Diversity, Prevalence, and Host specificity of Avian Plasmodium and Haemoproteus in a Western Amazon Assemblage

Authors: Svensson-Coelho, Maria, Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA, Blake, John G., Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA, Loiselle, Bette A., Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA, Penrose, Amanda S., Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA, Parker, Patricia G., Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA, et. al.

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DIVERSITY, PREVALENCE, AND HOST SPECIFICITY OF AVIAN *PLASMODIUM* AND *HAEMOPROTEUS* IN A WESTERN AMAZON ASSEMBLAGE

Maria Svensson-Coelho,[1,2] John G. Blake,[1,3] Bette A. Loiselle,[1,3]
Amanda S. Penrose,[1] Patricia G. Parker,[1] and Robert E. Ricklefs[1]

[1]*Department of Biology, University of Missouri–St. Louis, One University Boulevard, St. Louis, Missouri 63121, USA*

Abstract.—We used PCR and DNA sequencing to screen for haemosporidian parasites (*Haemoproteus* and *Plasmodium*) in 2,488 individual birds from 104 species and 22 families, primarily understory suboscine passerines, captured in a lowland Amazonian forest in Ecuador as a first major step to understanding the transmission dynamics of this cosmopolitan group of parasites in this region. To assess diversity of avian haemosporidia in our study site, we identified putative evolutionary lineages of haemosporidia using the mtDNA gene cytochrome *b* (cyt *b*). We sampled birds over 9 years, which allowed us to assess annual variation in haemosporidian prevalence. Additionally, we investigated among-species variation in prevalence and tested relationships between traits of hosts and prevalence of haemosporidia in a comparative analysis. Finally, we estimated host specificity of each recovered parasite lineage and compared several indices with different details of host information. Prevalence of haemosporidia was 21.7% when we combined years and ranged from 5.6% to 91.2% among well-sampled host species. Prevalence varied significantly among years, ranging from 14.5% in 2006 to 33.2% in 2009. The hypothesis that haemosporidian prevalence increases with level of sexual dimorphism and decreases with foraging height of a host species received some support. We identified 65 unique cyt *b* haplotypes, some of which we considered variation within the same evolutionary lineage. In total, we defined 45 putative evolutionary lineages based on 363 identified parasites. Fourteen haplotypes were identical to haplotypes found elsewhere, sometimes on different continents. Host specificity varied greatly among parasite lineages. Collectively, our findings indicate that within a local Neotropical assemblage of avian haemosporidia, community organization is highly complex and part of this complexity can be attributed to differences in host life history; diversity, particularly of *Plasmodium* spp., is high; and individual parasite lineages can differ greatly in both abundance and number of host species. *Received 15 August 2012, accepted 1 March 2013.*

Key words: avian blood parasites, avian malaria, community ecology, compound community, Neotropics, parasite diversity, parasite prevalence.

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Resumo.—Nós usamos PCR e sequenciamento de DNA para identificar infecções de parasitas hemosporídeos (*Haemoproteus* e *Plasmodium*) em 2.488 indivíduos pertencentes a 104 espécies e 22 famílias de aves (primariamente passeriformes suboscines) em uma localidade

[2]Present 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: mariasve@usp.br
[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, Florida 32611, USA.

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da Amazônia Equatoriana. Este estudo representa o primeiro grande passo para uma maior compreensão da dinâmica de transmissão envolvendo este grupo de parasitas cosmopolitas na região Amazônica. Para avaliar a diversidade de hemosporídeos de aves na localidade de estudo, nós identificamos supostas linhagens evolutivas destes parasitas usando o gene mitocondrial citocromo *b* (cyt *b*). Nós amostramos as aves durante nove anos, o que nos permitiu avaliar a variação anual na prevalência de hemosporídeos. Adicionalmente, nós investigamos a variação interespecífica na prevalência dos parasitas e usamos uma análise comparativa para testar possíveis relações entre atributos das aves hospedeiras e prevalência de hemosporídeos. Finalmente, nós estimamos a especificidade de cada linhagem de parasita em relação aos hospedeiros e comparamos diversos índices contendo detalhes de atributos dos hospedeiros. A prevalência de hemosporídeos foi de 21,7% após combinarmos amostras de diferentes anos e variou entre 5,6% e 91,2% entre espécies de hospedeiros com tamanho amostral satisfatório. A prevalência variou significantemente entre os anos, sendo 14,5% em 2006 e 33,2% em 2009. A hipótese que prediz um aumento na prevalência de hemosporídeos com o nível de dimorfismo sexual e uma diminuição na prevalência com a altura de forrageamento foi parcialmente suportada. Nós identificamos 65 haplótipos únicos de cyt *b*, sendo alguns considerados variações dentro de uma mesma linhagem evolutiva. No total, nós definimos 45 supostas linhagens evolutivas a partir de 363 parasitas identificados. Do total de haplótipos recuperados neste estudo, quatorze foram idênticos a haplótipos encontrados em outras regiões, incluindo continentes distintos. A especificidade a determinados hospedeiros variou de maneira significativa entre as linhagens de parasitas. Coletivamente, nossos resultados indicam que a organização desta comunidade Neotropical de hemosporídeos de aves é complexa e parte desta complexidade pode ser atribuída à diferença das características de estórias de vida dos hospedeiros; a diversidade, particularmente de *Plasmodium* spp., é elevada; e linhagens individuais de parasitas diferem grandemente entre si na abundância e número de espécies de hospedeiros.

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 multihost 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 multihost (restricted to birds) pathogens (Pérez-Tris et al. 2005). Avian haemosporidia are harmful to their hosts (Atkinson and van Riper 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 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 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, because they avoid 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, on the basis of 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 might 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 as one approaches the equator, and—more importantly—reaches an average low well above 13°C, one might expect prevalence to vary less in tropical than in temperate regions from year to year.

Avian haemosporidia are unevenly distributed 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, Križanauskienė et al. 2006, Latta and Ricklefs 2010). Several independent studies have addressed whether among-host species prevalence is related to ecological and 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 1997), leading to (1) a positive association between prevalence and foraging or nest height because vectors have been shown to be more abundant toward the canopy (Bennett and Fallis 1960), (2) greater prevalence in open-cup nesters than in cavity and dome nesters because covered nests offer protection 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 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, (5) some bird species might have impoverished immune systems compared with others, which might relate to measurable host traits. It has been demonstrated, for example, that host survival rate (a proxy for longevity) is positively related to cell-mediated immunity (Tella et al. 2002), which suggests that longer-lived birds have stronger immune systems. Furthermore, (6) the Hamilton-Zuk hypothesis, which applies to chronic parasites like avian haemosporidia, states that "if 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 1982:385). According to the same authors, this implies that species in which sexual selection is stronger should exhibit greater parasite prevalence.

Studies have found mixed support for whether ecological and life-history traits of hosts are related to the prevalence of blood parasites. For example, 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. Ricklefs (1992) found an inverse relationship between parasite prevalence and the length of the incubation period, which suggests that prolonged embryo development might permit the development of a more competent immune system. Scheuerlein and Ricklefs (2004) found that male plumage brightness and body size were associated with greater prevalence of *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and *Trypanosoma* combined, and that a longer life span was associated with higher prevalence of *Plasmodium*. Ricklefs et al. (2005) found a significant upward 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. In a sample from a site in the Brazilian cerrado, 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 with birds building closed nests, but no relationship between host body size and prevalence of either parasite genus. Finally, the positive association between *Haemoproteus* prevalence and social breeding was verified in Fecchio et al. (2013), but the same study found no significant associations between haemosporidian prevalence and nest type, nest height, weight, incubation time, or migratory behavior. Because of these inconclusive findings, the relationship between host ecology and life history and parasite prevalence deserves further attention.

Along with the prevalence of this group of pathogens, or of single pathogen species, a relevant parasite trait to consider in multihost pathogens is host specificity. The quantification of host specificity can include, at least, phylogenetic relationships among hosts utilized by a given parasite

lineage and frequencies 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 frequencies on alternative host species when estimating host specificity (Poulin 2007). Thus, we used our data set to compare different host-specificity indices to each other in order to determine how much the estimate of host specificity changes when excluding or including host phylogeny and excluding or including prevalence information of several haemosporidian lineages. Additionally, even for biologically realistic measures of host specificity that include phylogenetic and frequency information, one still has to decide what constitutes a specialist and a generalist parasite. We applied an index developed for community phylogenetics (Webb et al. 2002, Kembel et al. 2011) to the avian haemosporidian system to aid in this decision.

First, we used molecular techniques to describe the diversity of avian haemosporidia and their evolutionary relationships in a region that has not previously been explored with respect to these parasites. We compared recovered lineages to lineages from other parts of the world to determine whether haemosporidia in our study site are unique to the area. Next, we tested whether assemblage-wide and within-host species prevalence of *Plasmodium* and *Haemoproteus* varied annually and between our two closely situated sampling sites. Specifically, we predicted that the wetter plot would exhibit elevated haemosporidian prevalence. We made no *a priori* predictions for annual prevalence variation because we lack climate data for this period. Instead, we explored whether external factors need to be invoked to explain annual variation or whether it can be attributed to differential sampling of primary hosts. In addition, we tested, using a comparative multiple regression, whether haemosporidian prevalence is related to the host species' foraging height, nest type, abundance, level of sexual dimorphism, body mass, and apparent survival rate. On the basis of earlier literature described above, we predicted that prevalence would (1) increase with increasing foraging height, abundance, level of sexual dimorphism, and body mass; (2) decrease with increasing survival rate; and (3) be greater in bird species that build open nests. Finally, we compared several quantitative measures of host specificity and determined which lineages within this site can be considered significantly specialized and generalized.

Studies such as ours provide a first step toward understanding the transmission dynamics of multihost pathogens. Insights from community samples allow us to delineate possible coevolutionary scenarios and might have implications for avian conservation. For example, to protect populations most vulnerable to particular pathogens, we need to understand the distribution of those pathogens among species that might act as potential disease reservoirs. Additionally, our study illustrates how a common group of multihost pathogens is distributed in a local assemblage in the absence of human-induced disturbance, which can serve as a foundation for investigating the effect of human influence on parasite transmission dynamics in the wild.

Methods

Sampling.—We sampled birds 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). We chose these plots as replicated study areas because they were the most ecologically similar 100-ha sites within the research station that also were not crossed by any permanent trails. The area is relatively undisturbed, with the closest indigenous human settlement being ~10 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. Although both plots are dominated by *terra firme* forest and both partially flood during the rainy season (April–October), the Puma plot contains more permanently moist habitats than the Harpia plot (Loiselle et al. 2007, Sheth et al. 2009). Consequently, swamp habitats are found only in the Puma plot, although they make up a small proportion of the total area (Sheth et al. 2009).

More than 300 species of bird have been detected in these plots (Blake 2007). The avian assemblage is dominated by relatively sedentary suboscines (Passeriformes, suborder Tyranni), with respect to both 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. Our study encompassed primarily understory birds. Bird taxonomy follows the AOU's South American Classification Committee

(www.museum.lsu.edu/~Remsen/SACCBaseline.html). We set 96 nets (12 × 2.6 m, 36-mm mesh) per plot at ground level, arranged in eight rectangles (100 × 200 m) of 12 nets, placed ~50 m apart. We collected samples between 0600 and 1300 hours ECT and ringed all birds with numbered aluminum leg bands (Loiselle et al. 2007, Blake and Loiselle 2009). We conducted our work at the Tiputini Biodiversity Station in accordance with research permit number 13-IC-FAU-DFN (and subsequent renewals), Ministerio del Ambiente, Distrito Forestal Napo, Tena, Ecuador.

