendix A.



Family	Species	P11U	P12	P13U	P14	P15U	P25	P8	P9
Furnariidae	GLYSPI						2		
Rhinocryptidae	LIOTHO						1		
Thamnophilidae	DICCIN							1	
	GYMLEU						1		
	HYLNAE		2		1		1		
	HYPCAN						1		

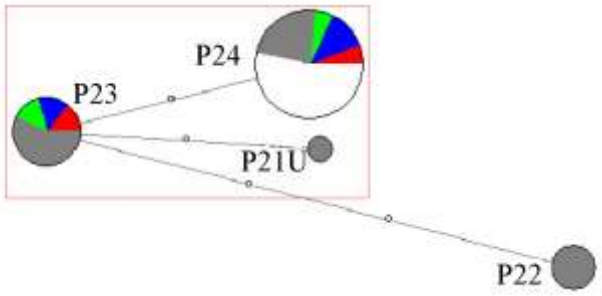
	MYRAXI	1
	MYRFOR	2 2
	MYRHAU	1 3
	MYRLON	1
	MYRMYO	1 2 2
	PHLERY	1 1 1
	SCHLEU	1 1 1
	THAARD	2 6
	THACAE	3
	THASCH	1
Troglodytidae	MICMAR	1
Tyrannidae	MYIBAR	7

Appendix D. Haplotype network and table listing host species of clade Plas2. Color-code as in Appendix A.



Family	Species	P10	P18U	P19U	P20
Furnariidae	PHIPYR			1	
Pipridae	LEPCOR				7
	PIPFIL				1
	PIPPIP				1
Thamnophilidae	MYRAXI	2			
Thraupidae	TACCRI				1
Vireonidae	HYLOCH		1		

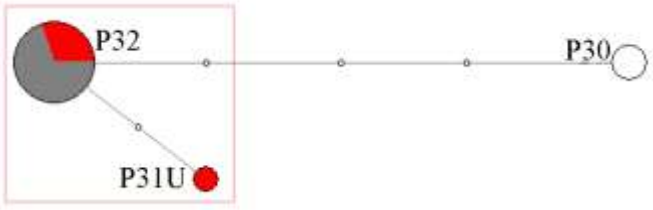
Appendix E. Haplotype network and table listing host species of clade Plas3. P23 is ~ 0.2% divergent to both P24 and P21 and shares species with P24. These three are considered the same lineage (P24L). P22, however, is the most distant to the others and it exhibits genus specificity (to *Automolus*). Therefore, P22 is considered a separate lineage. Color-code as in Appendix A.



Family	Species	P21U	P22	P23	P24
Formicariidae	FORANA	1			
	MYRCAM			1	
Furnariidae	AUTINF		2		
	AUTMEL		1		
	GLYSPI			1	
	XENMIN			1	
Momodidae	BARMAR				1
Pipridae	LEPCOR				3
	PIPERY				1
	PIPPIP				1
Thamnophilidae	CERSER			1	
	MYRHAU				1

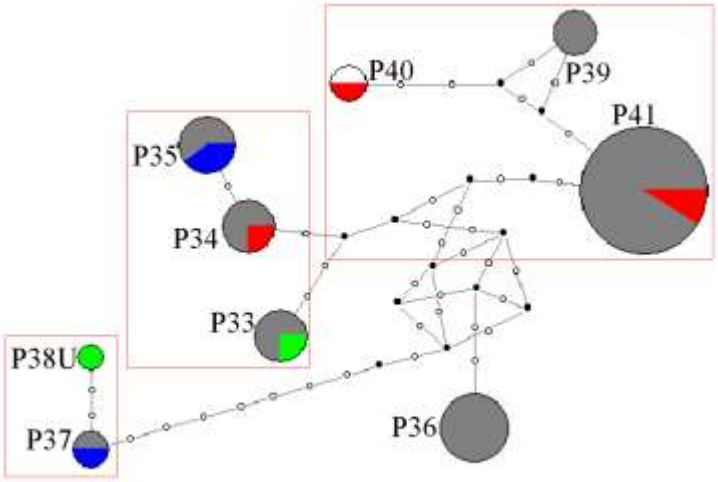
	MYRLON	1
	PITALB	1 1
	THAARD	1 2
	THACAE	1 1
	THAMUR	1
	WILPOE	1
Troglodytidae	HENLEU	1
Tyrannidae	MIOOLE	1
Vireonidae	HYLOCH	1

Appendix F. Haplotype network and table listing host species of clade Plas4. The two haplotypes sharing a species are 0.3% divergent. They are, in turn, 0.6% and 0.9% divergent from P30, found exclusively in manakins. P31 and P32 are considered the same evolutionary lineage (P32L), distinguished from P30. Color-code as in Appendix A.



Family	Species	P30	P31U	P32
Pipridae	LEPCOR	1		
	PIPFIL	1		
Thamnophilidae	GYMLEU		1	3
	HYLNAE			2
	MYRHAU			3
	PHLERY			1
	THAARD			1

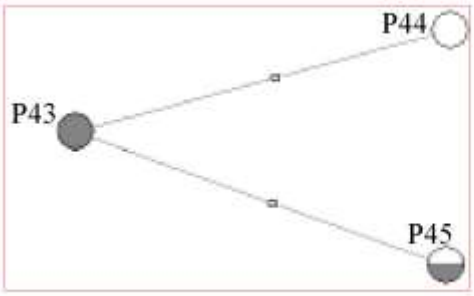
Appendix G. Haplotype network and table listing host species of clade Plas5. Color-code as in Appendix A.



Family	Species	P33	P34	P35	P36	P37	P38	P39	P40	P41
Formicariidae	FORANA					1				
	MYRCAM				1					
Furnariidae	GLYSPI									15
	XIPOCE									2
	XIPSPI		1						1	2
	AUTINF				3					
	AUTMEL				3					
	SCLCAU									3
Thamnophilidae	GYMLEU			2		1				
	HYLNAE	1					1			
	MYRAXI	3								

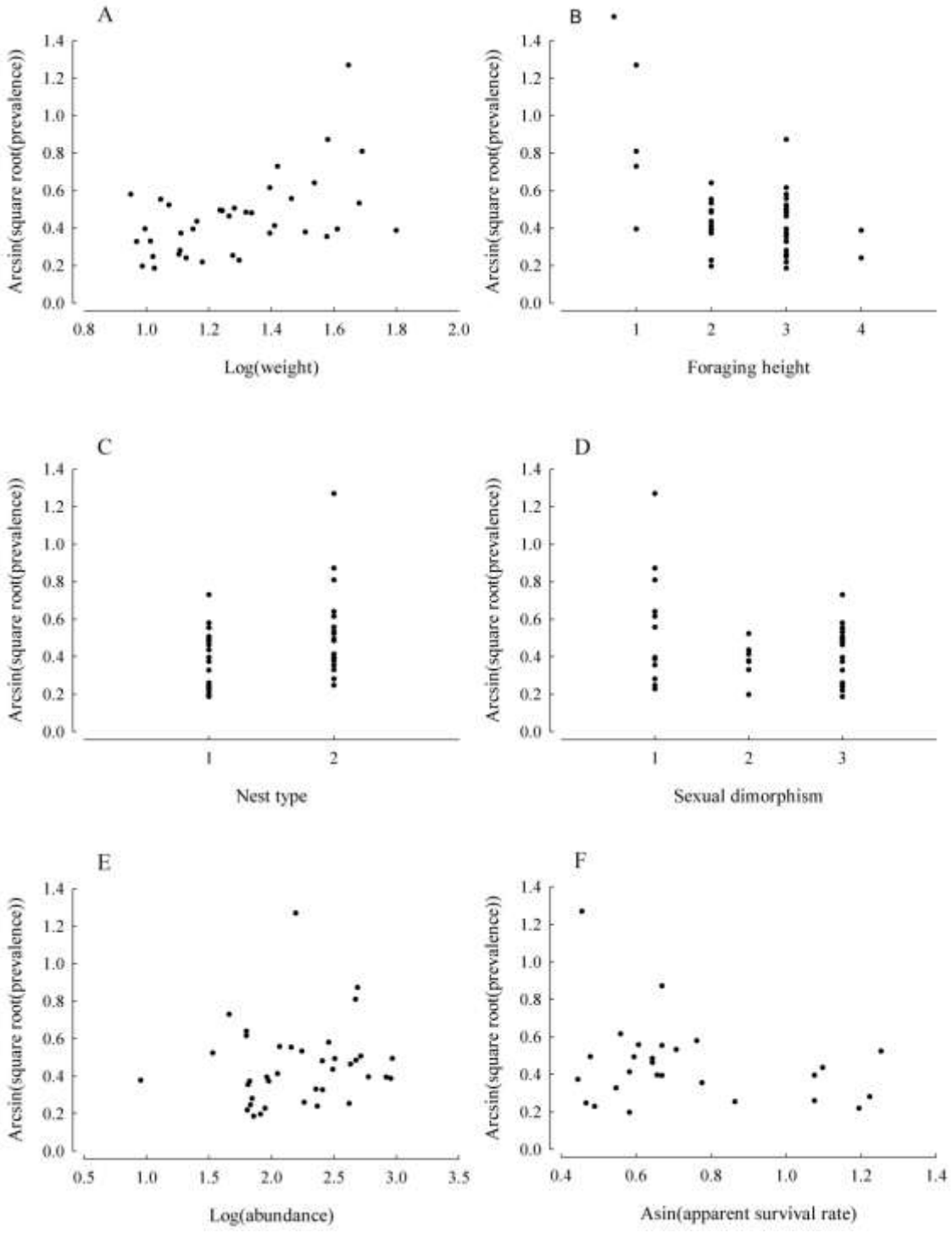
	MYRLON								1
	MYRMYO						2		
	RHEMEL			3					
	SCHLEU						1		
	WILPOE		3						
Vireonidae	HYLOCH							1	

Appendix H. Haplotype network and table listing host species of clade Plas6. Color-code as in Appendix A.



Family	Species	P43	P44	P45
Thamnophilidae	WILPOE			1
Cardinalidae	CYACYA			1
	HABRUB	2		
Fringillidae	EUPXAN		1	
Thraupidae	TANSCH		1	

Appendix I. Scatter plots of host body weight (A), foraging height (B), nest type (C), sexual dimorphism (D), abundance (E), and apparent survival rate (F) and arcsine square root transformed haemosporidian prevalence.



Appendix J. GenBank ID for host RAG-1 sequences used to control for host phylogeny in the gls analysis. XXXXX refers to sequences that have not yet been submitted to GenBank but are available upon request.

Family	Species	GenBank	Source
Momotidae	*Baryphthengus martii*	XXXXX	this study
Furnariidae	*Hyloctistes subulatus*	FJ461145	Moyle *et al*. 2009
	Automolus infuscatus	FJ461149	Moyle *et al*. 2009
	Automolus melanopezus	XXXXX	this study
	Xenops minutus	XXXXX	this study
	Glyphorynchus spirurus	FJ461160	Moyle *et al*. 2009
	Xiphorhynchus ocellatus	XXXXX	this study
	Xiphorhynchus guttatus	XXXXX	this study
Thamnophilidae	*Thamnophilus schistaceus*	XXXXX	this study
	Thamnophilus murinus	XXXXX	this study
	Thamnomanes ardesiacus	FJ461182	Moyle *et al*. 2009
	Thamnomanes caesius	FJ461176	Moyle *et al*. 2009
	Myrmotherula axillaris	FJ461183	Moyle *et al*. 2009
	Epinecrophylla fjeldsaai	XXXXX	this study
	Myrmotherula hauxwelli	XXXXX	this study
	Myrmotherula longipennis	XXXXX	this study
	Epinecrophylla erythrura	XXXXX	this study
	Hypocnemis hypoxantha	XXXXX	this study

Family	Species	GenBank	Source
	Myrmoborus myotherinus	XXXXX	this study
	Schistocichla leucostigma	XXXXX	this study
	Myrmeciza fortis	XXXXX	this study
	Pithys albifrons	XXXXX	this study
	Gymnopithys leucaspis	XXXXX	this study
	Rhegmatorhina melanosticta	FJ461208	Moyle *et al*. 2009
	Hylophylax naevius	XXXXX	this study
	Willisornis poecilinotus	FJ461204	Moyle *et al*. 2009
	Philydor erythrocercum	XXXXX	this study
	Sclerurus caudacutus	XXXXX	this study
Formicariidae	*Formicarius colma*	AY056993	Barker *et al*. 2002
	Formicarius analis	XXXXX	this study
Tyrannidae	*Mionectes oleagineus*	XXXXX	this study
	Myiobius barbatus	FJ501675	Tello *et al*. 2009
Pipridae	*Machaeropterus regulus*	XXXXX	this study
	Lepidothrix coronata	XXXXX	this study
	Chiroxiphia pareola	XXXXX	this study
	Pipra pipra	XXXXX	this study
	Pipra filicauda	FJ501714	Tello *et al*. 2009
	Pipra erythrocephala	FJ501713	Tello *et al*. 2009
Cardinalidae	*Cyanocompsa cyanoides*	XXXXX	this study

Appendix M. Sample size, number of infections, and prevalence of species in the Tiputini Biodiversity Station, Ecuador.

Family	Species code	Species	Sampled	Infected	Prevalence
Columbidae	GEOMON	*Geotrygon montana*	4		
Trogonidae	TRORUF	*Trogon rufus*	1	1	100
Momotidae	BARMAR	*Barypthengus martii*	4	2	50.0
	MOMMOM	*Momotus momota*	3	1	33.3
Galbulidae	GALALB	*Galbula albirostris*	5		
Bucconidae	BUCCAP	*Bucco capensis*	1	1	100
	MALFUS	*Malacoptila fusca*	4		
	MONMOR	*Monasa morphoeus*	2		
	NONBRU	*Nonnula brunnea*	5		
Capitonidae	CAPNIG	*Capito niger (auratus)*	2	1	50.0
Ramphastidae	PTEAZA	*Pteroglossus azara*	2		
	SELREI	*Selenidera reinwardtii*	2	1	50.0
Picidae	PICRUF	*Picumnus rufiventris*	1		
Furnariidae	ANCSTR	*Ancistrops strigilatus*	2	1	50.0
	AUTINF	*Automolus infuscatus*	75	44	58.7
	AUTMEL	*Automolus melanopezus*	15	6	40.0
	AUTOCH	*Automolus ochrolaemus*	4	2	50.0

Family	Species code	Species	Sampled	Infected	Prevalence
	AUTRUB	*Automolus rubiginosus*	3		
	CAMPRO	*Campylorhamphus procurvoides*	1		
	CAMTRO	*Campylorhamphus trochilirostris*	11	3	27.3
	CRAGUT	*Cranioleuca gutturata*	1		
	CRYBAR	*Crypturellus bartletti*	1		
	DENRUF	*Dendrexetastes rufigula*	1		
	DENFUL	*Dendrocincla fuliginosa*	7		
	DENMER	*Dendrocincla merula*	7	1	14.3
	DENCER	*Dendrocolaptes certhia*	4		
	GLYSPI	*Glyphorhynchus spirurus*	320	53	16.6
	HYLSUB	*Hyloctistes subulatus*	25	7	28.0
	PHIERY	*Philydor erythrocercus*	39	17	43.6
	PHIERT	*Philydor erythropterus*	3	3	100
	PHIPYR	*Philydor pyrrhodes*	13	3	23.1
	SCLCAU	*Sclerurus caudacutus*	27	5	18.5
	SCLMEX	*Sclerurus mexicanus*	5		
	SCLRUF	*Sclerurus rufigularis*	11		
	SYNRUT	*Synallaxis rutilans*	4		
	XENMIN	*Xenops minutus*	26	2	7.69
	XIPGUT	*Xiphorynchus guttatus*	14	4	28.6

Family	Species code	Species	Sampled	Infected	Prevalence
	XIPOCE	*Xiphorynchus ocellatus*	33	4	12.1
	XIPSPI	*Xiphorynchus spixii*	13	6	46.2
Thamnophilidae	CERSER	*Cercomacra serva*	4	3	75.0
	DICCIN	*Dichrozona cincta*	8	6	75.0
	EPIERY	*Epinecrophylla erythrura*	20	3	15.0
	EPIFJE	*Epinecrophylla fjeldsai*	28	3	10.7
	FREUND	*Frederickena unduligera*	5		
	GYMLEU	*Gymnopithys leucaspis*	62	11	17.7
	HYLNAE	*Hylophylax naevia*	85	16	18.8
	HYPCAN	*Hypocnemis cantator*	10	2	20.0
	HYPHYP	*Hypocnemis hypoxantha*	15	2	13.3
	MYRFOR	*Myrmeciza fortis*	31	11	35.5
	MYRMYO	*Myrmoborus myotherinus*	69	15	21.7
	MYRAXI	*Myrmotherula axillaris*	41	13	31.7
	MYRHAU	*Myrmotherula hauxwelli*	47	15	31.9
	MYRLON	*Myrmotherula longipennis*	31	5	16.1
	MYRMEN	*Myrmotherula menetriesii*	9		
	MYRORN	*Myrmotherula ornata*	2		
	NEONIG	*Neoctantes niger*	1		
	PHLERY	*Phlegopsis erythroptera*	12	6	50.0

