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Factors related to prevalence and parasitemia of avian malaria in an Ash-Breasted Sierra Finch population in an Andean dry forest.

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Factors related to prevalence and parasitemia of avian malaria in an Ash-Breasted Sierra Finch population in an Andean dry forest.

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RESUMEN

La malaria aviar es una enfermedad infecciosa de varios taxa de vertebrados. En las aves, esta infección está causada por los géneros cosmopolitas de apicomplejos intracelulares obligados en los géneros *Plasmodium* y *Haemoproteus,* de la familia Haemosporida. Diferentes estadios de los parásitos infectan los glóbulos rojos y hepatocitos de los huéspedes causando síntomas como anemia y fallo orgánico. Comprender la ecología de las infecciones de malaria puede proveer información importante sobre cómo manejar la conservación de las aves y como fenómenos como el cambio climático pueden afectar distintas comunidades y la epidemiología de estas infecciones. En el presente estudio se analizaron las infecciones de malaria en *Geospizopsis plebejus*, un ave de la familia Thraupidae que habita en los Andes y sobre el cual no se han reportado investigaciones similares. Para el diagnóstico de la infección, se utilizó como marcador una secuencia parcial del citrocromo b de la mitocondria de los parásitos, que fue amplificado de muestras de sangre mediante PCR. Este trabajo se complementó con datos de intensidad de infección o parasitemia en cada individuo. El estudio se llevó a cabo en 64 individuos de *G. plebejus* del Bosque Protector Jerusalem, un bosque arbustal semideciduo de los Andes. Se detectaron los linajes moleculares de los parásitos infectando al huésped, y se determinaron los factores abióticos y bióticos relacionados al estado e intensidad de infección utilizando una serie de Modelos Generalizados Lineales. Encontramos que la prevalencia y la parasitemia no siguieron una tendencia del resto del Neotrópico, de presentar mayor prevalencia de *Plasmodium* que de *Haemoproteus.* La prevalencia de *Haemoproteus* fue de 58% y la de *Plasmodium* fue de 13%. La parasitemia promedio fue mayor en *Haemoproteus* con 82 células infectadas por cada 10 mil eritrocitos, la de *Plasmodium* fue de 22 células infectadas por cada 10 mil eritrocitos. Nuestros modelos lineales generalizados no seleccionaron predictores significativos, excepto en el caso de la presencia de ácaros en las plumas, donde se encontró una relación positiva significativa con el estado de infección. Encontramos cinco linajes de parásitos infectando al hospedero: dos de *Plasmodium* y tres de *Haemoproteus*, de los cuáles uno es un nuevo linaje de citocromo b.

***Palabras clave: Plasmodium*, *Haemoproteus*, Malaria aviar, *Geospizopsis plebejus*, Citocromo b, Parasitemia, Prevalencia.**

ABSTRACT

Avian malaria is an infectious disease that affects various vertebrate taxa. In birds, this infection is caused by the cosmopolitan and obligate intracellular apicomplexan parasites of the genera *Plasmodium* and *Haemoproteus*, family Haemosporida. Different stages of the parasites infect red blood cells and hepatocytes of the host, causing symptoms like anemia and organ failure. Understanding the ecology of malaria infections can provide information for the management of the conservation of bird species, and how phenomena like climate change will affect different communities and the epidemiology of the disease. In this study, we analyze the infections in *Geospizopsis plebejus*, a finch from the Thraupidae family that inhabits the Andes, and from which similar studies have not been published. To diagnose the infection, we use the partial sequence of the cytochrome b of the parasite mitochondrion, amplified by PCR. This study was complemented with data of intensity of infection for every individual. For this study we used samples from 64 individuals of *G. plebejus* from a community of Bosque Protector Jerusalem, an Andean dry forest ecosystem. We detected the molecular lineages of the parasites infecting the hosts, and determined the abiotic and biotic factors related to the infection status and the intensity of infection through a series of Generalized Linear Models. We found that in our study, infections did not follow a known Neotropical trend of higher prevalence of *Plasmodium* over *Haemoproteus*. The prevalence of *Haemoproteus* was 58%, while the prevalence of *Plasmodium* was 13%. Median parasitemia was also higher in *Haemoproteus* with 82 infected cells per 10 thousand erythrocytes, while *Plasmodium* mean parasitemia was of 22 infected cells per 10 thousand erythrocytes. The models produced did not select significant predictors except in the case of the presence of ectoparasites in the feathers, where a positive significant relation was found with infection status. We report 5 cytochrome b lineages of parasites infecting the hosts: 2 *Plasmodium* lineages, and 3 *Haemoproteus lineages*, one of which is a new lineage.