Molecular screening and identification.—We obtained ~10 µL of blood by brachial venipuncture and stored it in 1 mL Longmire lysis buffer (Longmire et al. 1997). We extracted DNA by an ammonium acetate–isopropanol protocol (Svensson and Ricklefs 2009) or by phenol-chloroform. We screened the DNA samples for haemosporidia, along with positive and negative controls, 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 min. The presence of a 154-base-pair (bp) band provided evidence of infection. We amplified a 552-bp fragment of cytochrome *b* (cyt *b*) in a nested polymerase chain reaction (PCR) 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). The reactions for the cyt *b* amplification contained 1X buffer, 200 nM of each dNTP, 2 mM MgCl2, 20 ng BSA, 200 nM of each primer, and 0.5 unit of TaKaRa Taq (TaKaRa Bio, Shiga, Japan).

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, we used 0.5 µL of the outer PCR 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. We ran the outer reaction in 10 µL of reaction mix and the nested reaction in 20 µL of the mix. We sequenced products on an ABI 3100 Genetic Analyzer (Life Technologies, Carlsbad, California). In some cases, we had strong products from the 702-bp outer reaction and sequenced these instead of the nested product. We sequenced unique haplotypes in both directions. We edited cyt *b* sequences in SEQMAN II (DNASTAR, Madison, Wisconsin) and aligned haplotypes (≤663 bp long) in CLUSTAL X, version 2.0.10 (Larkin et al. 2007). We matched haplotypes found in the present study to known haplotypes in GenBank (www.ncbi.nlm.nih.gov), the MalAvi database (Bensch et al. 2009), and our local database as of 15 May 2012. When we detected double peaks in the chromatograms, we regarded these as mixed infections. We reconciled mixed infections 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. 2007a), some, for example *P. relictum* (Beadell et al. 2006, Martinsen et al. 2006, Valkiūnas 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 neighbor-joining 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. We constructed haplotype networks to more accurately view the connections within these shallow clades of parasites. We extracted sequences within the eight clades into separate files and deleted conserved sites. We then uploaded each group in the software NETWORK, version 4.6 (Fluxus Technology, Suffolk, United Kingdom), and estimated median-joining (MJ) haplotype networks (Bandelt et al. 1999) with the highest possible epsilon value (231) and a transition:transversion ratio of 2:1. Finally, we estimated the most parsimonious networks (Polzin and Daneschmand 2003); we 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, and DQ414649, respectively) composed the outgroup in a maximum likelihood (ML) 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, version 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, version 3.7 (Posada and Crandall 1998). Starting with a randomly generated tree and the Yule process of speciation, we ran 4× a minimum of 10 million generations (sampling every 1,000) or until the estimated sample size (ESS) was ≥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 14 February 2012. Our sequences overlapped the MalAvi data set by ~300 bp at most. We performed an ML analysis in RAXML BLACKBOX, described above, again using six mammalian *Plasmodium* spp. as outgroup.

Phylogenetic analysis of birds.—We estimated phylogenetic relationships among bird species with identified parasite infections 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 4,024 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 I). Otherwise, we sequenced 790 bp of RAG-1 from one to two individuals per species. We amplified RAG-1 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 our study by searching for conserved regions in an alignment of the RAG-1 sequences in Moyle et al. (2009). The PCRs contained 1X buffer, 200 nM of each dNTP, 2 mM $MgCl_2$, 40 ng BSA, 200 nM of each primer, and 0.5 unit of TaKaRa Taq (TaKaRa Bio, 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. We edited sequences as described for cyt *b* above and submitted them to GenBank (Appendix I). 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 $\Delta AIC \leq 2$. Starting with a randomly generated tree and a birth–death process of speciation, we ran 4× a minimum of 10 million generations (sampling every 1,000) or until the ESS was ≥500 for all parameters. All bird species included in the study were passerines, 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; and *Poicephalus meyeri*, JF807989) because 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. We had a sample size of 15 or more for 38 species, but because we had 14 captures for one species, we chose this as our minimum criterion when examining host-species-specific prevalence. Thus, we included 39 species in the "well-sampled species" data set. Prior to analysis, we arcsine square-root transformed prevalence and number of individuals to approximate normal distributions. We used the NESTED procedure in SAS, version 9.2 (SAS Institute, Cary, North Carolina), to partition the variation among groups (families), among subgroups (genera), and within genera. Nomenclatural changes that occurred after this analysis was completed were as follows: *Hylophylax poecilinota* to *Willisornis poecilinotus*, *Myrmotherula*

TABLE 1. Prevalence range (%) 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, 2001–2010.

Family	Combined	G_{adj}	df [a]	P	*Pla*	G_{adj}	df [a]	P	*Hae*	G_{adj}	df [a]	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.

erythrura to *Epinecrophylla erythrura*, and *M. fjeldsaai* to *E. fjeldsaai*. These changes did not qualitatively alter our results (not shown). 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 below), 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, version 3.2 (Hood 2010). For these analyses, we pooled prevalence among years and between plots. We considered prevalences of *Plasmodium* and *Haemoproteus* both together and separately.

Annual and plot variation in prevalence.—We used a three-way log-linear model, following Sokal and Rohlf (1995:743), in the package MASS in R, version 2.14 (R Development Core Team 2011), to test for two-way interactions between plot (a), year (b), and infection status (g). In addition to grouping all infected samples (identified and unidentified), we analyzed *Plasmodium* and *Haemoproteus* separately. This test is a stepwise procedure in which one first tests the null hypothesis that there is no three-way interaction by excluding the last term from the model $f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk}$. 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 interaction was significant, we used the G_{adj} test in POPTOOLS within each category (i.e., we estimated annual variation within each plot and plot variation within each year). Because we were interested in knowing whether prevalence varies among years and/or between plots, we tested only 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 J). Host abundance was estimated by recording the number of individual birds by sight and/or sound along transects in each plot over 4 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*. We estimated body size of each species by averaging the mass of all individuals within a species, which were measured in the field by J.G.B. and B.A.L. and ranged

between 8.90 g in *Myrmotherula axillaris* (Thamnophilidae) and 63.0 g in *Xiphorhynchus guttatus* (Furnariidae). We obtained foraging height, sexual dimorphism, and nest type from the *Handbook of the Birds of the World* (del Hoyo et al. 2003) and from J.G.B.'s personal observations. We categorized foraging height as 1 = ground, 2 = understory, 3 = midstory, and 4 = canopy. We categorized sexual dimorphism as 1 = no dimorphism, 2 = moderate dimorphism (e.g., different head patterns between male and female), and 3 = striking dimorphism (e.g., different body color, sexual ornaments). We categorized nest type as 1 = closed (domed, cavity) and 2 = open. We log transformed body size and abundance and arcsine square-root transformed prevalence prior to analysis. We used Grubb's test in GRAPHPAD (see Acknowledgments) to determine whether our data contained outliers and decided to remove the heavily parasitized *Formicarius colma* (Formicariidae) from this analysis ($z = 3.83$, $P < 0.05$). In addition, we excluded *Turdus albicollis* (Turdidae) from this analysis because it forages both on the ground and in the canopy and does not fall within either of the designated foraging-height categories. Thus, we included 37 species.

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). We used the maximum clade-credibility tree from the BEAST analysis 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 (AIC_c) (Johnson and Omland 2004) and selected those with $\Delta AIC_c \leq 4$ (Burnham et al. 2011) for a multimodel inference procedure in the package "MuMIn" (Barton 2011) in R. Multimodel inference averages the parameter values of each variable (partial beta coefficients in a multiple regression such as ours) after weighting them by the AIC_c weights (Burnham and Anderson 2002, Burnham et al. 2011). To determine whether either of these variables is significantly related 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. We estimated apparent annual survival rate, a proxy for longevity, from 12 years of recapture data (Blake and Loiselle 2008, J. G. Blake unpubl. data) following methods in Blake and Loiselle (2008). Apparent survival rate ranged between 0.42 in *F. colma* and 0.76 in *Chiroxiphia pareola* (Pipridae). We included 26 host species 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, we analyzed *Plasmodium* and *Haemoproteus* 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 were related 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 but within Tiputini Biodiversity Station. We removed the single *Baryphthengus martii* (Momotidae, order Coraciiformes) host individual, which was part of *Plasmodium* P24L's host range and the only nonpasserine 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; i.e., number of host species utilized) to complex (weighted mean pairwise distance [$MPD_{weighted}$], incorporating phylogenetic relationships and frequency distribution among hosts). We calculated five indices in the software package "Picante" (Kembel et al. 2010) in R: (1) host breadth, (2) an equivalent to Simpson's D (Magurran 2004; incorporating frequency but not phylogeny and calculated by $D = \Sigma p_i p_j$, 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 $MPD = 2\Sigma d_{ij}$, 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 $MPD_{weighted}$ (described in detail below). We estimated pairwise genetic distance between hosts (d_{ij}) from the RAG-1 sequences obtained as described above.

$MPD_{weighted}$ is equivalent to Rao's quadratic entropy index, Q (Rao 1982), which was 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, Ventim et al. 2012, Fecchio et al. 2013). $MPD_{weighted}$ 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 and within-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 1,000 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 <0.05. We could not perform the randomization procedure on strict host-species specialists. Instead, we determined the minimum sample size necessary 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*. Thus, the distribution of H17L on its hosts could 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 the probability that four random samples of this lineage should be on its preferred host is $0.43^4 = 0.03$. Thus, in four 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 four times or more.