Family	Species code	Species	Sampled	Infected	Prevalence
	PITALB	*Pithys albifrons*	98	6	6.12
	PYGSTE	*Pygiptila stellaris*	6	2	33.3
	RHEMEL	*Rhegmatorhina melanosticta*	22	3	13.6
	SCHLEU	*Schistocichla leucostigma*	18	8	44.4
	THAARD	*Thamnomanes ardesiascus*	76	16	21.1
	THACAE	*Thamnomanes caesius*	74	20	27.0
	THAAET	*Thamnophilus aethiops*	2		
	THAMUR	*Thamnophilus murinus*	18	5	27.8
	THASCH	*Thamnophilus schistaceus*	15	4	26.7
	WILPOE	*Willisornis poecilinota*	109	26	23.9
Formicariidae	CHANOB	*Chamaeza nobilis*	5	2	40.0
	FORANA	*Formicarius analis*	22	12	54.5
	FORCOL	*Formicarius colma*	34	31	91.2
	MYRCAM	*Myrmothera campanisona*	3	2	66.7
Conopophagidae	CONPER	*Conopophaga peruviana*	8		
Rhinocryptidae	LIOTHO	*Liosceles thoracicus*	1	1	100
Tyrannidae	CORTOR	*Corythopis torquatus*	10	3	30.0
	MIOOLE	*Mionectes oleagineus*	54	8	14.8
	MYIBAR	*Myiobius barbatus*	28	7	25.0

Family	Species code	Species	Sampled	Infected	Prevalence
	PLACOR	*Platyrinchus coronatus*	4		
	POECAP	*Poecilotriccus capitale*	1		
	TERERY	*Terenotriccus erythrurus*	1		
incertae sedis	SCHTUR	*Schiffornis turdinus*	10	3	30.0
Pipridae	CHIPAR	*Chiroxiphia pareola*	80	7	8.75
	CHLHOL	*Chloropipo holochlora*	1		
	LEPCOR	*Lepidothrix coronata*	147	24	16.3
	MACREG	*Macheropterus regulus*	31	6	19.4
	PIPERY	*Pipra erythrocephala*	91	10	11.0
	PIPFIL	*Pipra filicauda*	107	6	5.61
	PIPPIP	*Pipra pipra*	108	11	10.2
Vireonidae	HYLOCH	*Hylophilus ochraceiceps*	10	4	40.0
Sylviidae	MICCIN	*Microbates cinereiventris*	1		
Turdidae	CATMIN	*Catharus minimus*	8	2	25.0
	CATUST	*Catharus ustulatus*	5	2	40.0
	TURALB	*Turdus albicollis*	23	4	17.4
	TURLAW	*Turdus lawrencii*	8	1	12.5
Troglodytidae	HENLEU	*Henicorhina leucosticta*	13	4	30.8
	MICMAR	*Microcerculus marginatus*	5	3	60.0
	THRCOR	*Thryothorus coraya*	2		

Family	Species code	Species	Sampled	Infected	Prevalence
Fringillidae	EUPXAN	*Euphonia xanthogaster*	4	2	50.0
Parulidae	PHAFUL	*Phaeothlypis fulvicauda*	12	3	25.0
Thraupidae	LANFUL	*Lanio fulvus*	2		
	TACCRI	*Tachyphonus cristatus*	2	2	100
	TACSUR	*Tachyphonus surinamus*	1		
	TANSCH	*Tangara schrankii*	5	3	60.0
Cardinalidae	CYACYA	*Cyanocompsa cyanoides*	15	4	26.7
	HABRUB	*Habia rubica*	7	3	42.9
		TOTAL	2488	539	21.7

Appendix N. 100% matches of cyt *b* haplotypes (HAP) founding birds in Tiputini to sequences from three databases (DB). Host species and family of the match, general locality, reference (REF), GenBank ID (ID) and number of bases that overlap (BP) are shown. If the haplotype has been placed within a putative evolutionary lineage, the name of this lineage is shown in parenthesis below the haplotype name. If a haplotype has been identified to morphospecies, the name is listed in parenthesis below the 100% match name.

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
H1	4	H_DENPET01	MalAvi	*Dendroica petechia*	Parulidae	USA	S&L	AY640129	203
H2	1	H_APSPI01	MalAvi	*Zonotrichia capensis*	Emberizidae	Chile	MER	EF153652	273
				Aphrastura spinicauda	Furnariidae	Chile			
				Troglodytes musculus	Troglodytidae	Chile			
				Turdus falcklandii	Turdidae	Chile			
				Elaenia albiceps	Tyrannidae	Chile			
H3	1	H_LEPRUF01	MalAvi	*Leptotila rufaxilla*	Columbidae	Guyana	DUR	DQ241543	283
H4	1	H_PSADEC01	MalAvi	*Psarocolius decumanus*	Icteridae	Guyana	DUR	DQ241549	280
H5	1	OZ21	Ricklefs	*Saltator albicollis*	Cardinalidae	West Indies	FAL03	AY167242	438

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
		(*H. coatneyi*)							
				Coereba flaveola	Coerebidae	West Indies			
				Columbina passerina	Columbidae	West Indies			
				Loxigilla noctis	Emberizidae	West Indies			
				Loxigilla portoricensis	Emberizidae	West Indies			
				Melanospiza richardsoni	Emberizidae	West Indies			
				Tiaris bicolor	Emberizidae	West Indies			
				Quiscalus lugubris	Icteridae	West Indies			
				Margarops fuscus	Mimidae	West Indies			
				Piculus rubiginosus	Picidae	West Indies			
				Eulampis holosericeus	Trochilidae	West Indies			
				Glaucis hirsuta	Trochilidae	West Indies			
				Elaenia martinica	Tyrannidae	West Indies			
				Vireo altiloquus	Vireonidae	West Indies			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Vireo griseus	Vireonidae	USA			
				Vireo olivaceus	Vireonidae	USA			
		Toc-5	GenBank	*Dacnis cayana*	Thraupidae	Brazil	BEL	HQ287540	407
				Elaenia chiriquensis	Tyrannidae	Brazil			
H8		HN	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	FEC	JX501863	502
				Suiriri suiriri	Tyrannidae	Brazil			
				Neothraupis fasciata	Thraupidae	Brazil			
				Elaenia chiriquensis	Tyrannidae	Brazil			
H9	12	Toc-3	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	BEL	HQ287538	488
(H9L)				*Manacus manacus*	Pipridae	Brazil			
				Pipra fasciicauda	Pipridae	Brazil			
				Cantorchilus leucotis	Troglodytidae	Brazil			
				Corythopis torquatus	Tyrannidae	Brazil			
				Elaenia cristata	Tyrannidae	Brazil			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
		HEB	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	FEC	JX501908	
				Ammodramus humeralis	Emberizidae	Brazil			
				Aratinga aurea	Psittacidae	Brazil			
				Neothraupis fasceata	Thraupidae	Brazil			
				Elaenia chiriquensis	Tyrannidae	Brazil			
H10	10	Toc-1	GenBank	*Coereba flaveola*	Coerebidae	Brazil	BEL	HQ287536	486
				Coryphospingus pileatus	Emberizidae	Brazil			
				Pipra fasciicauda	Pipridae	Brazil			
				Sakesphorus luctuosus	Thamnophilidae	Brazil			
				Hemitriccus margaritaceiventer	Tyrannidae	Brazil			
		HG	GenBank	*Ammodramus humeralis*	Emberizidae	Brazil	FEC	JX501896	505
				Aratinga aurea	Psittacidae	Brazil			
				Elaenia chiriquensis	Tyrannidae	Brazil			
				Myiarchus swainsoni	Tyrannidae	Brazil			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Phaeomyias murina	Tyrannidae	Brazil			
				Suiriri suiriri	Tyrannidae	Brazil			
P8	9	Oz06J620	Ricklefs	*Passerina ciris*	Cardinalidae	West Indies	R&F	AF465555	293
(P25L)									
				Coereba flaveola	Coerebidae	West Indies		AF465554	
				Arremonops chloronotus	Emberizidae	West Indies		AF465553	
				Euneornis campestris	Emberizidae	West Indies			
				Icterus leucopteryx	Icteridae	West Indies			
				Baeolophus bicolor	Paridae	USA			
				Dendroica caerulescens	Parulidae	West Indies			
				Dendroica dominica	Parulidae	USA			
				Dendroica magnolia	Parulidae	USA			
				Geothlypis trichas	Parulidae	West Indies			
				Helmitheros vermivorus	Parulidae	USA, West Indies			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Mniotilta varia	Parulidae	USA, West Indies			
				Oporornis formosus	Parulidae	USA			
				Parula americana	Parulidae	West Indies			
				Wilsonia citrina	Parulidae	USA			
P21	1	P_BUTSTR01	MalAvi	*Butorides striatus*	Ardeidae	Guyana	DUR	DQ241528	293
P24	15	P_CYCYA01	MalAvi	*Cyanocompsa cyanoides*	Cardinalidae	Guyana	DUR	DQ241529	191
(P24L)				*Icterus cayanensis*	Icteridae	Guyana			
P37	2	P_GRW06	MalAvi	*Crateroscelis robusta*	Acanthizidae	Papua New	BEA	DQ659588	285
(P37L)		(*P. elongatum*)				Guinea			
				Alcedo atthis	Alcedinidae	Myanmar	ISH		
				Ardea herodias	Ardeidae	USA	BEA		
				Philesturnus carunculatus	Callaeidae	New Zealand	RB		
				Emberiza citrinella	Emberizidae	New Zealand	RB		
				Linurgus olivaceus	Fringillidae	Gabon	HEL07b		

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Linurgus olivaceus	Fringillidae	Cameroon	BEA09		
				Mohoua albicilla	Pachycephalidae	New Zealand	RB		
				Passer domesticus	Passeridae	New Zealand	RB		
				Passer domesticus	Passeridae	Bulgaria	ZET		
				Passer domesticus	Passeridae	Bulgaria	DIM		
				Passer domesticus	Passeridae	Bulgaria	MAR		
				Petroica australis	Petroicidae	New Zealand	RB		
				Ploceus melanogaster	Ploceidae	Cameroon	BEA09		
				Ailuroedus melanotis	Ptilonorhynchidae	Australia	BEA04		
				Strix varia	Strigidae	Minnesota	ISH		
				Acrocephalus arundinaceus	Sylviidae	Bulgaria	DIM		
				Acrocephalus arundinaceus	Sylviidae	Bulgaria	VAL08a		
				Acrocephalus arundinaceus	Sylviidae	Sweden	BEN		
				Acrocephalus scirpaceus	Sylviidae	Spain	FER		

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Hippolais icterina	Sylviidae	Sweden	HEL07b		
				Rimator malacoptilus	Timaliidae	Myanmar	ISH		
				Turdus merula	Turdidae	New Zealand	RB		
				Turdus philomelos	Turdidae	New Zealand	RB		
		P_PADOM11	MalAvi	*Cyanocompsa cyanoides*	Cardinalidae	Guyana	DUR	EU627843	285
				Pitylus grossus	Cardinalidae	Guyana	DUR		
				Saltator maximus	Cardinalidae	Guyana	DUR		
				Melospiza melodia	Emberizidae	USA	MART		
				Carpodacus mexicanus	Fringillidae	Georgia	KIM		
				Carpodacus mexicanus	Fringillidae	Wisconsin	KIM		
				Carpodacus mexicanus	Fringillidae	Idaho	KIM		
				Carpodacus mexicanus	Fringillidae	New York State	KIM		
				Carpodacus mexicanus	Fringillidae	California	KIM		
				Carpodacus mexicanus	Fringillidae	USA	MART		

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Agelaius ruficapillus	Icteridae	Uruguay	DUR		
				Cacicus cela	Icteridae	Guyana	DUR		
				Passer domesticus	Passeridae	Brazil	MAR		
				Passer domesticus	Passeridae	Colorado	MAR		
				Passer domesticus	Passeridae	Michigan	MAR		
				Passer domesticus	Passeridae	Missouri	MAR		
				Polioptila dumicola	Polioptilidae	Uruguay	DUR		
				Spheniscus demersus	Spheniscidae	Maryland	B&F		
				Aegolius acadicus	Strigidae	Vermont	MART		
				Strix varia	Strigidae	Minnesota	ISH		
		P. sp. E1	GenBank	*Tachycineta bicolor*	Hirundinidae	USA	S&L	AY640132	552
				Dendroica petechia	Parulidae	USA			
				Melospiza melodia	Emberizidae	USA			
				Carpodacus mexicanus	Fringillidae	USA			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
		P. relictum jb3	GenBank				B&F	AY733088	552
		P. elongatum P52	GenBank	*Ardea herodias*		USA	BEA	DQ659588	551
		MMK-2009a	GenBank	*Culex restuans*			KIM10	GQ471951	533
		Toc-32	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	BEL	HQ287549	510
		PQ	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	FEC	JX501787	552
				Neothraupis fasciata	Thraupidae	Brazil			
		Larus/RBG2/NZL	GenBank	*Larus scopulinus*		New Zealand	CLO	HM579784	415
		OZ01/haplotype 56	Ricklefs	*Cardinalis cardinalis*	Cardinalidae	USA	O&R	GQ141594	552
				Passerina ciris	Cardinalidae	West Indies			
				Passerina cyanea	Cardinalidae	USA			
				Pheucticus ludovicianus	Cardinalidae	West Indies			
				Coereba flaveola	Coerebidae	West Indies			
				Columbina passerina	Columbidae	West Indies			
				Geotrygon montana	Columbidae	West Indies			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Zenaida aurita	Columbidae	West Indies			
				Loxigilla portoricensis	Emberizidae	West Indies			
				Pipilo erythrophthalmus	Emberizidae	USA			
				Tiaris bicolor	Emberizidae	West Indies			
				Agelaius icterocephalus	Icteridae	West Indies			
				Icterus crysater	Icteridae	West Indies			
				Dumetella carolinensis	Mimidae	West Indies			
				Margarops fuscatus	Mimidae	West Indies			
				Margarops fuscus	Mimidae	West Indies			
				Mimus gilvus	Mimidae	West Indies			
				Dendroica caerulescens	Parulidae	West Indies			
				Dendroica discolor	Parulidae	West Indies			
				Dendroica dominica	Parulidae	USA			
				Dendroica petechia	Parulidae	West Indies			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Dendroica plumbea	Parulidae	West Indies			
				Icteria virens	Parulidae	USA			
				Limnothlypis swainsonii	Parulidae	West Indies			
				Mniotilta varia	Parulidae	West Indies			
				Wilsonia citrina	Parulidae	USA, West Indies			
				Eulampis holosericeus	Trochilidae	West Indies			
				Thryothorus ludovicianus	Troglodytidae	USA			
				Vireo altiloquus	Vireonidae	West Indies			
				Vireo griseus	Vireonidae	USA			
				Vireo modestus	Vireonidae	West Indies			
P44 (P43L)	2	PF	GenBank	*Volatinia jacarina*	Emberizidae	Brazil	FEC	JX501881	490
P45 (P43L)	2	OZ04	Ricklefs	*Cardinalis cardinalis*	Cardinalidae	West Indies	O&R	GQ141587	524

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Passerina cyanea	Cardinalidae	USA			
				Coereba flaveola	Coerebidae	West Indies			
				Euneornis campestris	Emberizidae	West Indies			
				Loxigilla noctis	Emberizidae	West Indies			
				Loxigilla violacea	Emberizidae	West Indies			
				Loxipasser anoxanthus	Emberizidae	West Indies			
				Tiaris bicolor	Emberizidae	West Indies			
				Tiaris olivacea	Emberizidae	West Indies			
				Volatinia jacarina	Emberizidae	West Indies			
				Icterus bonana	Icteridae	West Indies			
				Quiscalus lugubris	Icteridae	West Indies			
				Margarops fuscatus	Mimidae	West Indies			
				Mimus gilvus	Mimidae	West Indies			
				Dendroica adelaidae	Parulidae	West Indies			

HAP	N	100% matches	DB	Host species	Family	Locality	REF[a]	ID	BP
				Dendroica plumbea	Parulidae	West Indies			
				Icteria virens	Parulidae	USA			
				Mniotilta varia	Parulidae	USA			
				Euphonia jamaica	Thraupidae	West Indies			
				Nesospingus speculiferus	Thraupidae	West Indies			
				Turdus plumbeus	Turdidae	West Indies			
				Elaenia martinica	Tyrannidae	West Indies			
				Vireo olivaceus	Vireonidae	USA			
		P. sp. P6	GenBank	*Icterus cayanensis*	Icteridae	Uruguay	BEA	DQ659545	524

[a]Reference abbreviations: S&L (Szymanski and Lovette 2005), MER (Merino et al 2008), DUR (Durrant et al. 2006), FAL03 (Fallon et al. 2003a), BEL (Belo et al. 2011), FEC (Fecchio et al. 2012), R&F (Ricklefs and Fallon 2002), BEA (Beadell et al. 2006), ISH

(Ishtiaq et al. 2007), RB (Ruth Brown unpublished), HEL07 (Hellgren et al. 2007), BEA09 (Beadell et al. 2009), ZET (Zethindjiev unpublished), DIM (Dimitrov et al 2010), MAR (Marzal unpublished), BEA04 (Beadell et al 2004), VAL08 (Valkiūnas et al. 2008), BEN (Bensch et al. 2007), FER (Fernandez et al. 2010), MART (Martinsen et al. 2008), KIM (Kimura et al. 2006), B&F (Beadell and Fleischer 2005), KIM10 (Kimura et al. 2010), CLO (Cloutier et al. 2011), O&R (Outlaw and Ricklefs 2009).