Key words: *Plasmodium*, *Haemoproteus*, Avian Malaria, *Geospizopsis plebejus*, Cytochrome b, Parasitemia, Prevalence.

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INTRODUCTION

Avian Malaria is an infectious disease caused by Apicomplexan parasites belonging to the genera *Plasmodium* and *Haemoproteus* of the order Haemosporida (Ricklefs & Fallon, 2002). These parasites have a cosmopolitan distribution and infect almost all taxa of avian species (Kimura et al., 2010). Infection is transmitted by dipteran vectors like mosquitoes, biting midges, and black flies that inoculate the host with sporozoites at the moment of the bite (Woodford et al., 2018; Ricklefs et al., 2004) and recover the gametocytes from the bloodstream of infected hosts for sporogony in the salivary glands (Gwadz et al., 1989). Schizogony and gametogony take place inside the body of the host (Atkinson & Van Riper, 1991).

Haemoproteus has been found to display the highest prevalence of the two parasites in avian malaria infections, in most regions, and tends to be more host-specific (Clark et al., 2014; Bensch et al., 2000). However, while *Plasmodium* has a lower prevalence, it is believed to produce more detrimental effects on the host (Cannell et al., 2013; Ricklefs et al., 2004). There is also a general global pattern of higher diversity and richness of *Haemoproteus* lineages compared to those of *Plasmodium* (Clark et al., 2014). This higher diversity can be driven by host specificity given that host diversity tends to correlate with parasite richness (Kamiya et al., 2014; Zhang et al., 2014). However, in the Neotropics, one hotspot for avian richness, *Plasmodium* exhibits higher diversity and host-specialization than *Haemoproteus* (Möens & Pérez-Tris, 2016; Lacorte et al., 2013; Svensson-Coelho et al., 2013).

The Ash-breasted Sierra-finch (*Geospizopsis plebejus*) is a small and conspicuous finch of the Thraupidae family that inhabits the highlands of South America from the Ecuadorian Andes to Bolivia and Argentina (Jaramillo, 2019). Three subspecies are recognized: *G. p. ocularis* (P. L. Sclater, 1859) inhabits the Andes of Ecuador, *G. p. plebejus*

the Andes of Perú and Chile, and *G. p. naroskyi* in Argentina (Jaramillo, 2019). This species was separated along with its sister species *Geospizopsis unicolor* from the polyphyletic *Phrygilus* genus (previously *Phrygilus plebejus* Tschudi, 1844) based on mitochondrial markers (Campagna et al., 2011). The altitudinal range of *G. plebejus* spans from around 2,500 to 3,500 m a.s.l. (Campagna et al., 2011) where it can be found mainly on arid highlands, puna grasslands, inter-Andean shrublands, and paramo habitats (Freile & Poveda, 2019). To our knowledge, there have not been studies regarding haemosporidian parasites in *Geospizopsis plebejus*, but studies carried out in the Andes have included tanagers (Thraupidae, one of the most diverse Neotropical families) such as those of Möens et al. (2016), Harrigan et al. (2014), Rodríguez et al. (2009), and Munro et al. (2009). Among these, just Munro et al (2009) included a representative of *Geospizopsis* (G. *unicolor*), but did not find evidence of parasitic infection. The other two studies carried out in Ecuador that include Thraupidae as hosts are Möens & Pérez-Tris (2016) and Svensson-Coelho et al. (2013). Other Neotropical studies that included *Haplospiza unicolor,* the closest species to the *Geospizopsis,* found one infected individual of three sampled (Ribeiro et al., 2005), and two infected of 20 sampled (Bennett y Lopes, 1980).