We estimated pairwise correlations among all indices of lineages that infected 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 1. MPD and $MPD_{weighted}$ range between zero and the maximum possible pairwise distance (or less than this, after

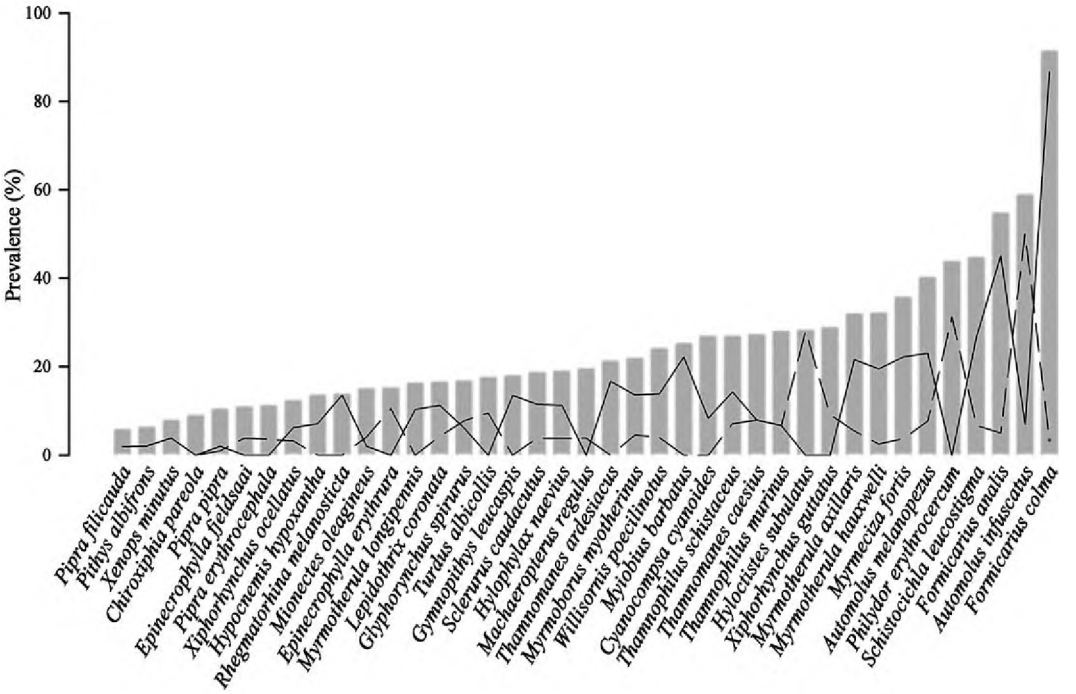


FIG. 1. Haemosporidian prevalence in well-sampled ($n \geq 14$) bird species (gray bars) captured at Tiputini Biodiversity Station, Ecuador, 2001–2010. Prevalence of *Plasmodium* (black line) and *Haemoproteus* (dashed line) is also shown.

incorporating prevalence for $MPD_{weighted}$), which varies, depending on the phylogeny, between 1 and n, where n is the number of host species utilized by a parasite species. We also determined whether any indices were significantly correlated with sample size. We log transformed sample size and host breadth 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, we screened 2,488 individual birds from 104 species for avian haemosporidia. Of these, 539 individuals (21.7%) of 73 species (70.2%) were infected (Appendix K).

Because of cyt *b* sequencing failure, we did not identify 176 haemosporidian infections to genus. We found *Plasmodium* in 223 of 2,312 birds (9.6%) and *Haemoproteus* in 149 of 2,312 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 that exhibited 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 analysis of variance, prevalence varied significantly among families but not among genera for *Plasmodium* (among families: $F = 6.3$, df = 6 and 11, $P = 0.005$; among genera: $F = 1.0$, df = 21 and 11, $P = 0.50$), but prevalence did not vary significantly at any level for *Haemoproteus* (among families: $F = 3.0$, df = 6 and 11, $P = 0.055$; among genera: $F = 1.0$, df = 21 and 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), so we could not test 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

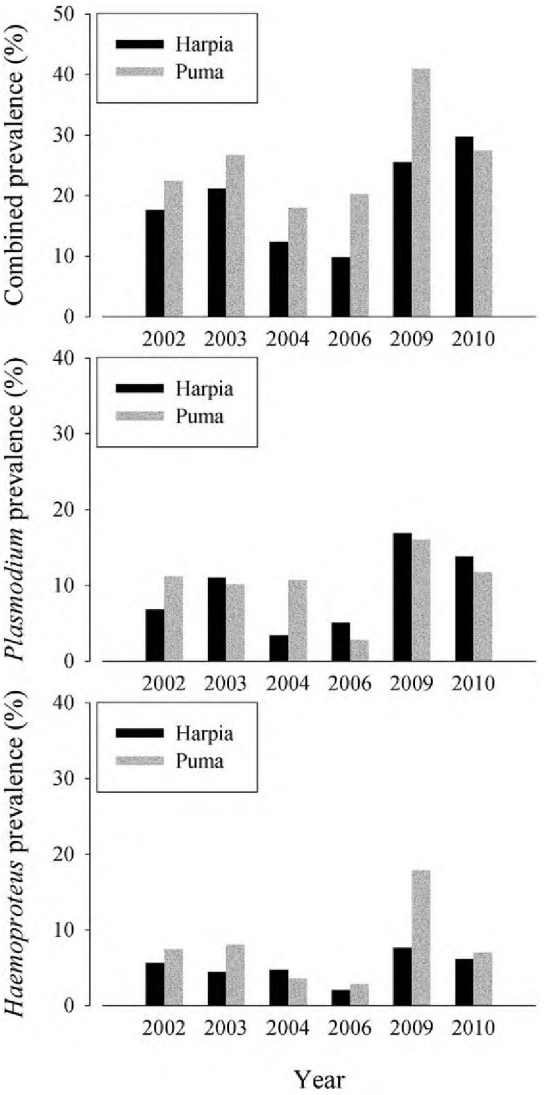


FIG. 2. Annual variation in assemblage-wide prevalence of any haemosporidian infection (top), *Plasmodium* infection (middle), and *Haemoproteus* infection (bottom) in six well-sampled years in two 100-ha plots (Harpia and Puma), Tiputini Biodiversity Station, Ecuador, 2002–2010.

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] at the Tiputini Biodiversity Station, Ecuador, 2002–2010.

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

[a] Because the three-way interaction was significant for the *Plasmodium* data set, 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.

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 1,225 (21.1%) birds were infected in the Puma plot and 197 of 1,222 (16.1%) birds were infected in the Harpia plot. Sample sizes per host species were significantly correlated between plots (Pearson's $r = 0.86$, df = 102, $P < 0.001$), which suggests 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 for which we had at least 6 samples year^{-1} and 3 years of data (Tables 3 and 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, we observed annual variation in prevalence 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.

TABLE 3. Species-level variation in the number of hosts infected (I) and uninfected (U) with *Plasmodium* among years at the Tiputini Biodiversity Station, Ecuador, 2002–2010. Abbreviations: AUTINF = *Automolus infuscatus*, GLYSPI = *Glyphorynchus spirurus*, HYLNAE = *Hylophylax naevius*, MYRAXI = *Myrmotherula axillaris*, THAARD = *Thamnomanes ardesiacus*, THACAE = *Thamnomanes caesius*, and 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 at the Tiputini Biodiversity Station, Ecuador, 2002–2010. Abbreviations: AUTINF = *Automolus infuscatus*, GLYSPI = *Glyphorynchus spirurus*, THACAE = *Thamnomanes caesius*, and 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.

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 seven other host species. P25L was found in 16 bird species,

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) at the Tiputini Biodiversity Station, Ecuador, 2002–2010. Prevalence of *Plasmodium* (*Pla*) and *Haemoproteus* (*Hae*) were analyzed separately. No three-way interactions were significant.

Family	Interaction	G	df [a]	P
Furnariidae	YR × Plot	15.7	10	0.109
Plasmodium	YR × I	14.6	10	0.147
	Plot × I	10.6	6	0.100
Thamnophilidae	YR × Plot	18.1	10	0.053
Haemoproteus	YR × I	22.5	10	0.013
	Plot × I	5.28	6	0.509
Pipridae	YR × Plot	10.2	14	0.746
Haemoproteus	YR × 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.

primarily in Thamnophilidae but also in other families. To determine whether the more specialized lineages vary accordingly to their preferred hosts' abundance, we plotted the abundance of parasite and number of primary host(s) individuals sampled (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 was sampled in 2006, so 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 exhibited significant phylogenetic signal, and among-species comparisons should therefore take into consideration the statistical non-independence of these data (Table 7).

TABLE 6. Annual variation in abundance (n) of individual haemosporidian lineages at the Tiputini Biodiversity Station, Ecuador, 2002–2010.

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.

Combined genera and *Haemoproteus* prevalence exhibited host phylogenetic signal, but *Plasmodium* prevalence did not.

For some data sets, several submodels had high AIC_c weights with $\Delta AIC_c \leq 4$ (Table 8), and we used multimodel inference to determine the beta coefficients (Table 9). Only two of the six host traits were related significantly to 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* ($b = 0.15$, df = 24, $P = 0.616$), *Haemoproteus* ($b = -0.36$, df = 24, $P = 0.31$), or combined ($b = -0.12$, df = 24, $P = 0.685$) 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, and 1 bird had three samples). Of these, 28 were infected with haemosporidia at least at one point, where 11 went from being uninfected to infected, 9 went from being infected to uninfected, and 8 were infected at both sampling occasions. In none of the latter eight cases did we manage to identify (by cyt *b* sequencing) the parasite lineages from both capture dates. We included recaptured individuals in other analyses only for 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 that exhibited multiple peaks. In eight host individuals that exhibited mixed infections, both parasite haplotypes were identified by matching the sequence to previously identified haplotypes from single infections. In 20 host individuals that exhibited mixed infections,

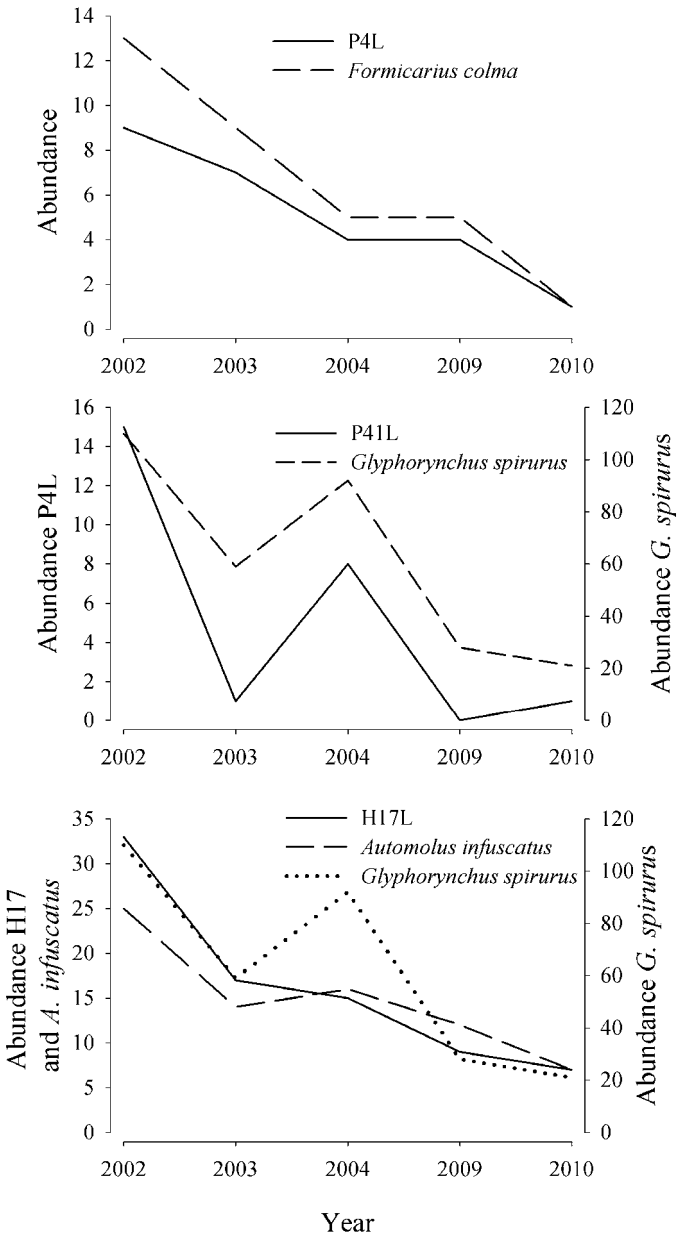


FIG. 3. Annual variation in the abundance of the most frequently recovered specialized parasite lineages and their primary host(s), Tiputini Biodiversity Station, Ecuador, 2002–2010.