Chapter 2: On the relationship between specialization and resource predictability in avian Haemosporida

Maria Svensson-Coelho, Bette A. Loiselle, John G. Blake, Robert E. Ricklefs

ABSTRACT

The hypothesis that host specialization of parasites is positively associated with traits of their hosts was tested in the avian haemosporidian system. Host specificity was measured as SES_{MPD}, and three host traits were investigated: local abundance, longevity, and body size. Mean trait values of hosts harboring specialist parasites were compared to those harboring generalists. Then, because we detected a significant signal of parasite phylogeny in the level of host specificity, we performed a multiple generalized least squares (GLS) regression analysis, controlling for parasite phylogeny, with all three host traits as independent variables and host specialization as the dependent variable. We used multimodel inference and model averaging to determine whether any combination of traits influence host specialization of parasites and determined whether partial beta coefficients of the regression (averaged from the best models) differed significantly from zero for each trait.

Although we found no significant relationships between host specificity and either of the host traits in the GLS regression analysis, hosts of specialist parasites were significantly more abundant than hosts of generalist parasites according to our trait means comparison. Thus, there was some evidence that malaria parasites specialize on what one might consider predictable resources. Because the support for this hypothesis was not

strong for all traits, we also discuss two alternatives: (1) that frequent encounter with multiple host species, mediated by blood-sucking insects, might promote generalization within this system; and (2) that interspecific interactions among the parasites themselves might lead to some parasites adopting a specialist strategy.

KEY WORDS

Avian malaria, community ecology, ecological specialization, species interactions

INTRODUCTION

In host-parasite systems, parasite transmission potential depends partly on the level of host specificity (Pedersen et al. 2005; Woolhouse et al. 2001), which can be broadly defined as " . . . the extent to which a parasite taxon is restricted in the number of host species used at a given stage in the life cycle" (Poulin 2007, p. 41). For a parasite population to be viable, transmission potential (R_0), which is the number of new infections per infected host individual, must be at least one (Anderson and May 1991). In general, this makes it difficult for parasites to become specialized on rare hosts, the alternatives being specialization on a common host or a capacity to infect many host species (Woolhouse et al. 2001). Whether or not a parasite utilizes multiple host species is to some extent influenced by the mode of transmission (Pedersen et al. 2005). For example, among zoonotic viruses, bacteria, and protozoan parasite groups, which by definition are shared between at least two species (humans and another animal), vector-borne pathogens are more often zoonotic than are those transmitted indirectly (e.g., ingested), which in turn are more often zoonotic than are those transmitted directly (e.g., sexually transmitted) (Woolhouse et al. 2001). There are exceptions, however. For example, within primates vector-borne protozoans are mostly host-specific (Pedersen et al. 2005). Moreover, one encounters both generalists and specialists within many parasite groups (e.g., monogenean parasites of fish or malaria parasites of birds) (Fallon et al. 2005; Sasal et al. 1999).

Avian haemosporidian ("malaria") parasites of the genera *Plasmodium* and *Haemoproteus* are vector-borne protozoans that reproduce asexually within bird hosts and both asexually and sexually within dipteran vectors (Culicidae mosquitoes,

Culicoides biting midges, and Hippoboscidae louse flies) (Valkiunas 2005). In any particular locality, one can expect to find malaria parasite lineages that occur on single host species as well as others infecting multiple, sometimes distantly related, host species (Fallon et al. 2005; Hellgren et al. 2009, Chapter 1; Ricklefs et al. 2005). In general, avian malaria parasite assemblages are dominated by generalists, as would be expected for vector-borne parasites (Woolhouse et al. 2001). Because specialization reduces the number of potential hosts, one might ask why specialists exist at all (Loiseau et al. 2012). There are several possibilities. First, what we perceive as specialized lineages could be poorly sampled generalists (sampling issue) (Poulin 2007). Both Ricklefs et al. (2005) and Svensson-Coelho et al. (Chapter 1) found a significant positive relationship between host breadth (calculated as number of host species) and sampling effort within local assemblages. Alternatively, specialization might be an adaptive response to qualities of potential hosts as well as interactions with other parasites species. If so, hypotheses formulated for explaining ecological specialization in general could help us find correlates of specialization in malaria parasites.

To parasites, hosts are resources (Poulin 2007). Thus, theory addressing ecological resource specialization can be extended to parasites. Futuyma and Moreno (1988) reviewed four hypotheses that address the causes of specialization: (1) environmental constancy, (2) foraging theory, (3) interspecific interactions, and (4) mating rendezvous. According the first hypothesis, specialization evolves in stable environments because there is no pressure on organisms to seek alternative resources. According to the second hypothesis, specialization evolves on the food resource yielding the highest fitness. In the third hypothesis, competition with other organisms excludes

poor competitors from resources and causes segregation of competitors among different resources. Alternatively, facilitation could promote coexistence on the same resource, in which case specialists would aggregate on few resources. Finally, in the fourth hypothesis, specialization evolves because of frequency-dependent runaway selection that causes rapid shifts in host association. That is, specialization evolves on a resource where mates are most readily found and most offspring are, consequently, produced. Because sexual reproduction in haemosporians occurs within dipteran vectors, this hypothesis cannot be tested on data from only the birds and parasites.

The environmental constancy and foraging theory hypotheses of resource specialization might be difficult to distinguish, and they can be combined into a more general resource predictability hypothesis (Combes 2001; Krasnov et al. 2006; Poulin 2007). Under this hypothesis, parasites should specialize on hosts that are "predictable" in space and time (Krasnov et al. 2006; Ward 1992) because such predictability increases parasite transmission and survival (Simková et al. 2006). Functional explanations for the evolution of specialized pathogens have been modeled under an optimal foraging framework (Ward 1992). Accordingly, generalists evolve when the frequency of encounter of the preferred host decreases and the relative mean fitness on non-preferred to preferred hosts is greater than a fitness threshold, w^*, which is directly related to the probability of settling on a preferred host. In other words, parasites are more likely to become and remain specialized on hosts that provide sufficiently large transmission opportunities, such that a viable parasite population can be maintained over time. Predictability, thus, implies anything that positively relates to the repeated encounter of a parasite to individuals of the same host species, or that in other ways increases fitness of

a preferred host such that Ward's (1992) fitness threshold is unlikely to be reached. Host species characteristics that allow for more parasite transmission opportunities, rendering a host more predictable as a resource base, include abundance, population stability, longevity, social behavior, and body size (reviewed in Poulin 2007, p. 62). Here, we investigate the relationship between parasite specialization and the abundance, longevity, and body size of hosts.

Parasites are more likely to encounter more abundant and larger hosts. Vectors of avian malaria parasites, assuming they bite hosts indiscriminately, are more likely to consecutively bite two members of an abundant host than two members of a rare host, which means that the parasites will encounter individuals of abundant hosts more often and might adapt better to the host's immune system. Parasite preference of abundant hosts is supported in that rare hosts tend not to harbor specialist parasites (Woolhouse et al. 2001). Larger-bodied hosts are likely to emit more CO_2 and olfactory cues, compounds vectors use for finding hosts (Gibson and Torr 1999). Thus, vectors should more often be attracted to larger hosts and parasites should more frequently encounter large hosts than small hosts, possibly leading to specialization. Host longevity should increase transmission opportunities for parasites that can remain within their hosts for a long time. Malaria parasite infections do indeed become chronic and can persist in their hosts well beyond the acute phase of infection (Valkiunas 2005).

In this study, we investigated a large sample of malaria parasites in a local assemblage of birds in Amazonian Ecuador with the primary objective of determining whether host specialization is related to host local abundance, longevity, and body size.

We expected not to find highly specialized parasite lineages on rare, short-lived, or small-bodied bird species.

METHODS

Data collection

Birds, primarily suboscines (Passeriformes, suborder Tyranni), were sampled during the dry season (January and March) between 2001 and 2010 in the Tiputini Biodiversity Station (hereafter "Tiputini"), Orellana Province, Ecuador (0°38´S, 76°08´W). The site is located in relatively undisturbed *terra firme* forest at *ca*. 200 m elevation. More than 300 species of bird have been recorded within two 100-ha plots in this site (Blake 2007), most of which are resident, although migratory birds such as *Catharus* spp. (Turdidae) are encountered on occasion. Ninety-six mist-nets (12 x 2.6 m, 36-mm mesh) were set at ground level, arranged in eight rectangles (100 x 200 m) of 12 nets, with nets placed *ca.* 50 m apart. Samples were collected between 0600 and 1300 h (Blake and Loiselle 2008).

Approximately 10 µL of blood was obtained by brachial venipuncture and stored in 1 mL Longmire lysis buffer (Longmire et al. 1997). Work at Tiputini was conducted in accordance with research permit number 13-IC-FAU-DFN (and subsequent renewals), Ministerio del Ambiente, Distrito Forestal Napo, Tena, Ecuador. Details of DNA extraction and parasite identification are provided in Chapter 1. Briefly, unique parasite lineages were identified by sequencing *ca.* 550 bp of the mtDNA cytochrome *b* (cyt *b*) gene. Recovered haplotypes were merged into putative evolutionary lineages based on genetic similarity and host species association (Chapter 1). A phylogeny of the parasite lineages in Tiputini was constructed using Maximum Likelihood and Bayesian

techniques (Chapter 1). Here, we present the ultrametric phylogeny estimated in BEAST v. 1.5 (Drummond and Rambaut 2007), using the GTR + gamma model of evolution (Fig. 1; for more details, see Chapter 1). In total, data from 363 individual parasites inside the two 100-ha plots, and 22 additional individual parasites from outside of the plots, are used in this study.

Host specificity

Host specificity of parasite lineages recovered four times or more was estimated by the standardized effect size of the mean pairwise genetic distance (SESMPD, Chapter 1), calculated by the formula

$$SES_{MPD} = \frac{MPD_{obs} - mean(MPD_{random})}{SD(MPD_{random})},$$

where MPD_{obs} is the observed $MPD_{weighted}$ (described below) and MPD_{random} is the *MPD* value calculated from 999 randomly generated host-parasite matrices (Kembel et al. 2011). We calculated $MPD_{weighted}$ as

$$MPD_{weighted} = 2 \sum_{i-1}^{S-1} \sum_{j=i+1}^{S-1} d_{ij} p_i p_j ,$$

where S is the number of hosts infected, d_{ij} is the pairwise genetic distance between hosts i and j, p_i is the proportion on host i and p_j is the proportion on host j. Strict host specialists received a slightly modified treatment. First, because of the zero d_{ij} term, no $MPD_{weighted}$ values are returned for strict host specialists. We consider all these strict host specialists to have an $MPD_{weighted}$ of zero. In addition, the SES_{MPD} returns an expected random distribution only for parasite lineages infecting more than one host species. We used the mean of the mean(MPD_{random}) and the mean of the SD(MPD_{random}) for parasite

lineages recovered more than four times when calculating the SES_{MPD} for strict host specialists, ensuring that strict specialists received the lowest SES_{MPD} values. We used the package *Picante* (Kembel et al. 2010) in R (R Development Core Team 2011) for all index calculations. The benefit of using the SES_{MPD} instead of $MPD_{weighted}$ in regression analyses is that it is not influenced by sample size (Chapter 1). In addition, by generating a null distribution, we are able to determine which parasite lineages are more specialized than expected by chance (i.e., the observed $MPD_{weighted}$ is in the leftmost tail of the null distribution) and which parasite lineages are more generalized than expected by chance (i.e., the observed $MPD_{weighted}$ is in the rightmost tail of the null distribution).

Host traits

Abundance, apparent survival rate (proxy for longevity) and average body weight (indicator of body size) have been estimated for the bird assemblage in Tiputini (Blake 2007; Blake and Loiselle 2008; Blake and Loiselle 2009, and unpublished data). Abundance was estimated over four years and survival rate was estimated from mark-recapture over 12 years (Chapter 1). Abundance and weight were log-transformed and survival rate was arcsine-transformed prior to analyses. We determined whether host traits were correlated with each other by calculating pairwise correlations in the software package *corrgram* (Wright 2006) in R.

Host traits and parasite specificity

We considered host species with at least four identified infections in a first assessment of the trait differences between hosts harboring specialized versus generalized parasites. The

means of the hosts of specialists were compared to the means of hosts of generalists using the non-parametric Mann-Whitney *U*-test in SPSS v. 15.0 for Windows (IBM Corporation, Armonk, New York). We considered specialist parasites those with a significantly narrow host distribution by the SES_{MPD}. Two of these specialists were found in more than one host species. We considered as hosts of those two specialist parasites only the hosts harboring more than 50% of the infections. For example, H17L was found on 24 host species, but 60% were found on *A. infuscatus* and *Glyphorhynchus spirurus* (Furnariidae). Each host species was placed in only one of the two categories, i.e., as harboring specialist or generalist parasites, with preference to the specialist category since few parasites were significantly specialized. Thus, *A. infuscatus,* for example, was considered a host of a specialist parasite, even though it also harbored some generalized parasites.

Phylogenetic conservatism of host specificity

If host specificity were to exhibit phylogenetic signal, i.e., if close relatives of parasites exhibit similar strategies (either specialist or generalist), one would have to control for parasite phylogeny when examining trait relationships (Felsenstein 1985). To determine whether host specificity is phylogenetically conserved, we used the test for serial independence (TFSI) described in Abouheif (1999). Briefly, data (here, values of host specificity) are ordered by phylogenetic relationships (such that values of sister taxa are closest to each other) and the serial sum of squared differences ($\sum d^2$) calculated. If there is no autocorrelation between phylogeny and specificity, the ratio (η) between $\sum d^2$ and the sum of squares ($\sum y^2$) should approach 2.0 (Sokal and Rohlf 1995, in Abouheif

1999). From the observed ratio, the *C*-statistic (1-η/2) can be calculated. Because the nodes of sister species can be rotated, the order of taxa within the phylogeny is not specified. The TFSI produces a distribution of the *C*-statistic calculated from all possible representations of the phylogeny. The mean of the observed *C*-statistic is then compared to the randomized mean *C*-statistic, which is obtained by randomly shuffling the taxon labels then calculating the *C*-statistic after each randomization. Here, we performed 999 such randomizations to produce our null distribution.

Comparative analysis of host traits and parasite specificity

Because of the high levels of generalization from both the host and parasite perspectives (i.e., many parasite species infect multiple hosts, which in turn harbor multiple parasite lineages), our dataset contained pseudoreplication. We used a method that incorporates data from all host species, recognizing that some species will occur within the dataset more than once and that results of this analysis should be interpreted with caution.

For each parasite lineage, each host trait (abundance, weight, and survival rate) was averaged, weighted by the frequency of association. For example, *Plasmodium* sp. 32L was recovered nine times: four times from *Gymnopithys leucaspis*, twice from *Myrmotherula hauxwelli*, twice from *Hylophylax naevius*, and once from *Thamnomanes ardesciacus* (all Thamnophilidae). The total abundances over the sampling years of these species are estimated as proportional to the 112, 144, 309, and 429 individuals captured, respectively. Thus, the weighted average abundance of hosts infected by P32L is 4/9*112 + 2/9*133 + 2/9*309 + 1/9*429 = 198. We transformed values before this averaging procedure, as described above.

A weighted generalized least squares (GLS) multiple regression , with the continuous SES_{MPD} as the dependent variable, was performed in the package *nlme* (Pinheiro et al. 2011) in R. This method examines the relationship between the dependent variable (host specificity) and the three independent variables, while controlling for the effect of parasite phylogeny on the dependent variable (Pagel 1997; Pagel 1999). The beta coefficients obtained are partial beta coefficients representing the relationships between host specificity and a host trait, while each comparison is controlled both for phylogeny and the influence of the other traits. A correlation matrix of phylogenetic relatedness among parasites is constructed and values of 1 are placed on the diagonal (for an example, see Schluter 2011). Any non-zero off-diagonal element is used to weight data for each parasite lineage. The closer these off-diagonal correlation coefficients are to 1, the less independent data from those two taxa are. In other words, data from two species with high correlation coefficients are treated as data from only a single taxon and data from two species with zero correlation coefficients are treated as independent. Thus, we can control for phylogeny but we cannot obtain partial beta coefficients between specialization and phylogeny (for information about how strong the signal of phylogeny is on host specialization, Abouheif's [1999] test suffices, and the sign of the C-statistic reveals whether close relatives tend to exhibit similar [+] or dissimilar [-] strategies). We used the ultrametric cyt *b* phylogeny of parasites from Chapter 1. Observations were weighted by the square root of parasite sample size, thus giving more importance to well-sampled lineages in the regression.

Using the package *MuMIn* (Barton 2011) in R, we estimated models of all possible combinations (not including interaction terms) of abundance, average body

weight, and apparent survival rate. We obtained the second order AIC (AICc), delta AICc (difference in AICc from the best model), and AICc probabilities, which are sometimes referred to as model weights (Johnson and Omland 2004). AICc was chosen as the rank criterion over AIC due to small sample size (Burnham and Anderson 2002; Burnham et al. 2011). We used model inference to obtain weighted means of our parameters of interest (the slopes of the regressions, beta) (Burnham and Anderson 2002; Burnham et al. 2011). Beta coefficients were weighted by the model probability (*w*). We chose to include models having a delta AICc of 2 or less. A *Z*-test was applied to each partial beta coefficient, after model averaging, to determine whether beta differed from a slope of zero. A non-zero slope indicates that the variable relates to SES_{MPD}.