A proper understanding of the factors that mediate the prevalence, richness, diversity, and intensity of parasites in different regions is important to determine the geographical zones, ecosystems, and species that will be affected by phenomena like climate change and its impact on vector-borne diseases ecology (Möens & Pérez-Tris, 2016). Factors related to parasitemia and prevalence can include biotic factors, like the ecology and physiology of the host, and abiotic factors like environmental conditions. Furthermore, parasite-vector interactions, that are complex and dependent on the environment, can also affect parasitemia and prevalence (Tripet et al., 2008). Some of those abiotic predictors of avian malaria are altitude, water availability (mediated by precipitation), minimum temperature, distance to

water reservoirs, and distance to livestock and poultry farms, which facilitates the aggregation of host birds and the availability of still water needed for the development of vectors (Gonzalez-Quevedo et al., 2014). Among the biotic predictors, the most important seem to be host density and vegetation type (Gonzalez-Quevedo et al., 2014), host age, host sex, host species (Knowles et al., 2011), host body mass (Atkinson & Van Riper, 1991), foraging and nesting behavior, body size, immune system state, and sexual selection (Svensson-Coelho et al., 2013). A previous investigation in the study area showed that sex (males), age (adults), sampling site, and higher monthly precipitation were good predictors of infection status, while only sampling site was a predictor or parasitemia (Cadena-Ortiz et al., 2019).

This study focused in a population of the Ash-breasted Sierra-finch sampled in an Andean dry forest of Ecuador, and some of the biotic and abiotic factors associated with prevalence and parasitemia of haemosporidian infections in this species. Given the relative lower diversity of birds (Cadena-Ortiz et al., 2015) in comparison to that of other Neotropical ecosystems like the Amazon rainforest (Svensson-Coelho et al., 2013), and a trend of higher prevalence of *Haemoproteus* found by Cadena-Ortiz et al. (2019) in *Zonotrichia capensis* in the same study area, we expect prevalence and parasitemia to be higher for *Haemoproteus* than for *Plasmodium*. We also expect water availability proxies like the daily or monthly precipitation variables to be good predictors, since the vectors are dependent on water in their early developmental stages and humidity is needed for the vectors to survive (LaPointe et al., 2012). Furthermore, we predict that males will show a higher prevalence and parasitemia due to the immunosuppressive effects of testosterone (Asghar et al., 2011).

METHODOLOGY

Study area and sampling process

Sample collection was carried out during the rainy season (November 2012–May 2013) by Cadena-Ortiz et al. (2019) at Bosque Protector Jerusalem (BPJ), an Andean dry forest in the valley of Guayllabamba, northern Ecuador (00° 00' 17.4" N, 078° 21' 34.7" W). BPJ is located at an altitude of 2,330 m a.s.l. and encompasses 1,110 ha of protected inter-Andean dry forest remnants (Cadena-Ortiz et al., 2015). Four collection sites separated by 300 m and encompassing different microclimates (Figure 1) were selected (Site 1: 00° 00' 17.4" N, 78° 21 '34.7" W; Site 2: 00° 00' 08.8" S, 78° 21' 25.3" W; Site 3: 00° 00' 21.4" N, 78° 21 '22.7" W; Site 4: 00° 00' 08.8" S, 78° 21' 29.8" W). Individuals were captured with mist nets and ringed, body allometric measurements were taken, as well as blood samples from the jugular vein.

Mitochondrial DNA extraction

Whole Genomic DNA was extracted from blood samples of 61 out of 74 individuals by Nicolás Peñafiel (unpuplished data) using a published in-house protocol (Peñafiel et al., *in press*). Partial coding sequences of the parasite's cytochrome b were amplified using the HaemF and HaemR2 primers (Waldenström et al., 2004). A non-nested PCR protocol was used, as follows, in 25 µl reaction volumes: 5 µl of genomic DNA, 1 X buffer, 3 mM $MgCl_2$, 0.4 mM dNTPs, 0.6 µM HaemF, 0.6 mM HaemR2, 0.05 U/ µL Platinum Taq polymerase. The amplification protocol used was: initial 3-minute denaturation at 94ºC; 37 cycles of 94ºC denaturation for 30 seconds, 50ºC annealing for 30 seconds, 72ºC extension for 45 seconds; and a 75ºC final extension for 10 seconds. Amplicons were visualized in a 1.2% agarose gel through electrophoresis stained with SYBR Safe (Invitrogen). Positive amplicons were purified with ExoSAP-IT (Affymetrix) and sequenced with the PCR primers.