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 nine 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 mixed infections to already

TABLE 7. Observed *C*-statistic values from 1,000 permutations of Abouheif's test for serial independence (TFSI), standard deviations (SD), 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*]), based on data collected at the Tiputini Biodiversity Station, Ecuador, 2001–2010. Survival is based on the reduced ($n = 26$) data set.

Variable	*C*	SD	*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	–0.067	–0.189	0.529
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 three data sets on which the generalized least-squares analysis of haemosporidian prevalence and host traits was performed, based on data collected at the Tiputini Biodiversity Station, Ecuador, 2001–2010 ($n = 37$ host species). Only models with $\Delta AIC_c \leq 4$ are shown. Abbreviations: FH = foraging height, W = weight, and SD = sexual dimorphism.

Data set	Variable	AIC_c	Delta AIC_c	AIC_c weights
Combined	SD	–30.22	0	0.44
	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

identified haplotypes, abundant haplotypes are likely to be overrepresented. We did not perform any analyses here comparing rare and common haplotypes; thus, this would have 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 nos. KC680657–KC680721). 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 nonunique haplotypes varied between 2 and 82 cases. *Plasmodium* was more abundant (217 individuals; 60%) than *Haemoproteus* (146 individuals; 40%) ($\chi^2 = 13.9$, 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. 2010), probably because we found no infected doves in Tiputini (out of 4 doves sampled).

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 two haplotypes each. One consisted of P3 and P4 (0.2% divergent), found in 27 *F. colma* individuals. The closest relative of this group (~3% divergent) was P2, found exclusively in eight *Formicarius analis* (Formicariidae) individuals (Fig. 4; and including one from outside the plots). The other group consisted of P5 and P6 (0.2% divergent), each recovered only once but from the same host species (*Chamaeza nobilis*, Formicariidae). *Chamaeza nobilis* was poorly sampled ($n = 3$; 2 of which were infected). The closest relative of 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 (Appendices A–H), we delineated a total of 45 putative evolutionary lineages, 15 *Haemoproteus* subgenus *Parahaemoproteus* and 30 *Plasmodium* (Fig. 4). Each lineage that consists 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

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 $\Delta AIC_c \leq 4$, based on data collected at the Tiputini Biodiversity Station, Ecuador, 2001–2010. Only significant beta coefficients are shown.

Data set	Variable	Beta	95% CI	z	P
Combined	Dimorphism	0.12	0.03 to 0.20	2.68	0.007
Plasmodium	Foraging	–0.16	–0.22 to –0.09	4.88	<0.001
	Dimorphism	0.16	0.07 to 0.26	3.32	<0.001

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 >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 segregated 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 L). The only lineages of these that appear to be restricted to South America 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. 2013) prior to the present 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 (*F. colma*, *G. spirurus*, and *A. infuscatus*).

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), *H. enucleator* (H18 grouped with ALCLEU01 identified morphologically in Beadell et al. 2006), *H. paruli* (H1 grouped with TABI02 identified morphologically in Ricklefs and Fallon 2002), and *Plasmodium elongatum* (P37 exactly matched GRW06 identified morphologically in Valkiūnas et al. 2008). *Haemoproteus enucleator* has not previously been found in South America (Valkiūnas 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. *Haemoproteus 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 in Tiputini these were distinguished both genetically and by host species association. None of our sequences was closely related to either *Plasmodium relictum* or *P. juxtanucleare*, both of which have been found in South America previously (Valkiūnas 2005). We collected blood smears from a fraction of birds during the last two sampling years, but in a preliminary assessment we only detected trophozoites of known positives, precluding morphological identification.

Two of the lineages at Tiputini matched lineages recovered from mosquito vectors in Gager et al.'s (2008) study (although each exhibited 1 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 *T. grayi* (Turdidae) and the vector *Aedeomyia squamipennis* (Culicidae) (in that study called PAN6) in Panama, 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 1 and 23 (H17L) host species, and hosts harbored between 1 and 9 (*G. spirurus* and *H. naevius*) parasite lineages (Fig. 4 and 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, 9 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 and 6).

Two parasite lineages were considered significantly specialized according to our SES_{MPD}, and we considered 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 in 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 were correlated significantly with each other (Fig. 7), and SES_{MPD} were correlated with both MPD indices. In addition, all indices except SES_{MPD} were correlated significantly with sample size (Fig. 7).

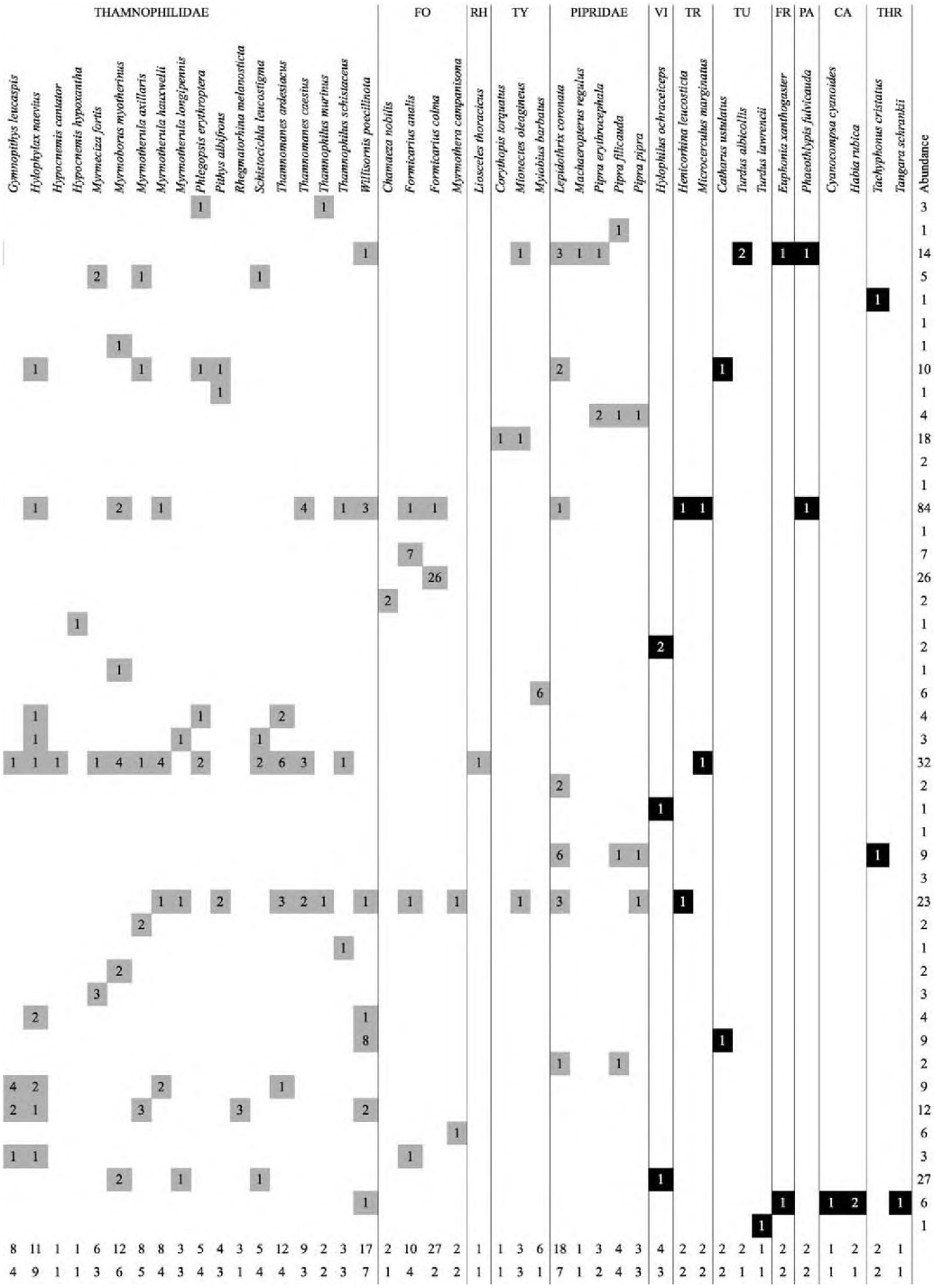
Discussion

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 *P. circumflexum* infection rates are consistently higher in hosts that are 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 the Harpia plot, corroborating the earlier studies on haemosporidian prevalence and moisture associations. However, 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 was substantially reduced when we analyzed prevalence variation within host species or abundance variation of individual parasite lineages. Nonetheless, our results did not provide convincing support for either of these two predictions. First, although two of three host species exhibited significant annual variation in *Haemoproteus* prevalence, in only one of seven host species did *Plasmodium* prevalence vary annually, indicating that in most cases prevalence remained homogeneous over years within host species. *Haemoproteus* but not *Plasmodium* prevalence varied annually within *G. spirurus* and *A. infuscatus*, perhaps an indication that fluctuations in vector abundance are responsible for annual variation in prevalence because the two genera utilize different vectors (Atkinson and van Riper 1991, Valkiūnas 2005). Second, abundance of individual parasite lineages appeared to be 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) disappeared when only data from its primary hosts were analyzed. This suggests that external factors have little influence on the abundance of individual parasite lineages.

FIG. 4. The maximum likelihood (ML) tree of haemosporidian lineages recovered from birds captured within two 100-ha plots at Tiputini Biodiversity Station, Ecuador, 2001–2010, 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 (PP) from the Bayesian analysis (right or bottom) are shown 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 nonpasserine bird. Suboscine passerines (Tyranni) are shown in gray, and oscine passerines (Passeri) are shown in black. Some families are abbreviated: MO = Momotidae, FO = Formicariidae, RH = Rhinocryptidae, TY = Tyrannidae, VI = Vireonidae, TR = Troglodytidae, TU = Turdidae, FR = Fringillidae, PA = Parulidae, CA = Cardinalidae, and THR = Thraupidae.