RESULTS

Host traits

Abundance, survival rate, and body weight were not significantly correlated with each other ($P > 0.05$; $N = 45$ for weight and abundance, and $N = 29$ for survival rate) (Appendix A).

Host specialization

Six parasite lineages were considered significantly specialized according to our SES_{MPD}: P2, P4L, P9, P29, P25L, and H17L (Fig. 1). Collectively, these parasites specialized on the hosts *A. infuscatus*, *G. spirurus* (both Furnariidae), *Formicarius analis*, *Formicarius colma* (both Formicariidae), *Myiobius barbatus* (Tyrannidae), *Myrmotherula fortis*, *M. hauxwelli*, *Myrmoborus myotherinus*, *T. ardesciacus*, *Thamnomanes caesius*, and

Willisornis poecilinotus (all six Thamnophilidae). H17L and P25L, however, infected more than these species, but not at great frequency (Chapter 1). No lineages exhibited significantly greater host breadth than expected under the random distribution at a two-tailed alpha level of 0.05 (Fig. 1).

Host traits and parasite specialization

Hosts of specialists were significantly more abundant than hosts of generalists (Mann-Whitney $U = 285.0$, $P = 0.008$). Host weight and survival rate did not differ between hosts of specialists and hosts of generalists ($U = 216.0$, $P = 0.458$; $U = 109.0$, $P = 0.542$ for weight and survival, respectively) (Table 1).

Phylogenetic independence

Based on Abouheif's (1999) test, SES_{MPD} was phylogenetically conserved ($C = 0.290$, $P = 0.030$), indicating that closely related parasites tend to exhibit similar level of host specificity (Fig. 1), forcing us to incorporate the non-independence of data in a comparative analysis of host specificity and host traits.

Comparative analysis of host traits and parasite specialization

In the weighted multiple GLS regression, where SES_{MPD} was the dependent variable representing host specificity of the parasites and weighted host trait values (local abundance, apparent survival rate, and average body weight) were independent variables, we found four models with a delta AICc less than 2 from the best model (Table 2). The evidence ratio of the models not containing abundance (calculated by dividing the

probability, *w*, of the better model with the probability of the worse model) was between 2.3 and 1.7, indicating that empirical support for models incorporating only survival and body weight was about twice that of the model also incorporating abundance. None of the partial beta coefficients between host specificity and host traits were significant, however.

DISCUSSION

Specialization and resource predictability within the avian malaria parasite system

When comparing the means of the three host traits (local abundance, average body weight, and apparent survival rate) between hosts harboring generalists and hosts harboring specialists, we found a significant relationship between host specificity and host abundance in the predicted direction (Table 1). That is, hosts harboring specialist parasites were on average more abundant than hosts harboring generalists. It should be noted that we oversimplified the definition of a "host of specialists" and a "host of generalists": several hosts harbored both specialist and generalist parasite lineages but were considered only as hosts of specialists. Nonetheless, because we expected generalists to utilize any host species, irrespective of host traits, the significantly greater mean abundance of the hosts of specialists confirms that specialist parasites do not use the less abundant hosts within this assemblage, supporting our prediction that specialist parasites do not utilize unpredictable resources.

In the weighted generalized least squares multiple regression analysis, however, in which we investigated the relationship between host specificity of parasites and the average trait values of hosts of each parasite lineage, while controlling for parasite

phylogeny, we did not find any support for the hypothesis that malaria parasites specialize on what might be considered predictable resources. Local abundance, which seemed a likely determinant of host specificity after our initial assessment, factored into only one of four well-supported models (Table 2), and the partial beta coefficient of abundance and host specificity was far from significant (Table 3).

Several host species were represented more than once in the dataset, potentially causing pseudoreplication. Rarely, however, did two parasite lineages preferentially infect the same host species. Exceptions were P41L and H17L, both of which were recovered multiple times from *G. spirurus*, and H17L and P36, both of which infect *A. infuscatus* (although P36 was recovered as often from *Automolus melanopezus* [Furnariidae]). *G. spirurus* and *A. infuscatus* are overrepresented in the host-parasite interaction matrix, carrying 11% and 12% of infections included in our analysis, respectively. Removing these two species from the analysis did not alter the outcome. The influence of abundance found when comparing the mean trait values of hosts harboring specialists to the mean trait values of hosts harboring generalists was not observed in the multiple regression analysis, even when we did not control for phylogeny (results not shown). In other words, the absence of significant relationships between host specificity and host traits cannot be explained by the fact that host specificity is phylogenetically conserved among parasites: even when not considering phylogeny in a standard multiple regression analysis we failed to detect any significant relationships. One possibility is that the relationship between host specificity and host traits is not linear and that it is only relevant that the host is predictable based on one trait. Evidence for this in our dataset is the fact that 18 of 34 (53%) of the bird species harboring generalized

parasites but only 1 of 11 (9%) of the bird species harboring specialized parasites are not in the upper quartile of either of the three host traits.

Hellgren et al. (2009) demonstrated that in an avian malaria parasite system, generalist lineages also reach higher prevalence on their hosts compared to specialists. This suggests that adopting a generalist strategy does not incur fitness costs, contradicting a large body of work of on specialization tradeoffs (e.g. Dethier 1954; Elena and Sanjuán 2003; Futuyma and Moreno 1988; MacKenzie 1996), but might instead be an adaptive response to the frequent encounter of multiple host species (Hellgren et al. 2009). Thus, the fitness threshold described by Ward (1992), which represents the relative encounter frequency of preferred and non-preferred hosts that must be reached before the fitness of a parasite expanding its host range will exceed its fitness as a specialist, might be low and easily reached by avian malaria parasites.

Interspecific interactions among parasite lineages: an alternative hypothesis for specialization

The potential benefit of adopting a generalist strategy within the avian malaria parasite system and scarcity of significant relationships between host specificity and host traits bring us back to the question posed by Loiseau et al. (2012) of why specialist malaria parasites exist at all. Alternative explanations to the predictability hypothesis of ecological specialization are that organisms (1) facilitate coexistence on or (2) competitively exclude each other from resources (Futuyma and Moreno 1988). In both cases we might expect specialization to evolve. Facilitation would cause parasite lineages to aggregate on few bird individuals and competition would cause competing parasites

not to co-occur on the same bird individual. At the bird population level, facilitation may result in parasite lineages utilizing only a few host species with substantial overlap of lineages on such hosts. In addition, multiple infections within single individual hosts should be commonplace. We have little evidence for this in our assemblage: 63 bird species are utilized as hosts by collectively 45 parasite lineages and identified multiple infections were infrequent (9.4% of infections had double peaks in their chromatograms, indicative of the presence of at least two parasite lineages).

Malaria parasites can both facilitate coexistence (de Roode et al. 2004) and competitively exclude each other within individual hosts (Cellier-Holzem et al. 2010; de Roode et al. 2004; de Roode et al. 2005). The outcome of these interactions varies depending on the host genotype (de Roode et al. 2004), parasite virulence (de Roode et al. 2004, 2005), and whether parasites are coinfected (i.e., transferred from the same mosquito individual hosting two strains) or superinfected (i.e., transferred from different mosquito individuals with several days delay) (Cellier-Holzem et al. 2010; de Roode et al. 2005). If the more specialized lineages are also better competitors (as was the case in *Plasmodium chabaudi* strains infecting mice; de Roode et al. 2004, 2005), they might exclude generalist parasites from their host.

Within our local assemblage, we sampled two closely related host species: *F. colma* and *F. analis*. Of 34 sampled birds of *F. colma*, we identified 28 infections to parasite cyt *b* lineage. Twenty-seven were lineage P4L, a *Plasmodium* sp. found exclusively on *F. colma*. One, however, was H17L, found in 24 species of bird but preferentially using *A. infuscatus*. Of 24 sampled *F. analis* we identified 11 infections. Eight were lineage P2, which is sister lineage to *F. colma*'s P4L and found exclusively on

F. analis. The other three infections (H17L, P24L, and P41L) were also found on multiple other host species. Generalist lineages (in this case, P24L and P41L) are, hence, capable of infecting both *F. colma* and *F. analis*, but specialist lineages dominate the infections of these two bird species. Without experimentally infecting these birds with different malaria parasite lineages, or sampling assemblages in more localities throughout the western Amazon, we cannot know with certainty whether P4L and P2 competitively exclude other lineages, or each other; nonetheless, these anecdotal examples in combination with previous studies documenting competition of malaria parasite strains suggests that interspecific interactions are important determinants of host specificity within this system.

It is possible that all factors discussed here (traits of hosts rendering them more or less predictable as a resource base and interspecific interactions among parasite lineages) contribute to the evolution of malaria parasite specialization. Our inability to distinguish among the different factors that influence specificity might be a result of the multiple factors involved, as well as the complexity of outcomes of interspecific interactions.

Vector biology

A caveat in our study is our lack of knowledge of the vectors of avian malaria parasites and how they transmit parasites among host individuals of the same or different species. A generalist feeder (i.e., a mosquito that bites indiscriminately) might facilitate generalization of the parasites because the likelihood that an individual mosquito will consecutively bite members of the same bird species might be small compared to the likelihood that a second meal will be obtained from a bird individual of a different

species, particularly in an area with such high host diversity as the Amazon. A specialist feeder, however, will undoubtedly facilitate specialization of the parasite on the same host species. From studies on two ornithophilic mosquito species, *Qulex pipiens pallens* and *Qulex sasai*, it is apparent that mosquitoes that are competent vectors of avian malaria parasites feed on a variety of host species (Ejiri et al. 2011; Kim and Tsuda 2010; Kim et al. 2009a; Kim et al. 2009b). Although one host species appears to be favored in any one locality (in that it dominates the blood meals of mosquitoes), the identity of the dominant host species varies among localities within the same mosquito species (Ejiri et al. 2011; Kim and Tsuda 2010; Kim et al. 2009b). In our analyses, we assumed that vectors of the parasites have no innate preference for host species, but we recognize that more studies on vector feeding preferences and transmission potential would be required to confirm the validity of this assumption.

CONCLUSIONS

Our objective was to evaluate the hypothesis that avian malaria parasites specialize on hosts that may be considered more predictable as a resource base. When comparing the mean trait values of hosts harboring specialists to the mean trait values of hosts harboring generalists, we found support for the prediction that local abundance positively influences host specificity (i.e., the mean abundance of hosts of specialists was significantly greater than the mean abundance of hosts of generalists). However, host specificity was significantly phylogenetically conserved among parasites, and phylogenetic correction to reduce pseudoreplication made these relationships non-significant. It is possible that there is a non-linear relationship between host specificity and host traits; after all, 91% of the

host species harboring a specialist parasite were in the upper quartile of at least one of the traits investigated here. Finally, specialization on predictable resources might be attenuated by other factors such as competition and facilitation among parasite lineages.

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TABLES

Table 1. Mean and standard deviation of local abundance, body weight, and apparent survival rate of hosts harboring generalist and hosts harboring specialist malaria parasites. We considered as specialist parasites one with a significantly narrow host range according to SES_{MPD}.

	Abundance	Weight	Survival
N	45	45	31
Generalists			
Mean	184	23.7	0.68
SD	199	14.3	0.18
Specialists			
Mean	404	26.4	0.61
SD	282	15.1	0.14

Table 2. AICc score, delta AICc (Δ) from the best model, and probability (*w*) of the four best models (delta AICc ≤ 2), with the independent variables host body weight (W), local abundance (A), and apparent survival rate (S) that were averaged to produce weighted partial beta coefficients (Table 3). *N* = 19 parasite lineages.

	AICc	Δ	*w*
W	66.5	0	0.34
S + W	67.1	0.57	0.26
S	67.2	0.65	0.25
A + W	68.2	1.68	0.15

Table 3. Averaged (using multimodel inference of models with a delta AICc ≤ 2) partial beta coefficients, *Z*-scores, *P*-values, and 95% confidence intervals from our weighted multiple generalized least squares regression using SES_{MPD} as the dependent and host body weight, local abundance, and apparent survival rate as the independent variables. *N* = 19 parasite lineages.

	Beta	*Z*	*P*	95% CI
Intercept	-1.94	0.508	0.612	-9.41 – 5.54
Abundance	0.652	0.700	0.484	-1.17 – 2.48
Survival	-1.42	0.852	0.394	-4.68 – 1.84
Weight	2.07	1.00	0.271	-1.62 – 5.75

FIGURES

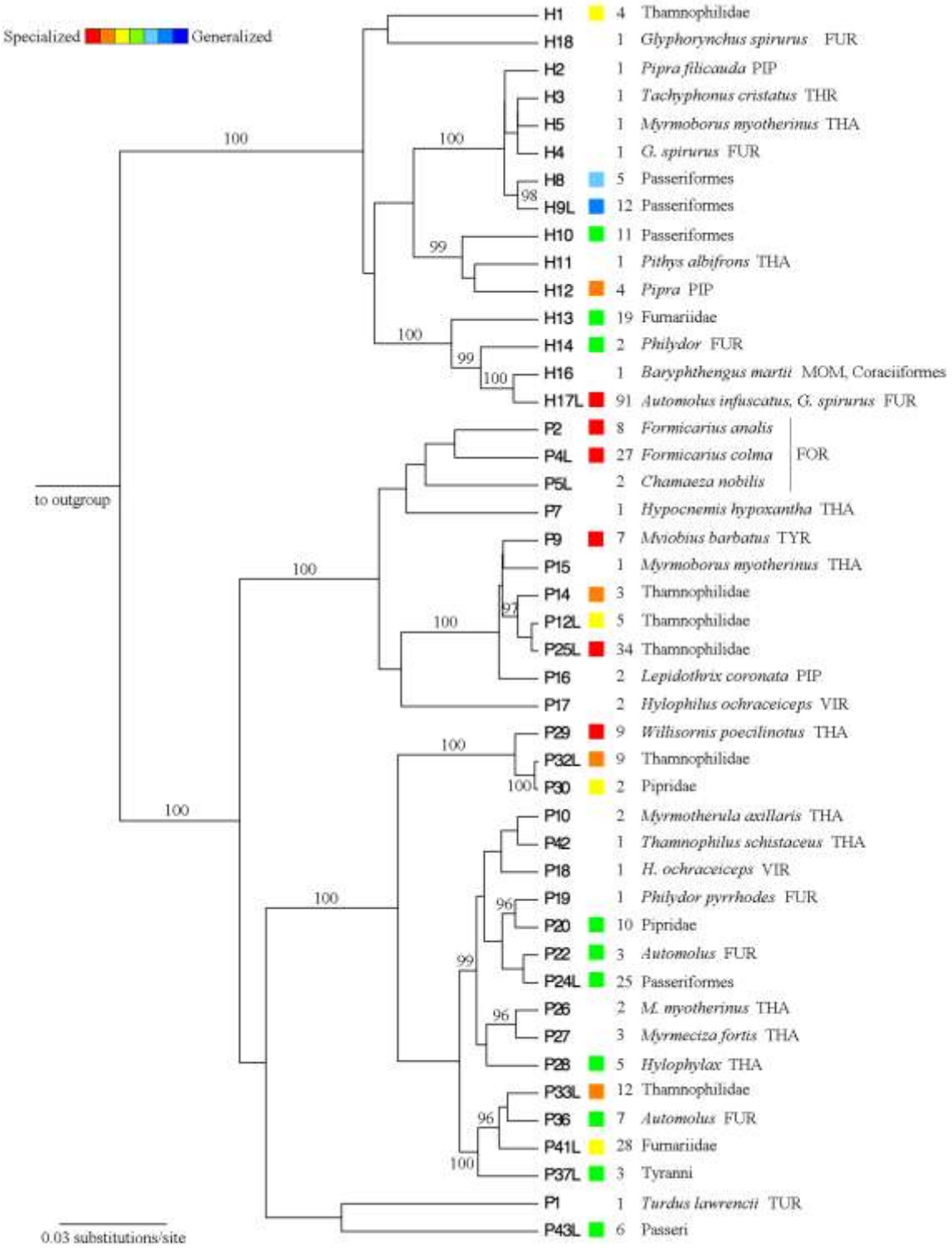
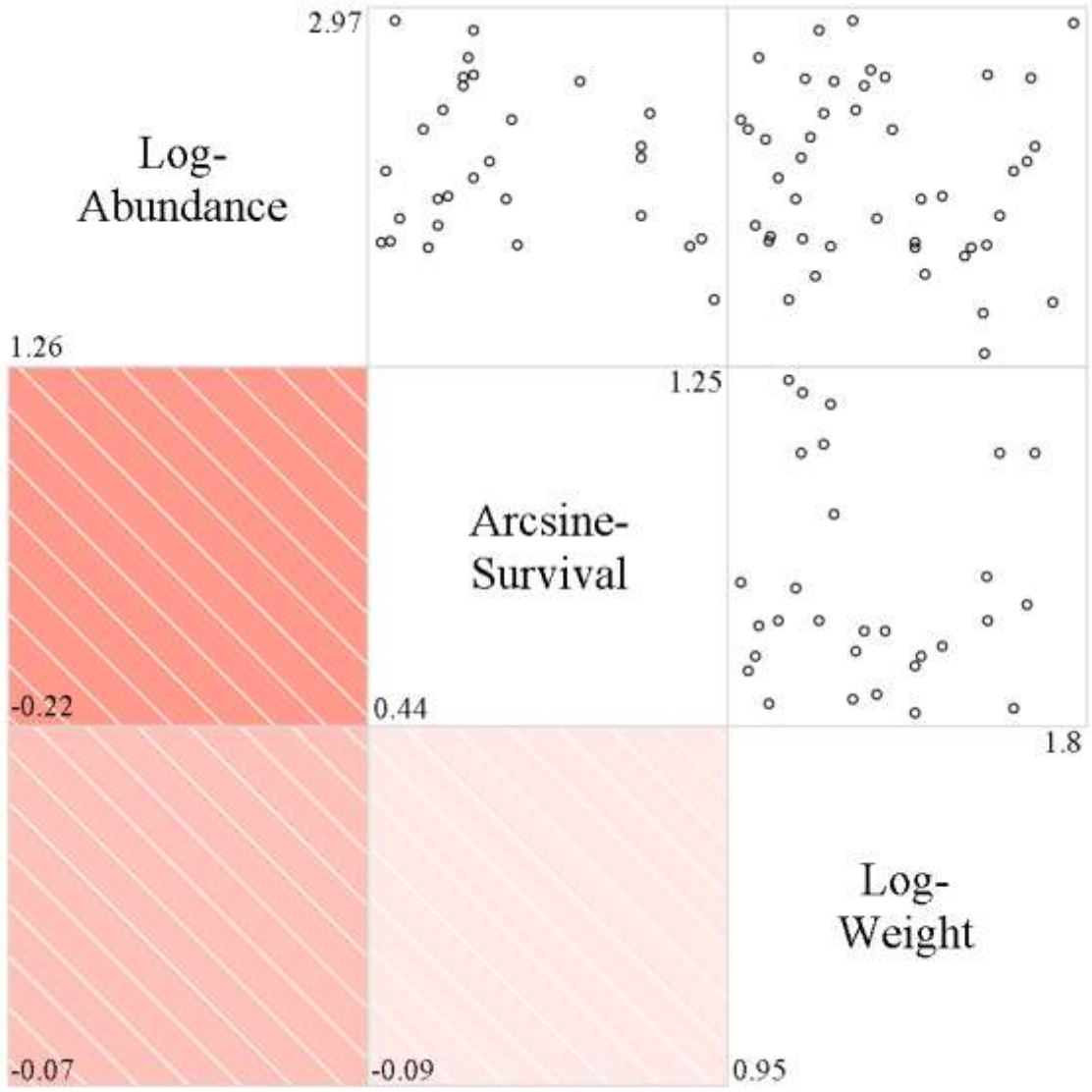