Molecular lineages

Consensus sequences for each sample were obtained in Genious 5.1.7. All sequences that did not appear to be coinfections (no double picks in chromatograms) were aligned using Clustal X2.1 (Thompson et al., 1997). GenBank and MalAvi's BLASTn (Bensch et al., 2009) were used to compare the sequences obtained to previously published ones. New lineages were defined as sequences with a match lower than 100% with those in the mentioned databases. Separate alignments for unique sequences of *Plasmodium* and *Haemoproteus*, as well as Neotropical lineages, were produced in Clustal X2. *Leucocytozoon fringillinarum* was used in both alignments as the outgroup (TFUS04; GenBank: JQ815435). The alignment was cropped to match the length of the outgroup in Mesquite 3.6 (Maddison & Maddison, 2018) and then translated to amino acids. No stop codons or indels were found.

Prevalence and parasitemia

Parasitemia or intensity of infection (infected cells per 10,000 erythrocytes) values were obtained from Giemsa-stained blood smear counts (Bahamonde, 2014). Prevalence, mean parasitemia, and their confidence intervals were calculated with Quantitative Parasitology 3.0 (Reiczigel et al., 2019). Prevalence confidence intervals were calculated using the Sterne method (Reiczigel, 2003), and parasitemia confidence intervals was obtained using the Bias-Corrected and accelerated (BCa) bootstrap interval method (Rózsa et al., 2000). Comparisons of prevalence between genera and lineages were performed through chi-square tests, while comparisons of mean parasitemia were performed through 2,000 bootstrap t-tests.

Phylogenetic relationships

PartitionFinder 2.1.1 was used in order to choose the best substitution model of molecular evolution for each alignment (Lanfear et al., 2016; Guindon et al., 2010). The

parameters used were: branch lengths linked, all models of evolution, corrected Akaike Information Criterion (AICc), and greedy scheme algorithm (Lanfear et al, 2012). The best partition schemes for *Plasmodium* (by codon position from first to third) were: TrN (Tamura-Nei) + I (invariable sites) + G (gamma distribution), K81UF (unequal-frequency Kimura 3-parameter) + G, and GTR (General Time Reversible) + G. The best partition schemes for *Haemoproteus* (by codon position from first to third) were: TIM (Transition Model) + I + G, TIM + G, and TrN + G. Phylogenetic trees were produced using Mrbayes 3.2.7 for Bayesian Inference (Ronquist & Huelsenbeck, 2003) and W-IQ-TREE for maximum Likelihood (Trifinopoulos et al., 2016). The analysis in Mrbayes was produced using 10 million generations, with sampling every 1000 trees, discarding 2000 and retaining 8000 trees. The analysis in W-IQ-TREE was performed with 10000 bootstrap replicates using Ultrafast Bootstrap (Hoang et al., 2017). The resulting trees were visualized using FigTree v1.4.2 (Rambaut, 2017) and edited with iTOL v.5 (Letunic & Bork, 2019).

Factors related to prevalence and parasitemia

Monthly precipitation data was collected from INAMHI's (Instituto Nacional de Meteorología e Hidrología) nearest weather station at Machinguí. For daily precipitation data we obtained the "*TRMM (TMPA) Precipitation L3 1 day 0.25 degree x 0.25 degree V7"* dataset (Huffman et al., 2010; Huffman et al., 2007). The dataset was provided by Goddard Space Flight Center's Precipitation Measurement Missions (Tropical Rainfall Measuring Mission, TRMM) and the Precipitation Processing System (PPS). Precipitation values for the sampling dates were produced by the TMPA (TRMM Multi-satellite Precipitation Analysis) algorithm archived in the NASA GES DISC (Huffman & Bolvin, 2015; GES DISC, 2016). The "*3B42RT* Derived Daily Product" for precipitation, high quality precipitation, and pre-gauge adjusted infrared precipitation for the nearest point to the study area were downloaded in NetCDF Format. From this data we produced precipitation variables for 1, 2, 3, and 7 days

before the sampling date and for the sampling date, as well as average and accumulative values for the same periods.

We used a series of Generalized Linear Model (GLM) analyses in R (R Core Team, 2013) to establish how abiotic and biotic factors are related to infection status (infected-no infected) and parasitemia. These analyses were performed using the package MASS (Venables & Ripley, 2002). Prevalence was analyzed through a logit model (logistic regression with binomial distribution) using the following parameters: family = binomial, link = logit. The response was the infection status found by PCR. The independent variables used were sex, age, physical condition (calculated as the residuals of the regression between body mass and tarsus length), sampling site, and different measurements of precipitation.