TABLE 10. Host specificity of lineages recovered twice or more at the Tiputini Biodiversity Station, Ecuador, 2001–2010, measured as the number of host species utilized, Simpson's *D*, MPD, $MPD_{weighted}$, and SES_{MPD}. Sample size (*n*) and significance based on the two-tailed *z* value are also shown. Lineages are sorted by phylogenetic placement in Figure 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

Previous studies that addressed climatic influences on avian haemosporidia were from the temperate region (e.g., Wood et al. 2007, Lachish et al. 2011). Our study, by contrast, is set on the equator in the Amazonian rainforest. Temperatures at 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. We found no significant relationships when considering *Haemoproteus* on their own, but we found support for our prediction that greater levels of sexual dimorphism are associated with greater combined and *Plasmodium* prevalence. We also found that, contrary to our prediction, prevalence of *Plasmodium* decreased 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

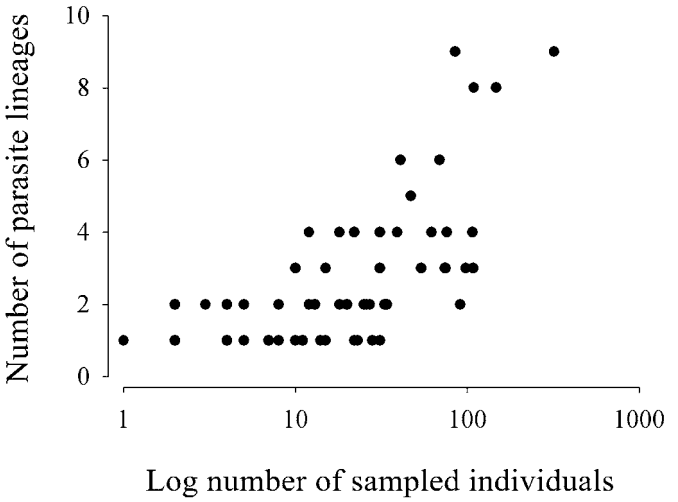


FIG. 5. Number of parasite lineages (parasite richness) per host species as a function of host sample size, Tiputini Biodiversity Station, Ecuador, 2001–2010. The relationship is significant (b = 22.8, t = 9.62, $P < 0.01$, $R^2 = 0.60$).

monomorphic species (Poulin and Forbes 2012). Scheuerlein and Ricklefs (2004) also found that haemosporidian prevalence on blood smears was positively associated 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 in our study 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 contradiction to the Hamilton-Zuk hypothesis because manakins exhibited

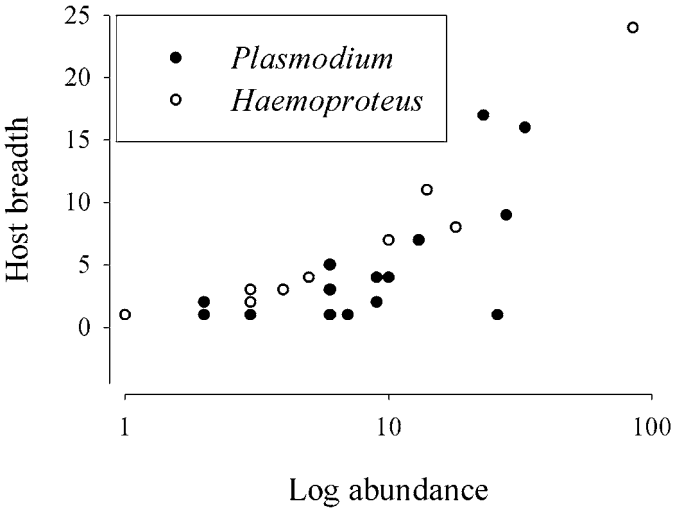


FIG. 6. Host breadth (number of host species) of individual parasite lineages as a function of parasite abundance, Tiputini Biodiversity Station, Ecuador, 2001–2010. The relationships between abundance and (1) *Plasmodium* and (2) *Haemoproteus* are significant (b = 2.0, t = 6.78, $P < 0.01$, $R^2 = 0.68$ and b = 2.9, t = 5.71, $P < 0.01$, $R^2 = 0.80$, respectively).

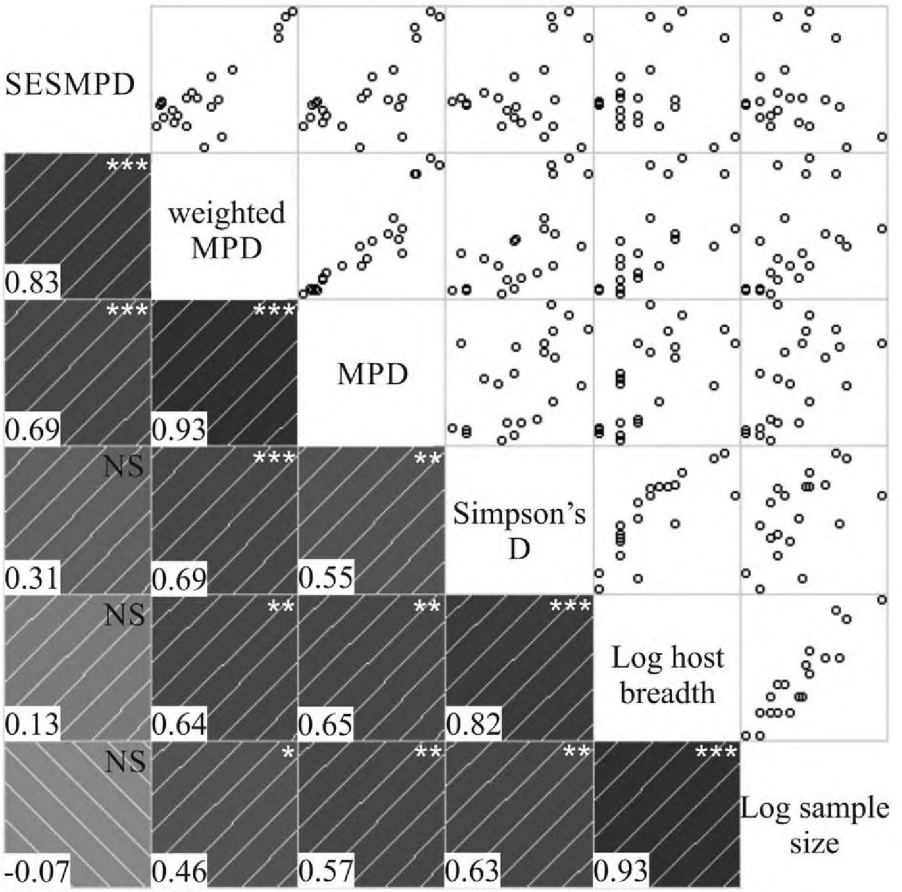


FIG. 7. Correlogram of four indices of host specificity (SES_{MPD}, $MPD_{weighted}$, MPD, and Simpson's *D*), host breadth (number of host species), and sample size of parasite lineages recovered from birds captured at Tiputini Biodiversity Station, Ecuador, 2001–2010. Pearson's correlation coefficient 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 = nonsignificant).

significantly lower average prevalence than that of the remaining bird assemblage (11.9% in Pipridae [$n = 6$] vs. 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 argued 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 (1997) found that prevalence of haemosporidia increased with increasing nest height,

and for the same reasons they provide (greater vector exposure near 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, 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 near 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, most 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 is observed within Pipridae, Thamnophilidae, and Furnariidae. In Pipridae, the species that forages 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 that forage from the ground to the midstory; thus, it is unlikely that individual parasite lineages show preference for birds that forage 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 affect 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 our study. Some measures of immunity vary more among than within host species (Tella et al. 2002). Tella et al. (2002) demonstrated positive relationships among cell-mediated immunity, longevity, and incubation period in a sample of 50 species of birds. However, immunity can also be acquired throughout a bird's life as a response to primary infection by 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 Canaries (*Serinus canaria*), 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 1991). Here, in fact, the three most generalized lineages were *Haemoproteus*, and all the strict host-species specialists were *Plasmodium*. Although counterintuitive, the parasite lineage with the greatest host breadth, *Haemoproteus* sp. H17L ($n = 91$), 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 a family of birds in order to be deemed significantly specialized by the SES_{MPD}. However, the identical haplotype to P8 (here grouped within P25L), OZ 06, recovered in the Missouri Ozarks (Ricklefs et al. 2005), infected primarily Parulidae (12 of 13 recoveries [92%] were found in 8 species within this family). Thus, P25L may be an example of a parasite with high alpha specificity but low beta specificity (Krasnov et al. 2011); that is, locally it may 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} may be a promising host-specificity index to use as a parasite trait in studies where sample size varies among parasite species.

Conclusions.—The general community patterns of avian haemosporidia in our undisturbed study area in the Amazon Basin were remarkably similar to those in previously investigated regions (e.g., Fallon et al. 2005; Ricklefs et al. 2005; Belo et al. 2011, 2012; Fecchio et al. 2013). Common patterns between our and other studies include presence of both *Haemoproteus* and *Plasmodium*, a broad range of host specificities, and most lineages being locally rare while a few lineages are common. Thus, avian haemosporidia seem to assemble into local communities similarly in different habitats and host communities.

The wide range of host specificity exhibited by different haemosporidian lineages suggests that transmission dynamics are highly variable within this group of closely related pathogens. In addition, on the basis of the host distributions of those lineages found both in Tiputini and elsewhere, we can conclude that they exhibit great plasticity in their host-species utilization. Even a lineage that has restricted host breadth locally, such as P25L, can utilize other hosts and reach high abundance on those hosts in other parts of its range. The broad spectrum of host specificity and range-wide plasticity in host-species association suggests that many avian haemosporidia have the capacity to readily switch to alternative hosts should one host become less available. From a conservation and management perspective, a decrease in one avian population might result in an increase in the prevalence of that population's haemosporidian parasites on other coexisting host species. Thus, the risk of parasite infection and, potentially, disease, is dynamic and may represent significant challenges to conservation and management.

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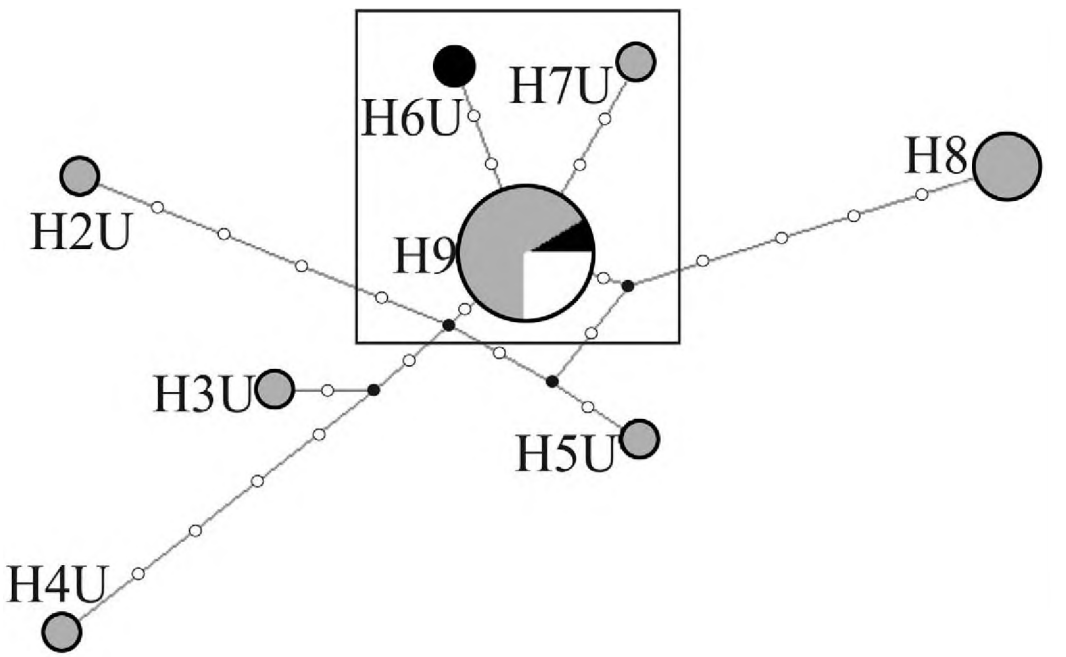
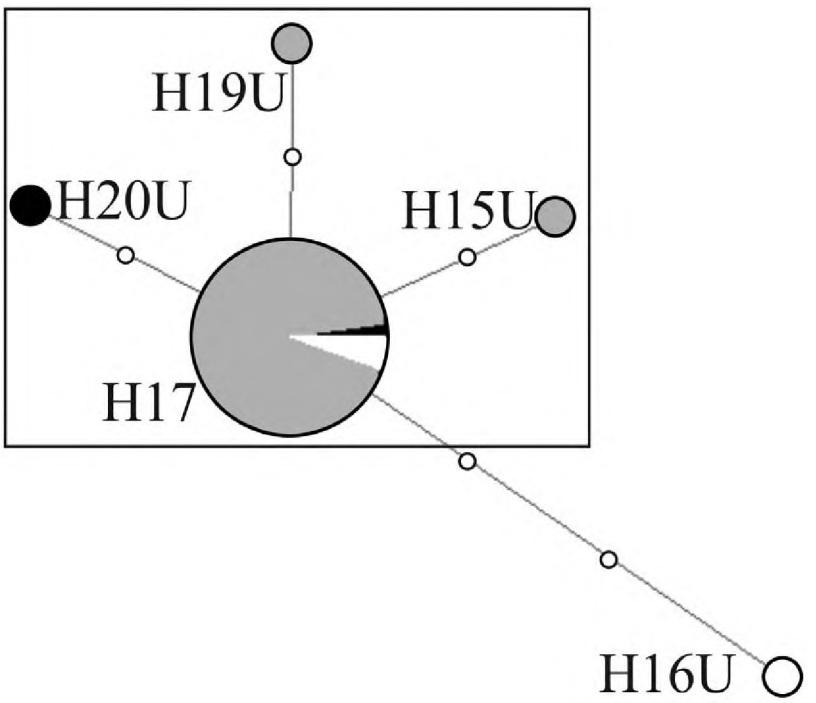
APPENDIX A. Haplotype network and table listing host species of clade Haem1, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code: white = nonshared families; light gray = nonshared species within families that are shared; and black = shared species. Empty circles in the network are mutations, and filled circles are median vectors. Haplotypes that are considered variation within one evolutionary lineage are boxed. Species codes refer to species in Appendix K.