Figure 1. Ultrametric BEAST phylogeny of haemosporidian parasite lineages at the Tiputini Biodiversity Station, Ecuador. *Plasmodium* lineages are preceded by a "P" and

Haemoproteus by an "H." Posterior probabilities are shown for branches with 95% support or more. The color column represents level of host specificity according to SES_{MPD}. Bright red indicates significant specialists and dark blue significant generalists at the 0.05 alpha level. Orange and blue indicate significant specialists and generalists, respectively, at the 0.1 alpha level. Color codes are shown only for parasites included in analysis (i.e., with $N \geq 4$). The number of recoveries is shown after the color columns. Listed on the right are hosts from which a lineage was most often recovered (note: this is not an exclusive listing of hosts utilized, but greater detail of all host species used by each parasite lineage can be found in Figure 4 of Chapter 1). Each host species and genus are followed by a three-letter family code (FOR = Formicariidae, FUR = Furnariidae, MOM = Momotidae, PIP = Pipridae, THA = Thamnophilidae, THR = Thraupidae, TUR = Turdidae, TYR = Tyrannidae, VIR = Vireonidae).

Appendix A



Correlogram of body weight, apparent survival rate, and local abundance of birds sampled in the Tiputini Biodiversity Station, Ecuador. The range of values are shown along the diagonal (lowest in bottom left corner, highest in top right). Pearson's correlation coefficient is shown in the bottom left corner of the lower panel (none were significant).

Chapter 3: Reciprocal specialization in multi-host avian haemosporidia: a temperate – tropical comparison

Authors: Maria Svensson-Coelho[1,2], John G. Blake[1,3], Bette A. Loiselle[1,3], Vincenzo A. Ellis[1], Robert E. Ricklefs[1]

[1]Department of Biology, University of Missouri – St. Louis, One University Blvd., St. Louis, Missouri 63121-4499 USA

[2]E-mail: lmsd5d@umsl.edu

[3]Present address: Department of Wildlife Ecology and Conservation and Center for Latin American Studies, 110 Newins-Ziegler Hall, P.O. Box 110430, University of Florida, Gainesville, FL 32611-0430 USA

Abstract

The structure of two local assemblages of birds and their haemosporidian parasites was examined, one tropical and one temperate, to describe the complexity of these community interactions as a function of latitude. Assuming coevolution proceeds towards reciprocal specialization (*i.e.*, an exclusive association between only one bird species and one haemosporidian species), the level of reciprocal specialization should be greater in the tropics, where coevolution is hypothesized to be more important in species diversification. Network analysis in combination with null models was applied to test this. There was no evidence for increased levels of reciprocal specialization in the tropical site; instead, reciprocal specialization was greater in our temperate site. In

addition, when analyzing specialization from the parasite and host perspectives separately, and found that the increased specialization in the temperate site could be explained by the greater specialization from the host perspective. Specialization from the parasite perspective was not different between the two sites. Considering that the tropics consist of more closely- related bird species, we suspected that phylogenetic relationships might explain why specialization appears greater in the temperate site. Rerunning the network analysis for data sets comparing host genera and families, rather than host species, with parasite lineages, the difference in reciprocal specialization disappeared at the bird family level. These results suggest that phylogenetic relationships among birds are at least partly responsible for the greater specialization in the temperate assemblage. In none of our analyses did specialization appear greater in the tropical site. Thus, the increased bird and haemosporidian species diversities in the tropics compared to the temperate areas are unlikely the results of selective pressures imposed by these particular parasites and hosts, respectively.

Key words

Avian malaria, biotic interactions, community ecology, latitudinal gradient

Introduction

Increase in species richness towards the tropics is nearly ubiquitous among organisms (Hillebrand 2004), although biologists have not agreed on explanations for this pattern (Mittelbach *et al.* 2007). Dobzhansky (1950) and Fischer (1960) suggested that intense selection from biological interactions might increase rates of speciation in the tropics, while organisms living at high latitudes are constrained by adaptation to the physical environment (MacArthur 1955, 1969, 1972, May 1973).

One indication that biotic interactions might comprise a stronger selective force in tropical compared to temperate latitudes would be increasing frequency of interactions at lower latitudes (Schemske 2009; Schemske *et al.* 2009). Local coevolution of interacting species might lead to population divergence and allopatric speciation, tending to drive species richness upwards (Schemske 2009). Coevolution can be defined as "the process of reciprocal evolutionary change between interacting species driven by natural selection" (Thompson 2005, pg. 3). When species coevolve in different directions in different local assemblages (Turner and Mallet 1996), a geographic mosaic of coevolutionary outcomes might promote further diversification within a region (Thompson 2005). Increasing strength of biotic interactions at lower latitudes has been suggested for plant-herbivore (*e.g.* Morrow and Fox 1989, Pennings and Silliman 2005, Dyer *et al.* 2007) and plant-pollinator (*e.g.* Johnson and Steiner 2000) systems, but within these same systems, several studies have also contradicted this pattern (*e.g.* Beaver 1979, Novotny *et al.* 2002, Moles *et al.* 2011). To our knowledge, latitudinal differences in biotic interaction strengths in host-pathogen systems have not been quantified. Here, we

address this issue by comparing the distribution and prevalence of haemosporidian ("malaria") parasites within a temperate and a tropical assemblage of forest birds.

Coevolution has long been recognized as an important generator of biological diversity (Ehrlich and Raven 1964). The population and evolutionary consequences of coevolution, however, are notoriously difficult to evaluate, particularly when more than two species are involved (*e.g.* Nuismer and Thompson 2006) and when the traits that control the relationship among coevolving species are not known (Thompson 2005). Nonetheless, coevolution might lead to discernible patterns of association between species within local assemblages. In its simplest form, coevolution leads to reciprocal specialization between species (*i.e.*, one-to-one associations between two species to the exclusion of others present in the assemblage) (Thompson 2005, pg. 35). Such reciprocal specialization might be an expected outcome of host-parasite coevolution because many genes are involved in vertebrate host immunity and in the evasion of host immunity by parasites. Accordingly, interacting host and parasite species are likely to evolve under negative frequency-dependent selection of coevolving polymorphisms (Thompson 2005, 2009), which would result in strong interactions primarily between one host and one parasite species. The principle of allocation suggests, however, that when the abiotic environment exerts strong selection on adaptations to extreme conditions, organisms are constrained in their evolutionary response to other factors. Thus, in temperate and boreal climates, host species would be less able to mount defenses against parasites than similar host species in more benign tropical environments. If such constraints influenced parasite-host coevolution, one might expect to find more specialized parasites in the

tropics, where biotic interactions predominate, and less specialized parasites, as well as more parasites per host species, in communities at higher latitudes.

Our objective was to examine the structure of two local assemblages of birds and their haemosporidian parasites, one tropical and one temperate, to describe the complexity of these community interactions as a function of latitude. Within localities, parasites form infracommunities (within host individuals), component communities (within individual host species), and the compound community (within a geographical location, Esch *et al.* 1990b). Parasite community organization has been investigated primarily at the component community level, and to a lesser extent in the infracommunity (reviewed in Esch *et al.* 1990a, Simberloff and Moore 1997). Nonetheless, to the extent that parasite species interact with each other through the evolved defenses of their hosts, the full impact of evolutionary relationships in different regions can be appreciated only at the level of the compound community. Although we prefer the term "assemblage" for the species that co-occur at a point in space (Ricklefs 2008), we retain "community" in terms such as "compound community" to be consistent with older literature.

Avian haemosporidia—globally common, abundant, and diverse vector-borne protozoan parasites of birds—are well-suited to investigate differences in the organization of compound communities of parasites between tropical and temperate regions. Avian haemosporidia are globally distributed, and many parasite species span temperate and tropical latitudes, such that clades of avian haemosporidia are not strongly geographically structured (Ricklefs and Fallon 2002, Beadell *et al.* 2006, Svensson *et al.* 2007). Moreover, host breadth varies widely among haemosporidian species within assemblages, and so strengths of interactions plausibly might vary among regions.

Although we know little about the genetic basis of host-parasite interactions in this system, it is undoubtedly complex. For example, genetic variability in the human malaria-causing agent, *Plasmodium falciparum*, is substantial: *ca.* 60 genes are members of the erythrocyte membrane protein 1 (PfEMP1) family (Horrocks *et al.* 2005), variants of which are expressed on the surface of infected host erythrocytes to hide the infected cell from the host immune system. Human hosts present a genetically-based resistance, or immunity, to infection: the nucleotide database of the European Bioinformatics Institute (EBI; http://www.ebi.ac.uk/imgt/hla/stats.html) contains over 7000 named alleles of our leukocyte antigen complex (HLA, or major histocompatibility complex, MHC), which is responsible for recognizing non-self antigens.

A useful tool for exploring assemblage structure within a system of interacting hosts and their parasites is network analysis, which has been applied in studies characterizing patterns of several systems of interacting species (*e.g.* Proulx *et al.* 2005, Bascompte and Jordano 2007, Poulin 2010). Networks are composed of nodes connected to each other by links and can represent interactions within single populations (*e.g.* social networks) or entire food webs. Hosts and parasites can be viewed in two-mode, or bipartite networks, where one level represents hosts and the other parasites. Nodes are species and links connect host species with parasite species, if they interact. Quantitative networks incorporate the strength of those links; the more individuals of a particular host species are infected with a parasite species, the stronger the link connecting them (*e.g.* Bascompte *et al.* 2006). A number of indices provide insight to the organization of such bipartite networks (Vázquez and Aizen 2003, Dormann *et al.* 2008, Dormann *et al.* 2009).

Because we were interested in reciprocal specialization, and hypothesized greater levels of reciprocal specialization in the tropics, we focus on indices that incorporate reciprocal specialization. That is, we used indices that take into account whether a species has exclusive access to its partner or not. These are the network-wide specialization, H_2', and species-level specialization, d' (Blüthgen *et al.* 2006). In a matrix where host species are arranged in rows and parasite lineages are arranged in columns, each cell represents the number of times a given parasite lineage has been recovered from a given host species. The H_2' index estimates how close an interaction network is to having all observed interactions arranged along the diagonal, which would represent perfect reciprocal specialization. Values of H_2' close to zero indicate that the interactions are randomly distributed and reciprocal specialization low, whereas values close to one indicate that the network contains mostly cases of one-to-one interactions. The d' index estimates specialization of each species within the network but returns higher values for species that have exclusive access to their partner. By obtaining the average d' for hosts and parasites separately, we gain insight to whether reciprocal specialization is more pronounced from the host or the parasite perspective. Because of the much greater species diversity in the tropical site, especially of birds, we compared our observed values to random expectations by generating null models and used the magnitude of the deviation from the null as a statistic for comparing tropical and temperate assemblages.

Our aim was to determine whether reciprocal specialization is greater within a more diverse tropical assemblage of birds and their haemosporidian parasites than in a less diverse temperate assemblage. We chose to apply network analysis to address this

question because of its capacity to simultaneously incorporate both the host and parasite perspectives into a single statistic.

Methods

Community matrices. Two local assemblages of birds (primarily passerines) and their haemosporidian parasites (Apicomplexa: Haemosporida: *Plasmodium* spp. and *Haemoproteus* spp.) were compared: Tiputini, a tropical assemblage in the western Amazon (0°38´S, 76°08´W), and Ozarks, a temperate assemblage in southern Missouri (37°14´N, 90°58´W). We screened 2488 individual birds belonging to 104 species in Tiputini and 1206 individual birds belonging to 51 species in the Ozarks for haemosporidia using methods described in Ricklefs *et al.* (2005) and Chapter 1. We recovered 539 infections (21.7% prevalence) in Tiputini and 429 infections (35.6% prevalence) in the Ozarks. We assigned 379 and 284 individual parasite infections to cytochrome *b* lineage in Tiputini and Ozarks, respectively. Data from both 100-ha plots in Tiputini (Puma and Harpia) were combined with 22 additional haemosporidian infections, obtained locally outside the plots. Data from the Ozarks locality were described in Ricklefs *et al.* (2005) and combined with 124 additional infections from 2011. We used molecular and host association data in both assemblages to assign infections to putative evolutionary lineages of parasites. Methods and details for the Tiputini data can be found in Chapter 1, and details for Ozarks lineage assignments can be found in Appendix 1 of this study. Here we placed “TI” in front of each lineage number from Tiputini to distinguish these from “OZ” lineages and an “H” or a “P” in

front of each lineage name to distinguish between *Haemoproteus* spp. and *Plasmodium* spp.

The Tiputini data comprise a matrix of 45 parasite lineages and 63 host species (matrix size $M = 45 + 63 = 108$ with $n = 379$ interactions and 167 realized links [5.9% of 2835 possible]) and the Ozark data comprise a matrix of 37 parasite lineages and 28 host species ($M = 65$, n = 284 and 102 realized links [9.8% of 1036 possible], Fig. 1, Table 1). Five lineages (POZ04/PTI43, POZ01/PTI37, POZ06/PTI25, POZ09/PTI17, HOZ21L/HTI5) were shared between the two regions and are referred to by their Ozarks code here. Bird species names in Ozarks follow the International Ornithological Committee (IOC) World Bird Names v. 2.11 and bird species names in Tiputini follow the South American Association Classification Committee (8/02/2012), both accessed through AviBase (D. Lapage, 2012, <avibase.bsc-eoc.org>).

Ozarks contains mostly migratory oscine passerines (dominated by the families Parulidae and Vireonidae), most of which migrate to the Caribbean basin in winter. Transmission on the breeding ground has been verified by recovery of most parasite lineages in resident and/or juvenile birds (Ricklefs et al 2005). Tiputini contains mostly resident, sedentary suboscine passerines (dominated by the families Furnariidae and Thamnophilidae). Sampling was conducted over several years (5 and 9 for Ozarks and Tiputini, respectively). In neither locality was there any evidence that prevalence of individual parasite lineages varied temporally, other than when primary host species were sampled unequally among years (Chapter 1, Ricklefs *et al.* 2005). Thus, years were combined.

We performed two sets of analyses: one in which all data were included and one in which only species with a sample size of four or greater were included. The "full" dataset consists of false specialists, *i.e.*, those that are poorly sampled. However, Dormann (2009) demonstrated that these singletons have very little effect on network statistics. The "reduced" dataset should perhaps give a more accurate representation of specialists because poorly sampled species might appear more specialized than they are (Blüthgen *et al.* 2008).