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



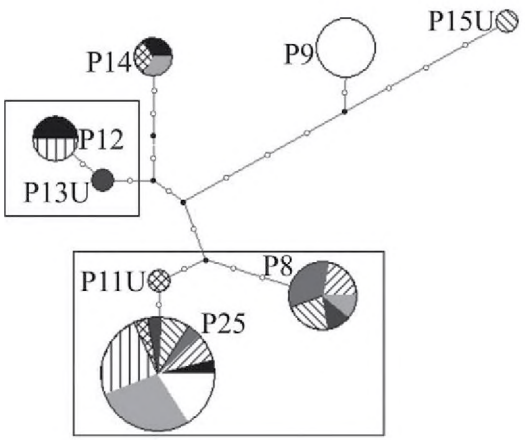
APPENDIX B. Haplotype network and table listing host species of clade Haem2, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code as in Appendix A.

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		
Momotidae	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		



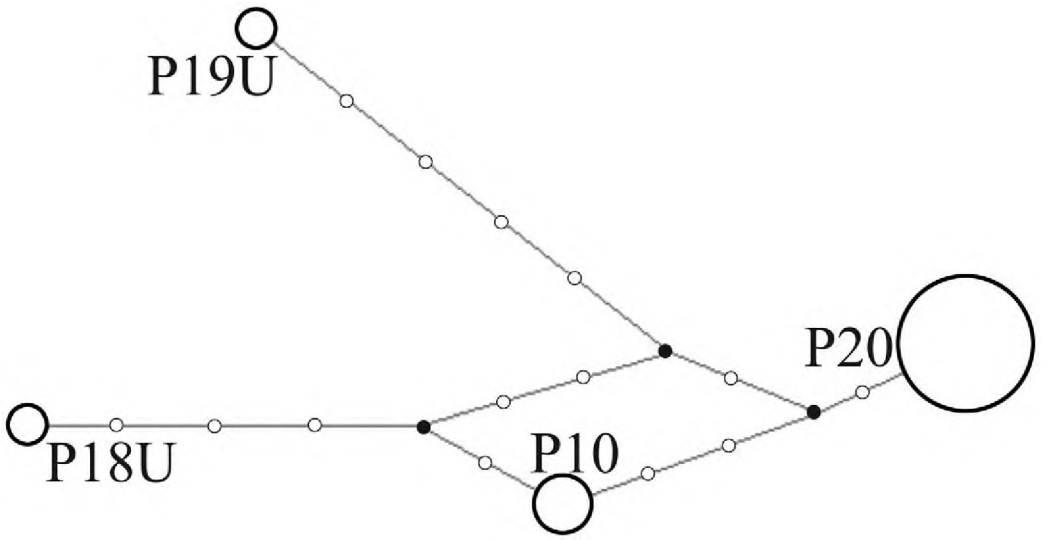
APPENDIX C. Haplotype network and table listing host species of clade Plas1, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code: white = nonshared families; light gray = nonshared species within families that are shared; and black, gray, dark gray, and hashed = shared species.

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



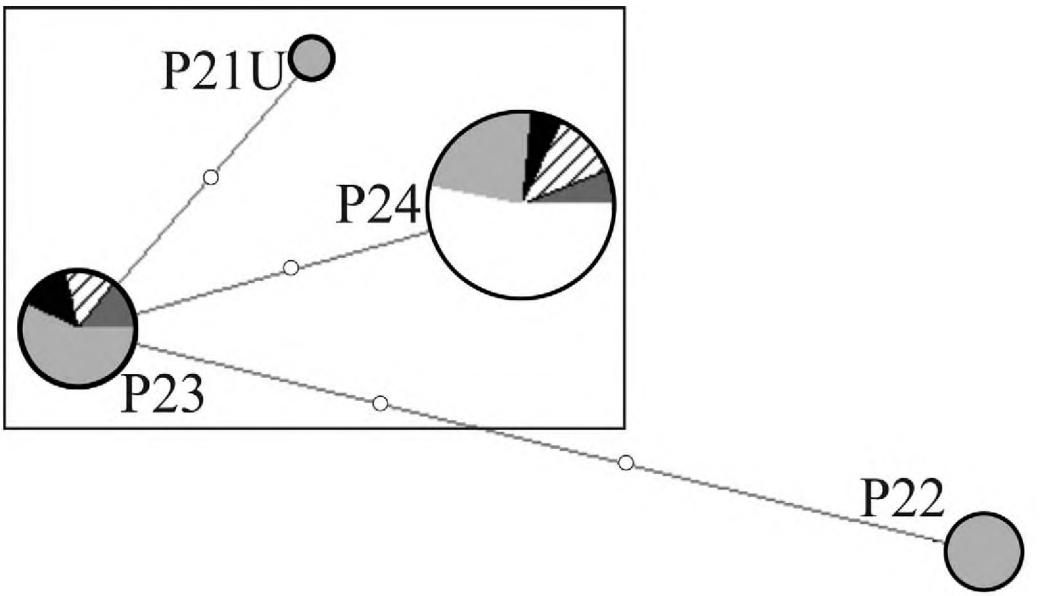
APPENDIX D. Haplotype network and table listing host species of clade Plas2, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code as in Appendix A.

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		



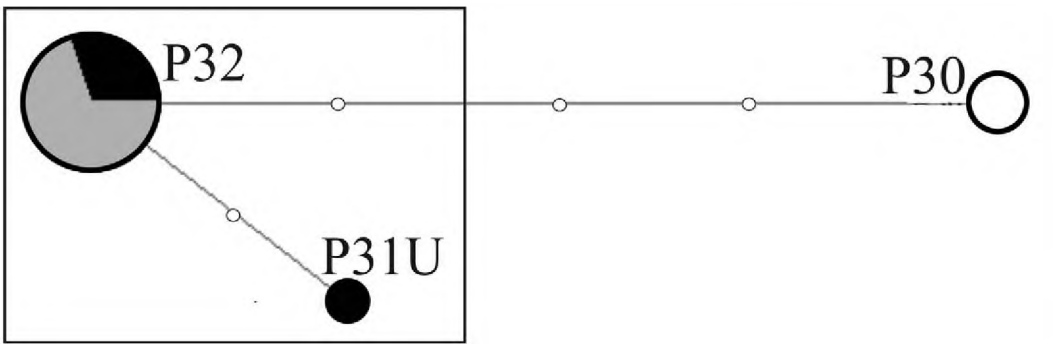
APPENDIX E. Haplotype network and table listing host species of clade Plas3, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code as in Appendix C.

Family	Sp.ecies	P21U	P22	P23	P24
Formicariidae	FORANA	1			
	MYRCAM			1	
Furnariidae	AUTINF		2		
	AUTMEL		1		
	GLYSPI			1	
	XENMIN			1	
Momotdidae	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



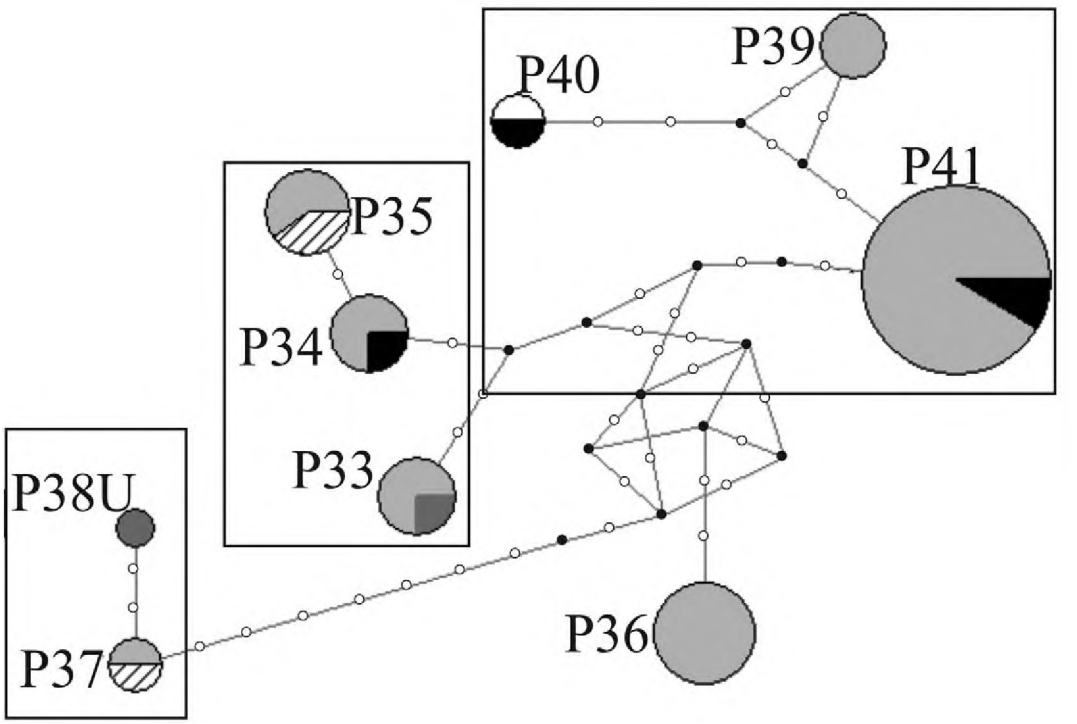
APPENDIX F. Haplotype network and table listing host species of clade Plas4, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color code as in Appendix A.