Network analysis. Several network analysis indices, many of which are correlated, provide insight into biological assemblage organization (see the 'bipartite' package manual, Dormann *et al.* 2008, for a list) (Dormann *et al.* 2009). To obtain a statistic that incorporates reciprocal specialization we used the standardized two-dimensional Shannon index of entropy (H_2'). This index simultaneously incorporates the entire assemblage matrix (in which hosts are rows and parasites columns) into a statistic of network-wide specialization (Blüthgen *et al.* 2006). The statistic ranges from zero (homogeneous distribution of host-parasite interactions) to one (all interactions are cases of reciprocal specialization). An assemblage with greater H_2' values, thus, is one in which biotic interactions are stronger, assuming species coevolve towards reciprocal specialization. To explore whether specialization is greater in the tropics from both the host and parasite perspective in turn, we use the standardized Kullback-Leibler index of entropy, d', which returns a measure of specialization for each species (Blüthgen *et al.* 2006). The species-specific d'values can then be averaged among parasites and hosts separately. Because it incorporates not only the frequency of a species on all its associates, but also the

frequency of the focal species in relation to other species that interact with each associate, d', too, incorporates reciprocal specialization.

When two assemblages differ structurally (that is, they have different number of species and/or different network dimensions), the observed values produced by network analysis indices in different assemblages are not always directly comparable. For example, connectance (proportion of realized links) will always be lower in larger networks (Dunne *et al.* 2002). Thus, in order to compare the hyperdiverse tropics to the relatively depauperate temperate region, we used null models (Gotelli and Graves 1996) to estimate the expected pattern under random interactions (considering the size, dimensions, and connectance of the original matrix) and compare the standardized effect sizes (SES, equivalent to *Z*-scores) between the sites in place of the observed index values. All analyses were performed in the package *bipartite* (Dormann *et al.* 2008) in R (R Development Core Team 2011).

Network-wide specialization – H_2'. The H_2' index is given by $H_2' = \frac{H_{2\max} - H_2}{H_{2\max} - H_{2\min}}$, where $H_2 = -\sum_{i=1}^{r}\sum_{j=1}^{c}(p_{ij} \cdot \ln p_{ij})$ (two-dimensional Shannon entropy index for the observed data), $H_{2\max} = -\sum_{i=1}^{r}\sum_{j=1}^{c}(q_i q_j \cdot \ln q_i q_j)$ (two-dimensional Shannon entropy index for data that are homogeneous with respect to row and column totals, *i.e.*, incorporating the relative abundance of parasite lineages and infected hosts), *r* is the number of rows, *c* is the number of columns, p_{ij} is the proportion of an interaction (*i.e.*, a cell value) relative to the interactions of the whole matrix, and $q_i q_j$ is the expected value within a cell from a

homogeneous interaction matrix. The theoretical $H_{2\min}$ is zero, and represents the case where all interactions are distributed along the diagonal (*i.e.*, the extreme opposite of $H_{2\max}$, where all interactions are homogeneous). Because row and column totals must remain constant, however, the actual $H_{2\min}$ will be greater than zero and must be estimated using a heuristic algorithm (Blüthgen *et al.* 2006). $H_{2\min}$ can be viewed as the maximum possible network-wide specialization, given the observed number of interactions in our network. Although the Shannon index is an entropy index and thus produces values that vary from disordered to structured, disorder in an interaction network is the same as generalization at both levels (*i.e.*, all hosts associate with all parasite lineages) and extreme structure in an interaction network is the same as complete reciprocal specialization (*i.e.*, each host associates with only one other parasite, which in turn associates with only that one host). Throughout this paper we use the terms specialization and generalization in place of structured and disordered, respectively. H_2' ranges from zero (generalized) to one (specialized). Assuming coevolution proceeds towards reciprocal specialization within this host-parasite system, we would expect greater network-wide specialization (an H_2' closer to 1) in our tropical than in our temperate site.

Species-level specialization – *d′*. The d' index is given by $d_i' = \frac{d_i - d_{\min}}{d_{\max} - d_{\min}}$, where $d_i = \sum_{j=1}^{c} (p_{ij}' \cdot \ln \frac{p_{ij}'}{q_j})$ (the Kullback-Leibler divergence measure of relative entropy for

observed data) and $d_{\max} = \ln \frac{m}{A_i}$ (the Kullback-Leibler divergence measure of relative entropy for data that are homogeneous with respect to row and column totals), *c* is the number of columns (*e.g.*, number of host species), p_{ij}' is the proportion of the number of interactions in relation to the respective row total (*e.g.*, abundance of parasite lineage *i*, A_i), and q_j is the proportion of all interactions by partner *j* in relation to all interactions in the matrix (*M*). As in the case of $H_{2\min}$, $d_{\min}$ is estimated using a heuristic algorithm (Blüthgen *et al.* 2006). d' ranges from zero (generalized) to one (specialized) (Blüthgen *et al.* 2006). Unlike other species-level specialization indices, *e.g.* S_{TD} (Poulin and Mouillot 2003), d' incorporates non-realized links and exclusiveness of host access through the q_j term. Thus, strict species specialists will have the same S_{TD} value (zero) but might have different d' values depending on how exclusive the interaction is. In other words, d' does not view parasite (or host) species in complete isolation from other parasite (or host) species, as does S_{TD}. Again, if biotic interactions are stronger in the tropics, we would expect to find greater average species-level specialization (d' closer to 1) from both the host and parasite perspective.

Null models. Our networks are relatively small (*M* = 108 and 65 for Tiputini and Ozarks, respectively), and H_2' and d' might not return comparable values because of the different basic network properties (*e.g.*, connectance) between our tropical and temperate sites. Therefore, null models were simulated to control for the effect of network size, dimension, and connectance (Vázquez and Aizen 2003, Vázquez *et al.* 2007, Dormann *et al.* 2009), allowing us to estimate expected network properties for assemblages where

interactions are random (Blüthgen *et al.* 2008). We used the null model described in Vázquez *et al.* (2007), which is similar to the "swap" model in Dormann *et al.* (2009), who demonstrated that this model produces values closer to the observed than other models not retaining the original connectance (*i.e.*, "Patefield" model) or connectance and marginal totals (*i.e.*, "shuffle" model) (Dormann *et al.* 2009). We created 1000 null matrices (method *vaznull*) with the *nullmodel* command in *bipartite*. For each index, we plotted the density curve to verify normal distribution of our null models.

We tested whether our results differed from random by calculating standardized effect sizes (SES) using the *Z*-transformation $(Obs - \mu_{null}) / sd_{null}$, where *Obs* is the observed index value, μ_{null} is the mean of the null distribution based on our 1000 replicates, and sd_{null} is the standard deviation of our null distribution (Ulrich and Gotelli 2007, and references therein). An SES with a magnitude greater than |1.96| indicates significant difference at an alpha level of 0.05, and an SES with a magnitude greater than |2.58| represents significant difference at an alpha level of 0.01 (Whitlock and Schluter 2009). Because we have no replication of tropical and temperate local assemblages, we could not statistically test for significant differences between Ozarks and Tiputini, but instead we compared the magnitudes of the SES obtained in each site. A difference greater than 1.96 or lower than –1.96 indicates that a given index value differs between the two sites, lending preliminary evidence to there being a difference between the sites. Consequently, if biotic interactions are stronger in the tropics, we would expect to find a greater deviation from random in the positive direction in the tropical assemblage compared to the temperate. In other words, subtracting the SES of the Ozarks assemblage

from the SES of the Tiputini assemblage, we would expect a value exceeding 1.96 ($SES_{Tiputini} - SES_{Ozarks} > 1.96$).

Sampling size effect on species level indices. Poorly sampled species might appear more specialized than they are (Poulin 2007, Blüthgen *et al.* 2008). Here, we plotted d' as a function of sample size of parasite lineages and host species and tested for significant pairwise associations using Pearson's correlation to determine whether d' is significantly correlated with sampling size.

Results

General characteristics of assemblages. Associations between birds and haemosporidian parasites appear similar in both sites (Fig. 1). Two "generalist" host species (*Glyphorynchus spirurus* and *Automolus infuscatus* [both Furnariidae] in the tropics and *Vireo olivaceus* [Vireonidae] and *Ictera virens* [Parulidae] in the temperate) and two to three generalist parasite species (*Haemoproteus* sp. HTI17, *Plasmodium* sp. OZ06, and *Plasmodium* sp. OZ01 in the tropics and *Haemoproteus* sp. HOZ10 and *Plasmodium* sp. POZ04 in the temperate) dominate in each site. The tropical site contains five strict host species specialized parasites (PTI04, PTI29 [although once also recorded in *Catharus ustulatus*, Turdidae], PTI20, PTI02, and PTI09, all *Plasmodium* sp.) whereas the temperate site contains only two such specialist parasites (HOZ05 and HOZ49, both *Haemoproteus* sp.). From the host perspective, three tropical host species harbor only one parasite species (*Xiphorhynchus spixii*, Furnariidae, [POZ01], *Myrmeciza fortis*, Thamnophilidae, [POZ06], and *Myiobius barbatus*, Tyrannidae, [PTI09]), and

Formicarius colma (Formicariidae) is nearly "specialized" on one parasite species (PTI04) bar one single host individual which harbored HTI17. In the temperate site, only one host species harbored only one parasite species (*Spizella passerina,* Emberizidae, [HOZ49]). Cases of perfect reciprocal specialization are rare in both sites (one in each site: *M. barbatus* – PTI09 in the tropical site and *S. passerina* – HOZ49 in the temperate site, although *F. colma* – PTI04 in Tiputini is nearly reciprocal). In both sites, associations appear highly complex with frequent species sharing from both host and parasite perspective (Fig. 1).

Network analysis and null model comparison. The mean H_2' of the 1000 simulated random networks, in which size, dimension, and connectance of the original networks were retained, was 0.32 and 0.22 in Tiputini and Ozarks, respectively. The mean *d'* of parasites was 0.43 and 0.27 and the mean *d'* of hosts was 0.33 and 0.25 in Tiputini and Ozarks, respectively. Thus, the Tiputini assemblage is expected to exhibit somewhat greater level of specialization because of its basic network properties. In all instances (tropical, temperate, full data set, restricted data set), observed properties of the bird – haemosporidian parasite networks differed significantly from random expectations (Table 2). Thus, in both the tropical and temperate site, despite there being few cases of perfect reciprocal specialization, network-wide specialization (H_2') is much greater than would be expected by chance, and the average species-level specialization (d') is also much greater than would be expected by chance, from both the bird and parasite perspective.

Species-level specialization (d') did not correlate with sample size of either host or parasite in either site (Tiputini parasites $r = 0.04$, $P = 0.82$; Tiputini hosts $r = 0.16$, $P =$

0.22; Ozarks parasites r = -0.10, P = 0.56; Ozarks hosts r = 0.17, P = 0.38). d' of parasites range from 0.09 in HOZ17 (n=7) to 1.0 in HOZ49 (n=4) in Ozarks (mean = 0.50 ± 0.27 standard deviation; 0.50 ± 0.28 for data set with n ≥ 4), and from 0.28 in PTI22 (n=3) to 1.0 in PTI5 (n=2) and PTI9 (n=7) in Tiputini (0.63 ± 0.23; 0.59 ± 0.25) (Table 1, Appendix 2). Species-level specialization of hosts range from 0.15 in *Pipilo erythrophthalmus* (Emberizidae, n=2) to 1.0 in *S. passerina* (n=4) in Ozarks (0.41 ± 0.24; 0.42 ± 0.22), and from zero in *Dichrozona cincta* (Thamnophilidae, n=2) and *Microcerculus marginatus* (Troglodytidae, n=2) to 1 in *Chamaeza nobilis* (Formicariidae, n=2) and *Myiobius barbatus* (Tyrannidae, n=7) in Tiputini (0.41 ± 0.24; 0.44 ± 0.23) (Table 1, Appendix 2). Again, a d' of 1.0 indicates that a parasite (or host) species specialize on a host (or parasite) species, which it shares with no other parasite (or host) species. A d' of zero indicates that a parasite (or host) species is an extreme generalist, and that it associates only with host (or parasite) species that are also extremely generalized.

Differences between the temperate and tropical assemblages. Although replicates are not available at this stage, based on the magnitude of the difference in SES between one tropical and one temperate site, there is greater network-wide specialization and greater average specialization on parasites from the bird perspective in the temperate site than in the tropical site. Meanwhile, there is no difference in average specialization on hosts from the parasite perspective (Table 2).

Species level specialization differences between the temperate and tropical assemblages. Five parasite species were recovered in both localities, two of which (POZ04 and POZ06) had a sample size of six or more in each site, giving us the opportunity to investigate whether the same parasite species is more specialized in the tropics than the temperate. No host species occurred in both sites. When comparing individual d' values to the mean d' of the null matrices, POZ06 is significantly more specialized than expected in both sites but POZ04 is significantly more specialized than expected only in Tiputini. In the Ozarks, the host distribution of POZ04 does not differ from random. Subtracting the SES of the Ozarks from the SES of Tiputini, we found that POZ04 seems more specialized in Tiputini than Ozarks, and POZ06 seems more specialized in Ozarks than Tiputini (Table 3).

Discussion

Organization of avian haemosporidian assemblages – spatially conserved properties. This study is a first attempt at describing patterns in the compound community of the avian haemosporidian system and to address the hypothesis that biotic interactions are stronger at lower latitudes using quantitative network analysis indices. Immediately evident is the much greater host species richness and somewhat greater parasite lineage richness in our tropical site (Fig. 1), concordant with the elevated tropical richness of most biological organisms (Hillebrand 2004). This elevated richness in our tropical site required us to control for connectance, which is highly correlated with species richness (Dunne *et al.* 2002, Olesen and Jordano 2002, Ollerton and Cranmer 2002). We did this using null models that retain the original connectance in random matrices (Vázquez *et al.*

2007). The use of null models allowed us to estimate how specialized an assemblage is beyond what one would expect under random linking of coexisting species (Vázquez and Aizen 2003, Vázquez *et al.* 2005, Vázquez *et al.* 2007, Blüthgen *et al.* 2008).

The consistent deviations from null expectations in both assemblages in the positive direction (Table 2) suggest that birds and their haemosporidian parasites exhibit high levels of exclusiveness that cannot be explained by unequal sampling of species, but rather is likely the consequence of coadaptation and trait matching (Blüthgen *et al.* 2008). Trait matching in the avian haemosporidian system most likely involves immune defense (host) and immune evasion (parasite) proteins. Genetic analyses of these proteins might help evaluate whether trait matching is indeed responsible for the seemingly high level of local coadaptation in avian haemosporidia (see Zangerl and Berenbaum 2003 for a test of phenotype matching of an herbivore and its host plant).

Differences between the tropical and temperate sites in strengths of biotic interactions. Based on a comparison of two well-sampled assemblages separated by nearly 40° in latitude, we obtained results opposite to our prediction: reciprocal specialization is greater in the temperate than in the tropical site (Table 2). Interestingly, because specialization from the parasite perspective is the same in both sites (SES of average d' = 5.58 and 5.56 in Tiputini and Ozarks, respectively; Table 2), the tendency towards greater reciprocal specialization in the temperate site can be explained by the greater specificity from the host perspective (SES of average d' = 3.16 and 5.81 in Tiputini and Ozarks, respectively). Specificity from the host perspective is equivalent to per host (species) parasite diversity. Thus, our results imply that the average host species

in the tropical site harbors a more diverse parasite assemblage than the average host species in the temperate site, even after taking into account that there are more parasite lineages in the tropical site.

More studies have investigated specificity from the enemy (herbivore or pathogen) perspective than from the host perspective across latitudes, and there is little consensus about whether specialization is greater in the tropics. Whereas greater specificity towards lower latitudes has been documented in some herbivores (Dyer *et al.* 2007) and parasites (Rohde 1978, Krasnov *et al.* 2008), others have found no difference in specificity of herbivores (Novotny *et al.* 2006) and other parasite taxa (Rohde 1978), and still others have found the reversed pattern in herbivores (Beaver 1979), namely greater specificity in temperate regions. A meta-analysis of resource niche breadth along a latitudinal gradient in various taxa also demonstrated that decreased niche breadth (equivalent to high specialization) at lower latitudes is far from universal (Vázquez and Stevens 2004).

In our study, a possible explanation for the similar level of specificity of haemosporidian parasites between a tropical and a temperate site might lie in the phylogenetic relationships of the birds. The tropical region contains more congeneric bird species than does the temperate region. The same is true for mammals, and Krasnov *et al.* (2008) found that whereas host specificity of fleas on small mammals was unrelated to latitude when they treated host species as independent observations, they did recover the predicted negative relationship between latitude and host specificity when they incorporated the phylogenetic relationships of the hosts (*i.e.*, at lower latitudes their index of host specificity was lower, indicating greater host specificity). Most plant groups are

also more species rich at lower latitudes, and Novotny *et al.* (2002) found that when they analyzed specificity of herbivorous insects at the plant-genus level instead of the plant-species level in a tropical site, host specificity increased. That is, a high number of what would be considered generalists on plant species were considered specialists on plant genera. Despite this increase in specificity at the plant genus level, Novotny *et al.* (2006) failed to recover the predicted negative relationship between latitude and host specificity within the same system (*i.e.* in Lepidoptera, Hymenoptera, Coleoptera, and orthopteroid herbivores).