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



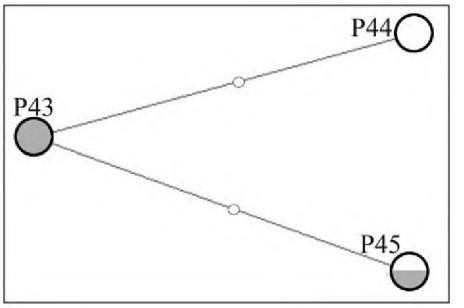
APPENDIX G. Haplotype network and table listing host species of clade Plas5, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. Color-code as in Appendix C.

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, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010. 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. GenBank ID for host RAG-1 sequences of birds sampled at Tiputini Biodiversity Station, Ecuador, 2001–2010, used to control for host phylogeny in the GLS analysis and for calculating host specificity.

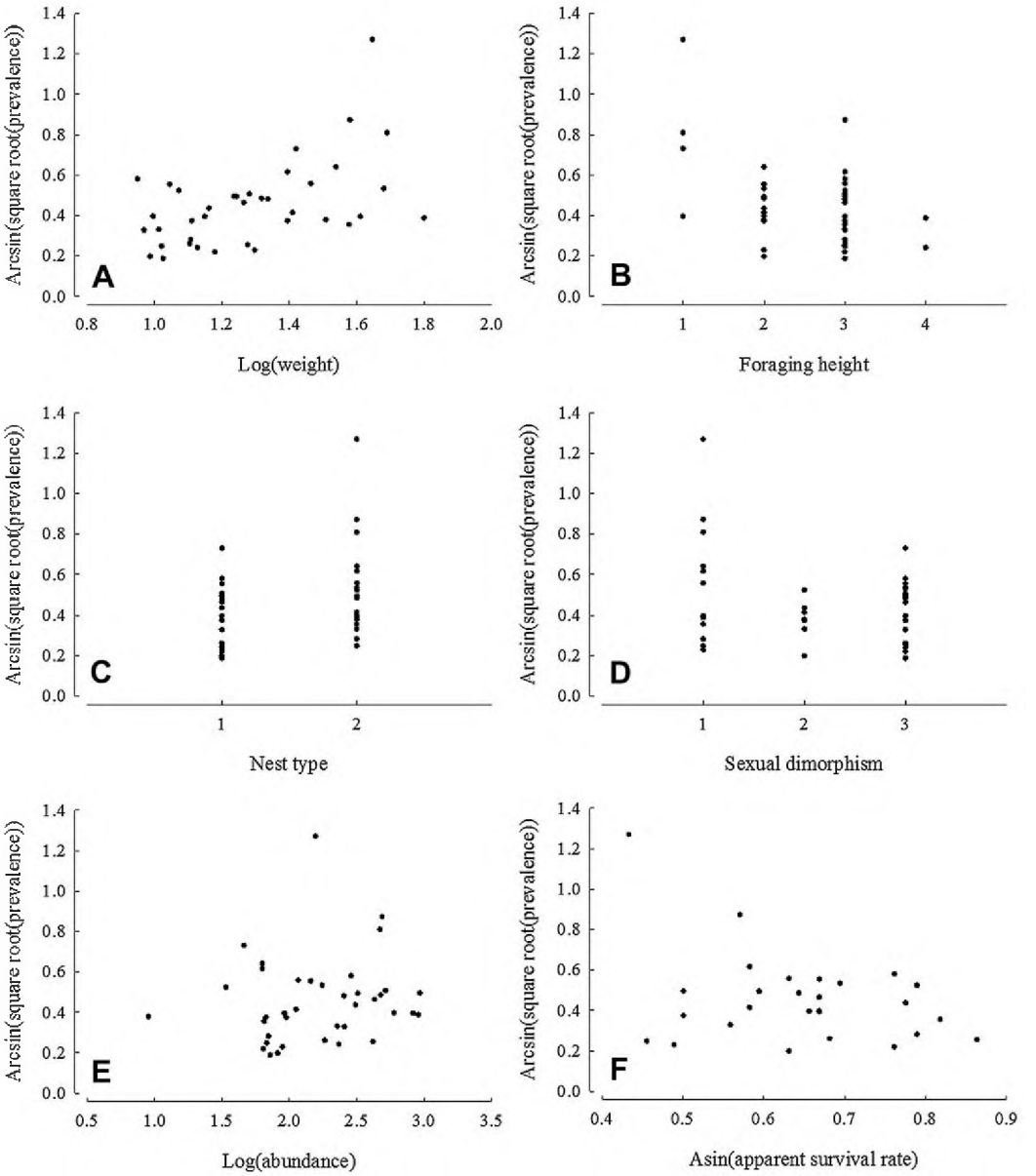
Family	Species	GenBank ID	Source
Furnariidae	*Ancistrops strigilatus*	KC668168	Present study
	Automolus infuscatus	FJ461149	Moyle et al. 2009
	Automolus melanopezus	KC668169	Present study
	Automolus ochrolaemus	KC668170-71	Present study
	Campylorhamphus trochilirostris	KC668172-74	Present study
	Glyphorynchus spirurus	FJ461160	Moyle et al. 2009
	Hyloctistes subulatus	FJ461145	Moyle et al. 2009
	Philydor erythrocercum	KC668225-26	Present study
	Philydor erythropterum	KC668223-24	Present study
	Philydor pyrrhodes	KC668228-29	Present study
	Xenops minutus	KC668249-50	Present study
	Xiphorhynchus guttatus	KC668251-52	Present study
	Xiphorhynchus ocellatus	KC668253-55	Present study
	Xiphorhynchus spixii	KC668256-57	Present study
Thamnophilidae	*Cercomacra serva*	KC668175-76	Present study
	Dichrozona cincta	FJ461184	Moyle et al. 2009
	Epinecrophylla erythrura	KC668183	Present study
	Epinecrophylla fjeldsaai	KC668184-85	Present study
	Gymnopithys leucaspis	KC668190-91	Present study
	Hylophylax naevius	KC668195-96	Present study
	Hypocnemis cantator	KC668199	Present study
	Hypocnemis hypoxantha	KC668200-1	Present study
	Myrmeciza fortis	KC668213-14	Present study
	Myrmoborus myotherinus	KC668219-20	Present study
	Myrmotherula axillaris	FJ461183	Moyle et al. 2009
	Myrmotherula hauxwelli	KC668215-16	Present study
	Myrmotherula longipennis	KC668217-18	Present study
	Phlegopsis erythroptera	KC668230	Present study
	Pithys albifrons	KC668233-34	Present study
	Rhegmatorhina melanosticta	FJ461208	Moyle et al. 2009
	Schistocichla leucostigma	KC668235-36	Present study
	Sclerurus caudacutus	KC668237-38	Present study
	Thamnomanes ardesiacus	FJ461182	Moyle et al. 2009
	Thamnomanes caesius	FJ461176	Moyle et al. 2009
	Thamnophilus murinus	KC668243-44	Present study
	Thamnophilus schistaceus	KC668245-46	Present study
	Willisornis poecilinotus	FJ461204	Moyle et al. 2009
Formicariidae	*Chamaeza nobilis*	KC668177-78	Present study
	Formicarius analis	KC668188-89	Present study
	Formicarius colma	AY056993	Barker et al. 2002
	Myrmothera campanisona	KC668211-12	Present study
Rhinocryptidae	*Liosceles thoracicus*	KC668204	Present study
Tyrannidae	*Corythopis torquatus*	FJ501622	Tello et al. 2009
	Mionectes oleagineus	KC668209-10	Present study
	Myiobius barbatus	FJ501675	Tello et al. 2009
Pipridae	*Chiroxiphia pareola*	KC668179-80	Present study
	Lepidothrix coronata	KC668202-3	Present study
	Machaeropterus regulus	KC668205-6	Present study
	Pipra erythrocephala	FJ501713	Tello et al. 2009
	Pipra filicauda	FJ501714	Tello et al. 2009
	Pipra pipra	KC668231-32	Present study

(continued)

APPENDIX I. Continued.

Family	Species	GenBank ID	Source
Vireonidae	*Hylophilus ochraceiceps*	KC668197-98	Present study
Turdidae	*Turdus albicollis*	KC668247-48	Present study
Troglodytidae	*Henicorhina leucosticta*	KC668193-94	Present study
	Microcerculus marginatus	KC668207-8	Present study
Fringillidae	*Euphonia xanthogaster*	KC668186-87	Present study
Parulidae	*Phaeothlypis fulvicauda*	KC668221-22	Present study
Thraupidae	*Tachyphonus cristatus*	KC668239-40	Present study
	Tangara schrankii	KC668241-42	Present study
Cardinalidae	*Cyanocompsa cyanoides*	KC668181-82	Present study
	Habia rubica	KC668192	Present study

APPENDIX J. Scatter plots of host body weight (A), foraging height (B), nest type (C), sexual dimorphism (D), abundance (E), and apparent survival rate (F) and haemosporidian prevalence, based on data collected at Tiputini Biodiversity Station, Ecuador, 2001–2010.



APPENDIX K. Sample size, number of infections, and prevalence of species at Tiputini Biodiversity Station, Ecuador, 2001–2010.

Family	Species code	Species	Sampled	Infected	Prevalence
Tinamidae	CRYBAR	*Crypturellus bartletti*	1		
Columbidae	GEOMON	*Geotrygon montana*	4		
Trogonidae	TRORUF	*Trogon rufus*	1	1	100
Momotidae	BARMAR	*Baryphthengus 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*	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
	AUTRUB	*Automolus rubiginosus*	3		
	CAMPRO	*Campylorhamphus procurvoides*	1		
	CAMTRO	*Campylorhamphus trochilirostris*	11	3	27.3
	CRAGUT	*Cranioleuca gutturata*	1		
	DENRUF	*Dendrexetastes rufigula*	1		
	DENFUL	*Dendrocincla fuliginosa*	7		
	DENMER	*Dendrocincla merula*	7	1	14.3
	DENCER	*Dendrocolaptes certhia*	4		
	GLYSPI	*Glyphorynchus spirurus*	320	53	16.6
	HYLSUB	*Hyloctistes subulatus*	25	7	28.0
	PHIERY	*Philydor erythrocercum*	39	17	43.6
	PHIERT	*Philydor erythropterum*	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	*Xiphorhynchus guttatus*	14	4	28.6
	XIPOCE	*Xiphorhynchus ocellatus*	33	4	12.1
	XIPSPI	*Xiphorhynchus 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 fjeldsaai*	28	3	10.7
	FREUND	*Frederickena unduligera*	5		
	GYMLEU	*Gymnopithys leucaspis*	62	11	17.7
	HYLNAE	*Hylophylax naevius*	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

(continued)

Appendix K. Continued.

Family	Species code	Species	Sampled	Infected	Prevalence
	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
	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 ardesiacus*	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
	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	*Machaeropterus 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		
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	2,488	539	21.7

APPENDIX L. One hundred percent matches of cyt *b* haplotypes (HAP) found in birds at Tiputini Biodiversity Station, Ecuador, 2001–2010, 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 parentheses below the haplotype name. If a haplotype has been identified to morphospecies, the name is listed in parenthesis below the 100% match name. Species names are based on the IOC World Bird List (Gill and Donsker 2013).