In vertebrates, close relatives presumably have more similar immune systems than distant relatives, which likely explains why multihost pathogens of primates are shared among close relatives more often than among distant relatives (Davies and Pedersen 2008). Thus, single haemosporidian parasite lineages might be able to infect multiple bird species if they are close relatives. Because of the greater number of closely related birds in the tropics, the average specialization as viewed from the parasite perspective might in fact increase in the tropics if the phylogenetic relationships of hosts are taken into consideration. In the tropical site, eleven genera contained more than one species of bird: one in the Formicariidae (*Formicarius* [2 species], three in the Furnariidae (*Automolus* [3], *Philydor* [3], *Xiphorhynchus* [3]), one in the Pipridae (*Pipra* [3]), five in the Thamnophilidae (*Epinecrophylla* [2], *Hypocnemis* [2], *Myrmotherula* [3], *Thamnomanes* [2], *Thamnophilus* [2]), and one in the Turdidae (*Turdus* [2]). In all, 63 bird species harboring haemosporidia in Tiputini belonged to 47 bird genera. In the Ozarks, only three genera contained more than one species of bird: one in Parulidae (*Setophaga* [7]), one *incertae sedis* close to Thraupidae (*Piranga* [2]), and one in Vireonidae (*Vireo* [3]). In

all, 28 bird species in the Ozarks belonged to 19 bird genera. Similarly, we can step up to the family level and find 13 families in the tropics and 12 families in the temperate site (Table 4). Only 10 of 28, and 10 of 27 parasite lineages infected hosts within the same genus in Tiputini and Ozarks, respectively; and these were usually lineages also infecting multiple other (non-congeneric) host species (8 of 10 and 7 of 10 were genus generalists in Tiputini and Ozarks, respectively). Furthermore, when combining parasite lineage information for bird genera and bird families and rerunning the network analysis we still failed to detect the predicted higher level of specialization from the parasite perspective in the tropical site (Table 5, 6).

Similarly, the greater specificity from the bird perspective in the temperate site could be a consequence of phylogenetic relationships among parasites. If the immune system of the birds cannot distinguish between closely related parasites, one would expect greater per-host parasite diversity in an assemblage where there are more closely related parasites. However, parasite lineages from the temperate and tropical sites are intermingled in a phylogenetic tree, and those clades consisting of only Tiputini lineages do not appear more lineage rich or to have shorter branches than those clades consisting of only Ozarks lineages (Fig. 2). We did find, however, that the difference in specialization from the host perspective disappeared when we compared association matrices of bird families and parasite lineages (Table 2, 6), suggesting that phylogenetic relationships among the hosts themselves to some extent determines how many parasite lineages the average host species will harbor. In other words, although single bird species on average harbor more parasite lineages in the tropics than in the temperate site, as families, birds harbor on average equally many parasite lineages in the two sites. Thus,

the observation that the average tropical bird species exhibits greater parasite diversity than the average temperate bird species, but that parasite diversity between the average tropical and temperate bird family does not differ, may be a result of more sharing of parasite lineages among hosts within the same family. This would be consistent with the hypothesis that increased host species richness within groups (*e.g.* within a phylum, a class, or a family) leads to increased parasite richness per host (Combes 2001). This has also been documented in, for example, monogenean and copepod parasites of marine fishes (Caro *et al.* 1997, Raibaut *et al.* 1998).

Conclusions. Based on a comparison of two local assemblages of birds and their haemosporidian parasites, we found no support for the hypothesis that biotic interactions are stronger in the tropics, therefore producing greater specialization. Thus, the increased bird and haemosporidian species diversities in the tropics compared to the temperate are unlikely the results of selective pressures imposed by these particular parasites and hosts, respectively. This does not mean that specialization within this system is generally low: it is not. Rather, the avian haemosporidian system is relatively specialized, much more so than expected by chance. We can only conclude—with caution because we compare only one assemblage in the temperate to only one assemblage in the tropics—that within the avian haemosporidian system, coadaptation is equally prominent in tropical and temperate regions.

We found some evidence that specificity from the bird perspective, that is, within-host-species parasite diversity, is higher (*i.e.*, parasite diversity lower) in the temperate than in the tropical site, which contradicted our prediction that specialization should be

greater in the tropics. This difference, however, disappeared at the bird family level, suggesting that the incorporation of phylogenetic information, which would control for the fact that the tropics harbors more closely related hosts, would result in equal levels of specialization in the tropical and the temperate site. More studies using network analysis on replicated assemblages within regions, preferably incorporating phylogenetic relationships of both birds and parasites, would be valuable in testing the generality of our results.

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TABLES

Table 1. Network properties of a tropical (Tiputini) and temperate (Ozarks) local assemblage of birds and haemosporidian parasites. Dimensions are the total number of species in the network, interactions is the sum of cell values in the matrix, connectance is the proportion realized links, H_2' is network-wide specialization, d'_P and d'_H are mean values, accompanied with standard deviations (s.d.) of species-level specialization from the parasite and host perspectives, respectively.

	Dimensions	Interactions	Connectance	H_2'	d'_P	d'_P(s.d.)	d'_H	d'_H (s.d.)
Tiputini	108 (45 x 63)	379	0.059	0.54	0.60	0.24	0.45	0.24
Ozarks	65 (37 x 28)	284	0.098	0.60	0.49	0.26	0.55	0.19
N ≥ 4								
Tiputini	37 (17 x 20)	257	0.194	0.63	0.60	0.22	0.50	0.21
Ozarks	31 (16 x 15)	234	0.250	0.64	0.49	0.25	0.54	0.20

Table 2. Standardized effect sizes (SES) of observed values in Table 1 and the mean of 1000 random matrices (null model). The difference is calculated by $SES_{Tiputini} - SES_{Ozarks}$, since we expected greater deviations from null models in the tropics. SES of |1.96| is significant at the 0.05 level (*), |2.58| at the 0.01 level (**), and |3.29| at the 0.001 level (***).

	H_2'	d'_P	d'_H
Tiputini	9.66***	5.58***	3.16**
Ozarks	11.63***	5.56***	5.81***
$SES_{Tiputini} - SES_{Ozarks}$	-1.96*	0.02	-2.64**
$N \geq 4$			
Tiputini	8.60***	7.38***	5.24***
Ozarks	14.05***	7.31***	8.51***
$SES_{Tiputini} - SES_{Ozarks}$	-5.45***	0.07	-3.27**

Table 3. *d'* values of parasite lineages recovered at least six times in both sites. Asterisks indicate significant deviation from the mean *d'* of the 1000 simulated random networks at the 0.05 (*), 0.01 (**), and 0.001 (***) level of significance.

Lineage	Tiputini	Ozarks	N (T / O)	$SES_{Tiputini} - SES_{Ozarks}$
POZ04	0.81**	0.24	6 / 6	14.72***
POZ06	0.50*	0.56**	34 /13	-5.44***

Table 4. Bird families with haemosporidian infections in the Tiputini and Ozarks, and the number of bird genera, bird species, parasite infections, and parasite lineages. Two species in the Ozarks are currently listed as *incertae sedis* (*i.s.*), close to Thraupidae.

	Tiputini				Ozarks			
Family	Genus	Species	Infections	Lineages	Genus	Species	Infections	Lineages
Momotidae	1	1	2	2				
Cardinalidae	1	1	1	1	2	2	22	7
Emberizidae					2	2	6	3
Fringillidae					1	1	1	1
Icteridae					1	1	2	2
Paridae					1	1	6	4
Parulidae	1	1	3	3	8	12	106	20
Polioptilidae					1	1	1	1
i.s. (Thraupidae)					1	2	18	5
Thraupidae	4	4	7	4				
Troglodytidae	2	2	4	3	1	1	6	5
Turdidae	2	3	5	4	1	1	2	2
Vireonidae	1	1	5	4	1	3	113	19
Formicariidae	3	4	43	7				
Furnariidae	9	15	137	16				
Pipridae	3	5	32	9				
Rhinocryptidae	1	1	1	1				

Thamnophilidae	16	22	128	24				
Tyrannidae	3	3	11	4	1	1	1	1

Table 5. Standardized effect sizes (SES) of observed values of H_2' and average *d'* of host genus – parasite lineage association matrices and the mean of 1000 random matrices (null model). The difference is calculated by $SES_{Tiputini} - SES_{Ozarks}$, since we expected greater deviations from null models in the tropics. SES of |1.96| is significant at the 0.05 level (*), |2.58| at the 0.01 level (**), and |3.29| at the 0.001 level (***).

	H_2'	d'_P	d'_H
Tiputini	8.77***	4.99***	2.98**
Ozarks	11.06***	4.48***	5.72***
$SES_{Tiputini} - SES_{Ozarks}$	-3.50***	0.57	-2.92**

Table 6. Standardized effect sizes (SES) of observed values of H_2' and average *d'* of host family – parasite lineage association matrices and the mean of 1000 random matrices (null model). The difference is calculated by $SES_{Tiputini} - SES_{Ozarks}$, since we expected greater deviations from null models in the tropics. SES of |1.96| is significant at the 0.05 level (*), |2.58| at the 0.01 level (**), and |3.29| at the 0.001 level (***).

	H_2'	d'_P	d'_H
Tiputini	10.23***	4.56***	3.20**
Ozarks	9.23***	3.80***	4.30***
$SES_{Tiputini} - SES_{Ozarks}$	1.01	0.75	-1.10

FIGURES

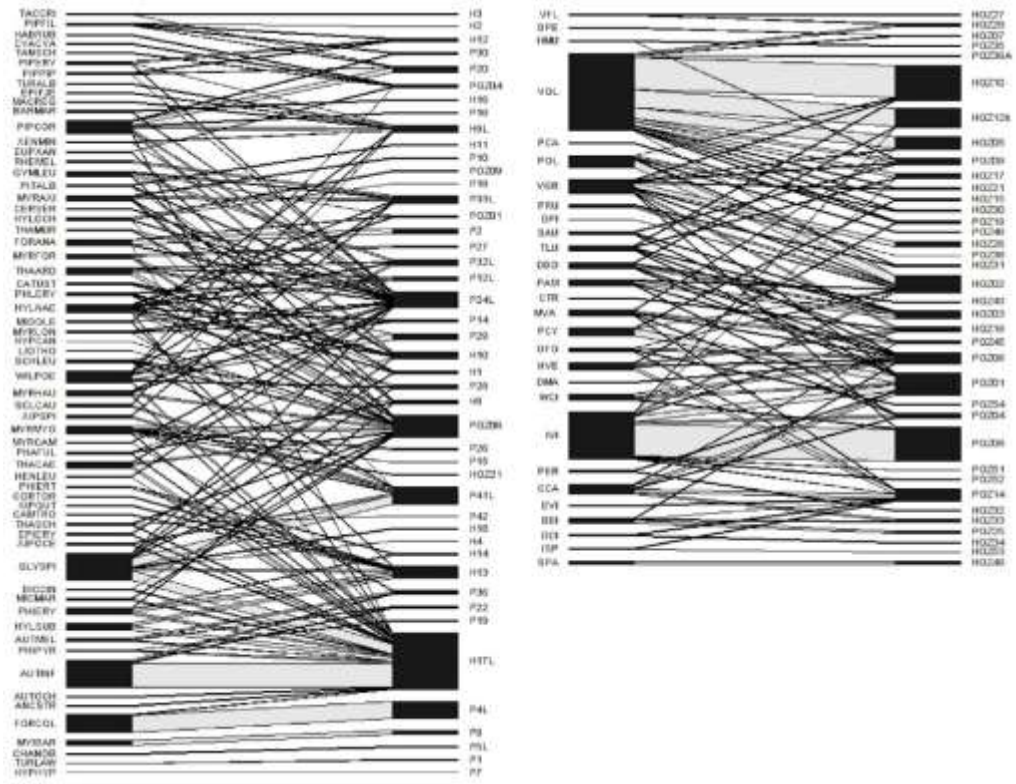


Figure 1. Networks of bird species (left nodes) and haemosporidian parasite lineages (right nodes) in a tropical site (left) and a temperate site (right). The thickness of links is proportional to abundance within each diagram.

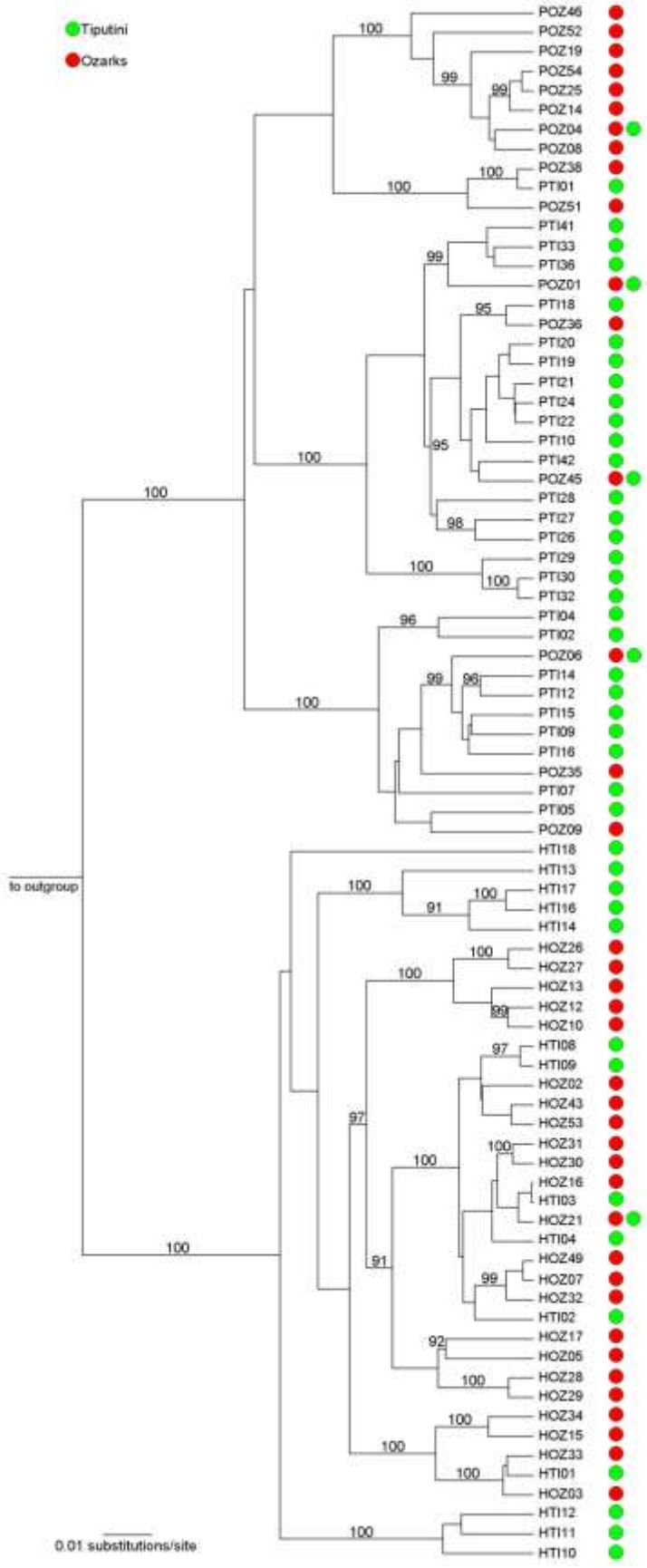

Tiputini
Ozarks
POZ46
POZ52
POZ19
POZ54
POZ25
POZ14
POZ04
POZ08
POZ38
PTI01
POZ51
PTI41
PTI33
PTI36
POZ01
PTI18
POZ36
PTI20
PTI19
PTI21
PTI24
PTI22
PTI10
PTI42
POZ45
PTI28
PTI27
PTI26
PTI29
PTI30
PTI32
PTI04
PTI02
POZ06
PTI14
PTI12
PTI15
PTI09
PTI16
POZ35
PTI07
PTI05
POZ09
HTI18
HTI13
HTI17
HTI16
HTI14
HOZ26
HOZ27
HOZ13
HOZ12
HOZ10
HTI08
HTI09
HOZ02
HOZ43
HOZ53
HOZ31
HOZ30
HOZ16
HTI03
HOZ21
HTI04
HOZ49
HOZ07
HOZ32
HTI02
HOZ17
HOZ05
HOZ28
HOZ29
HOZ34
HOZ15
HOZ33
HTI01
HOZ03
HTI12
HTI11
HTI10
to outgroup
0.01 substitutions/site

Figure 2. Ultrametric phylogeny generated in BEAST (Drummond and Rambaut 2007) of avian haemosporidian cytochrome *b* lineages from a temperate site (Ozarks) and a tropical site (Tiputini). Posterior probabilities > 90 are shown on branches. Methods for generating this phylogeny were described in Chapter 1.

Appendix 1.

We used a combination of haplotype clustering and host distribution to delineate potential evolutionary lineages following methods outlined in Chapter 1. Six clusters were not subjected to the MJ procedure because they contained only 2 or 3 haplotypes each. Of these clusters, four consisted of haplotypes that did not segregate into separate host species. Thus, we grouped the haplotypes HOZ12MSC1 with **HOZ33** into one lineage because both were recovered from *Cardinalis cardinalis*; HOZ12MSC2, HOZ12MSC3, and **HOZ05** into one lineage because all three were recovered from *Vireo olivaceus*; **HOZ28** and HOZ29 because they were poorly sampled (n=2 and 1) and found in the same host genus (*Vireo*); and **POZ06** and POZ12MSC5 into one lineage because they were both found in *Geothlypis fomosa*. Of the other two clusters, either (1) at least one haplotype was well sampled and no host species sharing detected or (2) although poorly sampled, the haplotypes were recovered from different host families, leading us to consider these separate lineages for now. POZMSC8 (renamed **POZ51**), found in one IVI was thus kept separate from **POZ38,** found in one *Vireo griseus* (i.e. different families) and **HOZ26**, found in 6 *V. griseus*, from **HOZ27,** found in one *Vireo flavifrons* (i.e., well-sampled HOZ26 with no species overlap). POZMSC9 was renamed **POZ52**. Bold-type signifies retained names.

All figures are color-coded as follows:

White – non-shared families

Grey – non-shared species within families that are shared

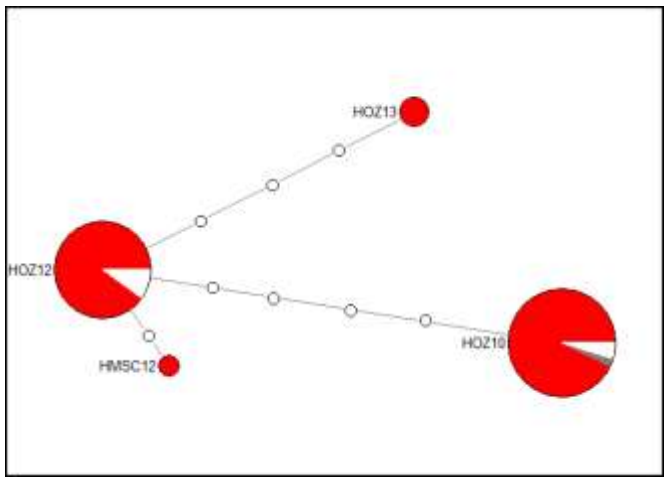
Color – shared species

Bars or circles along lines are mutations

Host species are coded as follows:

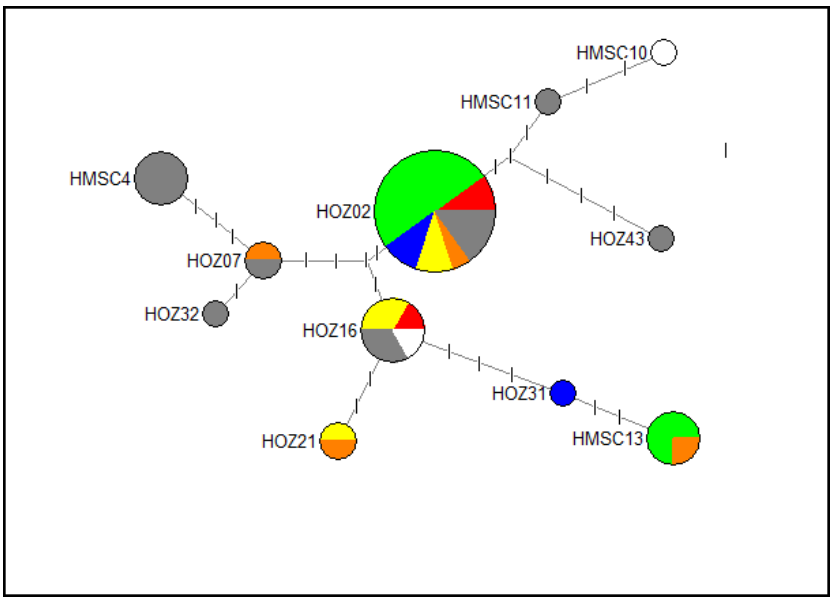
Species code	Family	Species
BBI	Paridae	*Baeolophus bicolor*
CCA	Cardinalidae	*Cardinalis cardinalis*
CTR	Fringillidae	*Carduelis tristis*
DDI	Parulidae	*Setophaga discolor*
DDO	Parulidae	*Setophaga dominica*
DPE	Parulidae	*Setophaga petechia*
DPI	Parulidae	*Setophaga pinus*
EVI	Tyrannidae	*Empidonax virescens*
HVE	Parulidae	*Helmitheros vermivorus*
ISP	Icteridae	*Icterus spurious*
IVI	Parulidae	*Icteria virens*
MVA	Parulidae	*Mniotilta varia*
OFO	Parulidae	*Geothlypis formosa*
PAM	Parulidae	*Setophaga americana*
PCY	Cardinalidae	*Passerina cyanea*
PER	Passerellidae	*Pipilo erythrophthalmus*
POL	Cardinalidae	*Piranga olivacea*
PRU	Cardinalidae	*Summer Tanager*
SPA	Emberizidae	*Spizella passerina*
TLU	Troglodytidae	*Thryothorus ludovicianus*
VAL	Vireonidae	*Vireo altiloquus*

VFL	Vireonidae	*Vireo flavifrons*
VGR	Vireonidae	*Vireo griseus*
VOL	Vireonidae	*Vireo olivaceus*
WCI	Parulidae	*Wilsonia citrina*



Family	Species	HOZ10	HOZ12	HOZ12MSC12	HOZ13
Parulidae	DDO		1		
Parulidae	IVI		1		
Thraupidae	POL	1			
Troglodytidae	TLU	1			
Vireonidae	VGR	1			
Vireonidae	VOL	39	18	1	2

Figure 1 (Haem1). OZ12 has been recovered from many VAL in the West Indies whereas OZ10 is almost exclusively from VOL and only in Missouri; only 3 of 25 OZ12 are from Missouri. Thus, we merged OZ12, OZ13, and HMS12 but kept OZ10 separate. 2 lineages: **HOZ10** and **HOZ12** (including HMSC12 and HOZ13).


HMSC10
HMSC11
HMSC4
HOZ02
HOZ43
HOZ07
HOZ32
HOZ16
HOZ31
HOZ21
HMSC13

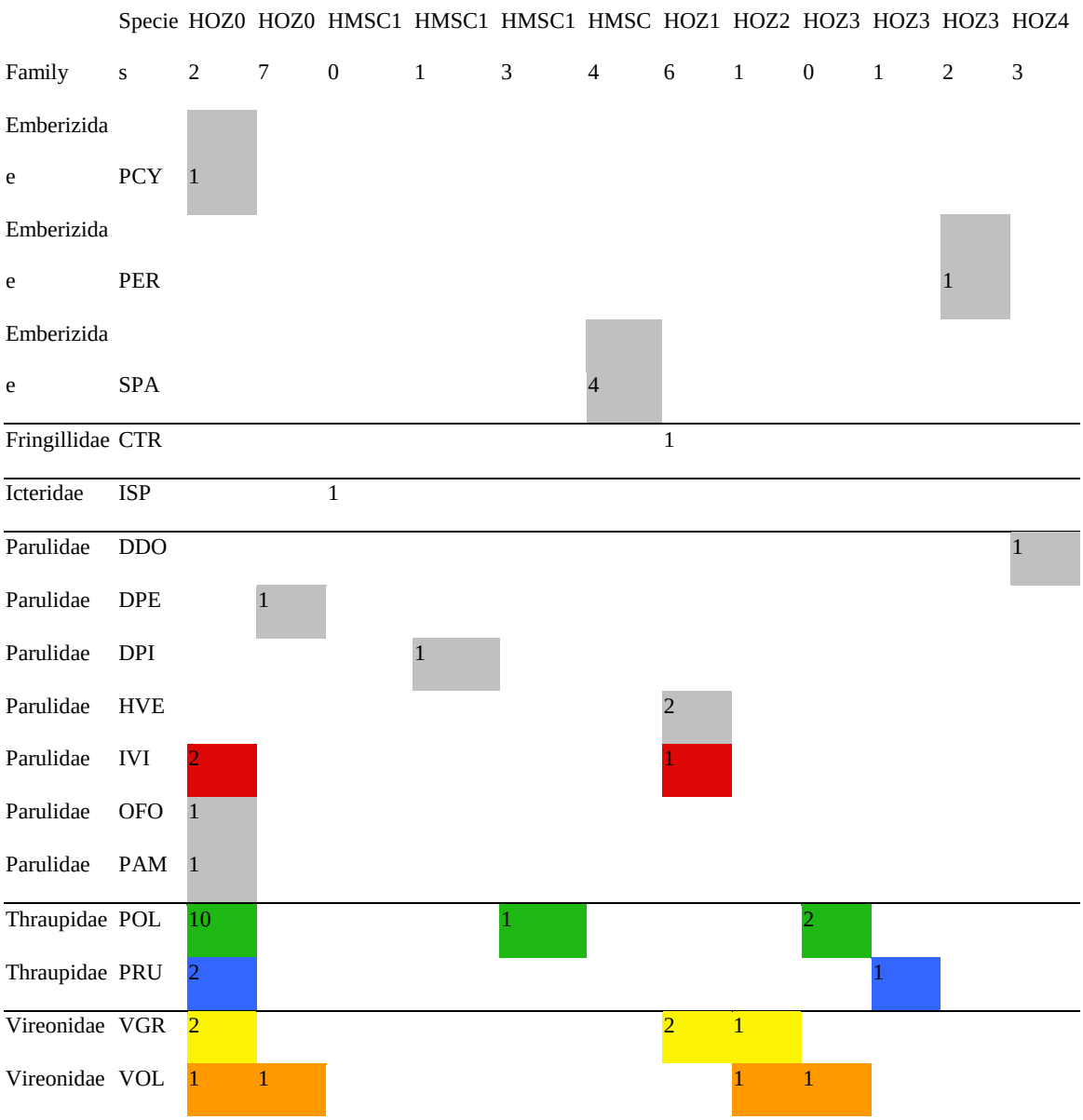
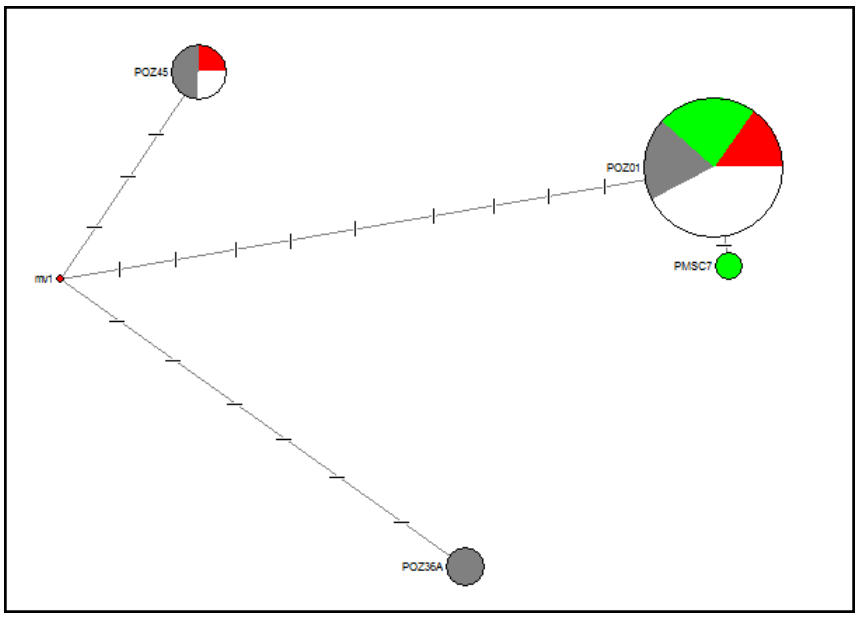


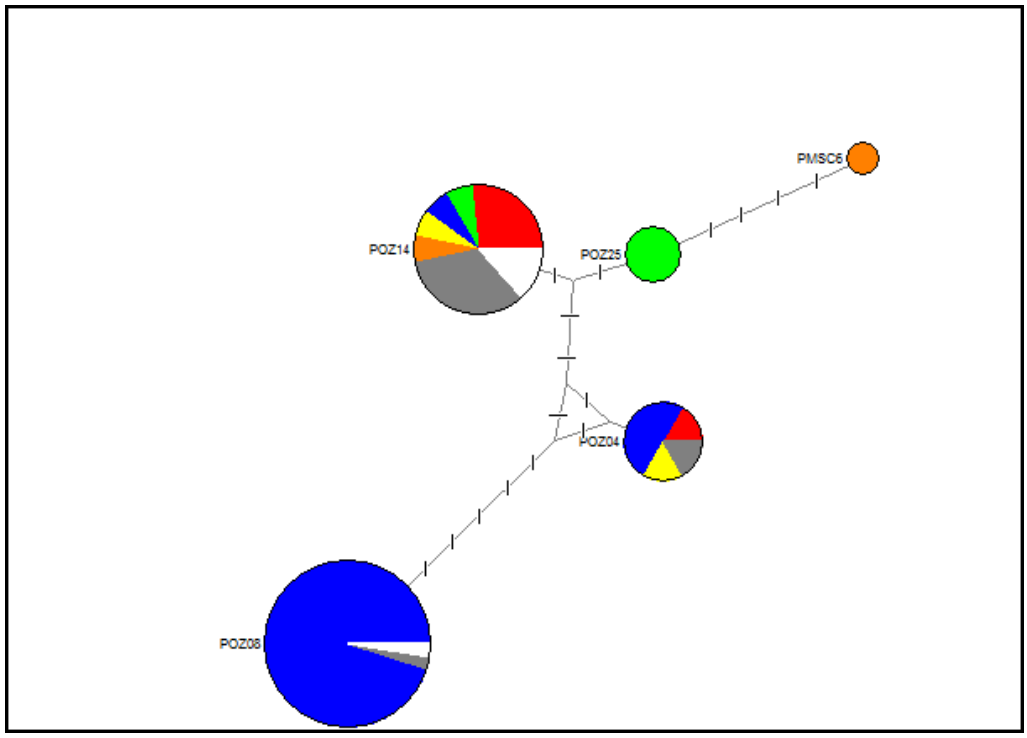
Figure 2 (Haem2). HMSC4 and HMSC10 are kept separate because of distance and host species. HOZ31 and HMSC13 are only recovered from *Piranga* tanagers and are merged with each other but kept separate from the others. HOZ43 is 5 mutations apart from HOZ02, and although poorly sampled, is considered a unique lineage. HMSC11 is only 3 mutations apart from HOZ02, and because of this proximity and low sampling size, we merge it with HOZ02. HOZ07 (n=12) is mostly from white-crowned sparrows (n=10) and is kept separate. HOZ32 (n=3) was recovered from a towhee, bananaquit, and

Hispaniolan spindalis, but since it is closest to HOZ07, it is kept separate. OZ02, OZ16, and OZ21 have broad and overlapping host and geographic distributions. OZ16 was recovered only from North America (except 1 from Grenada). Because of the close nesting with other, clearly separate lineages, all three are considered unique lineages. For example, in one ML phylogeny, OZ02 and OZ21 are together with LA07 (very abundant on emberizids in the West Indies), LA22 (almost restricted to *Cardinalis* and *Icterus* in Venezuela), PR03 (restricted to *Dendroica*, esp. *adelaidae* in PR). Since these other lineages are closely related, but have distinctive host distributions, it seems better to keep OZ02, OZ16, and OZ21 separate. 9 lineages: **HOZ02** (includes HMSC11), **HOZ07**, **HOZ16**, **HOZ21**, **HOZ31** (includes HMSC13), **HOZ32**, **HOZ43**, HMSC10 (renamed **HOZ53**), and HMSC4 (renamed **HOZ49**).



Family	Species	POZ01	PMSC7	POZ36	POZ45
Cardinalidae	CCA	4			1
Emberizidae	PCY	8			
Emberizidae	PER	1			
Parulidae	DDO	1			
Parulidae	IVI	6	1		
Parulidae	MVA				2
Parulidae	WCI	3			
Troglodytidae	TLU	2			1
Vireonidae	VGR	1			
Vireonidae	VOL			2	

Figure 3 (Plas1). Here, haplotypes are more divergent than in other networks (more than ca. 1%). Thus, only OZ01 and PMSC7 are merged with each other, the rest kept separate. 3 lineages: **POZ01** (includes PMSC7), **POZ36**, and **POZ45**.



Family	Species	POZ04	POZ08	PMSC6	POZ14	POZ25
Cardinalidae	CCA	1			4	
Icteridae	ISP				1	
Paridae	BBI				1	3
Parulidae	DDI				1	
Parulidae	HVE				4	
Parulidae	IVI	3	39		1	
Parulidae	MVA	1			1	
Parulidae	OFO			1	1	
Parulidae	PAM	1				
Parulidae	WCI		1			

Tyrannidae	EVI		1
Vireonidae	VGR	1	

Figure 4 (Plas2). Based on information gained in other geographic areas, OZ04 (n=198) are mostly West Indian; OZ08 (n=40) are almost all from IVI and none are from the West Indies; OZ14 (n=28), none from West Indian residents; OZ25 (n=5) are all from BBI. Thus, we keep these separate from each other. 5 lineages: **POZ04**, **POZ08**, PMSC6 (renamed **POZ54**), **POZ14**, and **POZ25**.

SUMMARY TABLE

Lineage	Haplotypes
HOZ33	HOZ33 , HMSC1
HOZ05	HOZ05 , HMSC2, HMSC3
HOZ28	HOZ28 , HOZ29
POZ06	POZ06, PMSC5
HOZ12	HOZ12, HOZ13, HMSC12
HOZ02	HOZ02, HMSC11
HOZ31	HOZ31, HMSC13
POZ01	POZ01 , PMSC7

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Appendix 2. Scatterplots showing d' as a function of sample size. Correlations were non-significant (haemosporidia in Tiputini [A] $r = 0.04$, $P = 0.82$; Ozarks [B] $r = -0.10$, $P = 0.56$; birds in Tiputini [C] $r = 0.16$, $P = 0.22$; Ozarks [D] $r = 0.17$, $P = 0.38$).