HAP	N	100% matches	DB	Host species	Family	Locality	REF [a]	ID	BP
H1	4	H_DENPET01	MalAvi	*Setophaga petechia*	Parulidae	USA	S&L	AY640129	203
H2	1	H_APSPI01	MalAvi	*Zonotrichia capensis*	Emberizidae	Chile	MER	EF153652	273
				Aphrastura spinicauda	Furnariidae	Chile			
				Troglodytes aedon	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*	*incertae sedis*	WI [b]	FAL03	AY167242	438
		(*H. coatneyi*)		*Coereba flaveola*	Coerebidae	WI			
				Columbina passerina	Columbidae	WI			
				Quiscalus lugubris	Icteridae	WI			
				Allenia fusca	Mimidae	WI			
				Colaptes rubiginosus	Picidae	WI			
				Loxigilla noctis	Thraupidae	WI			
				Loxigilla portoricensis	Thraupidae	WI			
				Melanospiza richardsoni	Thraupidae	WI			
				Tiaris bicolor	Thraupidae	WI			
				Eulampis holosericeus	Trochilidae	WI			
				Glaucis hirsuta	Trochilidae	WI			
				Elaenia martinica	Tyrannidae	WI			
				Vireo altiloquus	Vireonidae	WI			
				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*	Thraupidae	Brazil	FEC	JX501863	502
				Neothraupis fasciata	Thraupidae	Brazil			
				Suiriri suiriri	Tyrannidae	Brazil			
				Elaenia chiriquensis	Tyrannidae	Brazil			
H9	12	Toc-3	GenBank	*Manacus manacus*	Pipridae	Brazil	BEL	HQ287538	488
(H9L)				*Pipra fasciicauda*	Pipridae	Brazil			
				Volatinia jacarina	Thraupidae	Brazil			

(continued)

Appendix L. Continued.

HAP	N	100% matches	DB	Host species	Family	Locality	REF [a]	ID	BP
				Cantorchilus leucotis	Troglodytidae	Brazil			
				Corythopis torquatus	Tyrannidae	Brazil			
				Elaenia cristata	Tyrannidae	Brazil			
		HEB	GenBank	*Volatinia jacarina*	Thraupidae	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
				Pipra fasciicauda	Pipridae	Brazil			
				Sakesphorus luctuosus	Thamnophilidae	Brazil			
				Coryphospingus pileatus	Thraupidae	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			
				Phaeomyias murina	Tyrannidae	Brazil			
				Suiriri suiriri	Tyrannidae	Brazil			
P8	9	Oz06J620	Ricklefs	*Passerina ciris*	Cardinalidae	WI	R&F	AF465555	293
(P25L)				*Coereba flaveola*	Coerebidae	WI		AF465554	
				Arremonops chloronotus	Emberizidae	WI		AF465553	
				Icterus leucopteryx	Icteridae	WI			
				Baelophus bicolor	Paridae	USA			
				Setophaga caerulescens	Parulidae	WI			
				Setophaga dominica	Parulidae	USA			
				Setophaga magnolia	Parulidae	USA			
				Geothlypis trichas	Parulidae	WI			
				Helmitheros vermivorus	Parulidae	USA, WI			
				Mniotilta varia	Parulidae	USA, WI			
				Geothlypis formosa	Parulidae	USA			
				Setophaga americana	Parulidae	WI			
				Setophaga citrina	Parulidae	USA			
				Euneornis campestris	Thraupidae	WI			
P21	1	P_BUTSTR01	MalAvi	*Butorides striata*	Ardeidae	Guyana	DUR	DQ241528	293
P24	15	P_CYCYA01	MalAvi	*Cyanocompsa cyanoides*	Cardinalidae	Guyana	DUR	DQ241529	191

(continued)

APPENDIX L. Continued.

HAP	N	100% matches	DB	Host species	Family	Locality	REF [a]	ID	BP
(P24L)				*Icterus cayanensis*	Icteridae	Guyana			
P37	2	P_GRW06	MalAvi	*Crateroscelis robusta*	Acanthizidae	PNG[b]	BEA	DQ659588	285
(P37L)		(*P. elongatum*)		*Acrocephalus arundinaceus*	Acrocephalidae	Sweden	BEN		
				Acrocephalus scirpaceus	Acrocephalidae	Spain	FER		
				Hippolais icterina	Acrocephalidae	Sweden	HEL		
				Alcedo atthis	Alcedinidae	Myanmar	ISH		
				Ardea herodias	Ardeidae	USA	BEA		
				Philesturnus carunculatus	Callaeidae	NZ[b]	RB		
				Emberiza citrinella	Emberizidae	NZ	RB		
				Linurgus olivaceus	Fringillidae	Gabon	HEL		
				Linurgus olivaceus	Fringillidae	Cameroon	BEA09		
				Mohoua albicilla	*incertae sedis*	NZ	RB		
				Passer domesticus	Passeridae	NZ	RB		
				Rimator malacoptilus	Pellorneidae	Myanmar	ISH		
				Petroica australis	Petroicidae	NZ	RB		
				Ploceus melanogaster	Ploceidae	Cameroon	BEA09		
				Ailuroedus melanotis	Ptilonorhynchidae	Australia	BEA04		
				Strix varia	Strigidae	USA	ISH		
				Turdus merula	Turdidae	NZ	RB		
				Turdus philomelos	Turdidae	NZ	RB		
		P_PADOM11	MalAvi	*Cyanocompsa cyanoides*	Cardinalidae	Guyana	DUR	EU627843	285
				Melospiza melodia	Emberizidae	USA	MART		
				Haemorhous mexicanus	Fringillidae	USA	KIM		
				Chrysomus ruficapillus	Icteridae	Uruguay	DUR		
				Cacicus cela	Icteridae	Guyana	DUR		
				Saltator grossus	*incertae sedis*	Guyana	DUR		
				Saltator maximus	*incertae sedis*	Guyana	DUR		
				Passer domesticus	Passeridae	Brazil	MAR		
				Passer domesticus	Passeridae	USA	MAR		
				Polioptila dumicola	Polioptilidae	Uruguay	DUR		
				Aegolius acadicus	Strigidae	USA	MART		
				Strix varia	Strigidae	USA	ISH		
		P. sp. E1	GenBank	*Melospiza melodia*	Emberizidae	USA	S&L	AY640132	552
				Haemorhous mexicanus	Fringillidae	USA			
				Tachycineta bicolor	Hirundinidae	USA			
				Setophaga petechia	Parulidae	USA			

(continued)

Appendix L. Continued.

HAP	N	100% matches	DB	Host species	Family	Locality	REF [a]	ID	BP
		P. elongatum P52	GenBank	*Ardea herodias*	Ardeidae	USA	BEA	DQ659588	551
		MMK-2009a	GenBank	*Culex restuans*			KIM10	GQ471951	533
		Toc-32	GenBank	*Volatinia jacarina*	Thraupidae	Brazil	BEL	HQ287549	510
		PQ	GenBank	*Volatinia jacarina*	Thraupidae	Brazil	FEC	JX501787	552
				Neothraupis fasciata	Thraupidae	Brazil			
		Larus/RBG2/NZL	GenBank	*Chroicocephalus scopulinus*	Laridae	NZ	CLO	HM579784	415
		OZ01/hap 56	Ricklefs	*Cardinalis cardinalis*	Cardinalidae	USA	O&R	GQ141594	552
				Passerina ciris	Cardinalidae	WI			
				Passerina cyanea	Cardinalidae	USA			
				Pheucticus ludovicianus	Cardinalidae	WI			
				Coereba flaveola	Coerebidae	WI			
				Columbina passerina	Columbidae	WI			
				Geotrygon montana	Columbidae	WI			
				Zenaida aurita	Columbidae	WI			
				Pipilo erythrophthalmus	Emberizidae	USA			
				Chrysomus icterocephalus	Icteridae	WI			
				Icterus chrysater	Icteridae	WI			
				Icteria virens	*incertae sedis*	USA			
				Dumetella carolinensis	Mimidae	WI			
				Margarops fuscatus	Mimidae	WI			
				Allenia fusca	Mimidae	WI			
				Mimus gilvus	Mimidae	WI			
				Setophaga caerulescens	Parulidae	WI			
				Setophaga discolor	Parulidae	WI			
				Setophaga dominica	Parulidae	USA			
				Setophaga petechia	Parulidae	WI			
				Setophaga plumbea	Parulidae	WI			
				Limnothlypis swainsonii	Parulidae	WI			
				Mniotilta varia	Parulidae	WI			
				Setophaga citrina	Parulidae	USA, WI			
				Loxigilla portoricensis	Thraupidae	WI			
				Tiaris bicolor	Thraupidae	WI			
				Eulampis holosericeus	Trochilidae	WI			
				Thryothorus ludovicianus	Troglodytidae	USA			
				Vireo altiloquus	Vireonidae	WI			
				Vireo griseus	Vireonidae	USA			
				Vireo modestus	Vireonidae	WI			

(continued)

APPENDIX L. Continued.

HAP	N	100% matches	DB	Host species	Family	Locality	REF [a]	ID	BP
P44 (P43L)	2	PF	GenBank	*Volatinia jacarina*	Thraupidae	Brazil	FEC	JX501881	490
P45 (P43L)	2	OZ04	Ricklefs	*Cardinalis cardinalis*	Cardinalidae	WI	O&R	GQ141587	524
				Passerina cyanea	Cardinalidae	USA			
				Coereba flaveola	Coerebidae	WI			
				Euphonia jamaica	Fringillidae	WI			
				Icterus bonana	Icteridae	WI			
				Quiscalus lugubris	Icteridae	WI			
				Icteria virens	*incertae sedis*	USA			
				Margarops fuscatus	Mimidae	WI			
				Mimus gilvus	Mimidae	WI			
				Setophaga adelaidae	Parulidae	WI			
				Setophaga plumbea	Parulidae	WI			
				Mniotilta varia	Parulidae	USA			
				Euneornis campestris	Thraupidae	WI			
				Loxigilla noctis	Thraupidae	WI			
				Loxigilla violacea	Thraupidae	WI			
				Loxipasser anoxanthus	Thraupidae	WI			
				Tiaris bicolor	Thraupidae	WI			
				Tiaris olivacea	Thraupidae	WI			
				Volatinia jacarina	Thraupidae	WI			
				Nesospingus speculiferus	Thraupidae	WI			
				Turdus plumbeus	Turdidae	WI			
				Elaenia martinica	Tyrannidae	WI			
				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. 2013), R&F (Ricklefs and Fallon 2002), BEA (Beadell et al. 2006), ISH (Ishtiaq et al. 2007), RB (Ruth Brown unpubl. data), HEL (Hellgren et al. 2007b), BEA09 (Beadell et al. 2009), MAR (Marzal et al. 2011), BEA04 (Beadell et al 2004), BEN (Bensch et al. 2007), FER (Fernández et al. 2010), MART (Martinsen et al. 2008), KIM (Kimura et al. 2006), KIM10 (Kimura et al. 2010), CLO (Cloutier et al. 2011), and O&R (Outlaw and Ricklefs 2009).

[b] WI = West Indies, PNG = Papua New Guinea, and NZ = New Zealand.