Parasitemia was analyzed through a log-linear model (Poisson regression model) using the following parameters: family = poisson, link = log. The Pearson chi^2 statistic was obtained using the P_disp function (LOGIT package) and overdispersion of the data was found. To correct for overdispersion we replaced the Poisson model by a negative binomial regression model using the following parameters: link = log, maxit = 150. The same response and variables were used.

RESULTS

Relative abundance and recaptures of *Geospizopsis plebejus* in the study area

The total number of *Geospizopsis plebejus* individuals captured was 85 of a total of 871 birds sampled (9.76 %). A total of nine of those individuals are recaptured (10.59 %), and two of those were recaptured twice: the first one first in Site 4, then in Site 2, and again in Site 4; and the second one twice in Site 4. Of the single recaptures four individuals were recaptured in the same site, and one from site 2 was recaptured in Site 4.

Molecular lineages

Of the captured individuals, PCR was performed in 64 birds. We found three *Haemoproteus* lineages—GenBank/MalAvi MK216030/ZOCAP08; JQ988544; and a new lineage, GEPLE01. Also, we found two *Plasmodium* lineages—GenBank/MalAvi, KF537281/ZOCAP11; and MK077679/ ZOCAP15. The species that corresponded to those lineages were inferred from the BLASTn search results and confirmed by their position on the phylogenetic trees (Figure 2 and Figure 3). The *Haemoproteus* lineages JQ988544 and MK216030/ZOCAP08 were grouped with *H. coatneyi*, while the new lineage was grouped with *H. paruli*. The *Plasmodium* lineage KF537281/ZOCAP11 grouped with *P. homopolare* and lineage MK077679/ ZOCAP15 grouped with *P. cathemerium*.

Prevalence and Parasitemia

Prevalence was calculated only on individuals for which PCR was performed (n = 64). Parasitemia was calculated for the individuals diagnosed positive by PCR (n = 52). Confidence Intervals were calculated for total prevalence and parasitemia, as well as for species and lineages (Table 1). Individuals with ambiguous PCR results (n = 4) and recaptures were excluded. Prevalence and parasitemia were higher in *Haemoproteus* than in

Plasmodium (prevalence: exact p-value [two-tailed] < 0.00001; parasitemia: t-statistic= 3.384, bootstrap p-value [two-sided] = 0.0005). Total prevalence was 81.3% and mean parasitemia was 62.38%. When separating by haemosporidian species and molecular lineages, only sequences that were not suspected to be multiple infections were used. *Haemoproteus* prevalence was 57.8% and *Plasmodium* prevalence was 12.5%. *Haemoproteus* mean parasitemia was 82.14 and *Plasmodium* mean parasitemia was 22. The prevalence of the molecular lineages was as follows: JQ988544 (*Haemoproteus*) = 43.8% (n = 28), MK216030/ ZOCAP08 (*Haemoproteus*) = 1.6% (n = 1), GEPLE01 (*Haemoproteus*) = 12.5% (n = 8), KF537281/ZOCAP11 (*Plasmodium*) = 9.4% (n = 6), MK077679/ ZOCAP15 (*Plasmodium*) = 3.1% (n = 2). The mean parasitemia of the molecular lineages was as follows: JQ988544 = 41.3, MK216030/ ZOCAP08= 3.42, GEPLE01 = 2.77, KF537281/ZOCAP11 = 2.73, MK077679/ ZOCAP15 = 2.

Factors associated with Prevalence and Parasitemia

For analyzing different models, we used sex, age, and physical condition as biotic (intrinsic) predictors, and precipitation and collection site as abiotic predictors. Given that most recaptures took place in the same site, we use the sampling sites as proxies of the different microclimates in the study area.

We produced various models for infection status. The models using monthly precipitation did not find any significant predictor (Table 2). We also produced models using different precipitation variables. The first model used daily precipitation as predictor but it was not significant and model selection by backward stepwise AIC method eliminated all the variables. The model with precipitation one day before sampling was not significant, but model selection produced a model that included only the precipitation one day before sampling, which was significant (estimate = -0.1, p-value = 0.0414; Figure 4). However, this

model explained only 6.8 % of the variance. We also produced a model using the accumulative value of precipitation one month before the sampling day (estimate = -0.026, p-value = 0.03) and the average precipitation of the month before the sampling day (estimate = -0.8, p-value = 0.03). All models showed a negative relation between precipitation and infection status. Given the great variance showed by the models in the biotic variables, we additionally explored a model that included the abiotic variables and presence of acarian ectoparasites (Figure 5). This model showed a significant positive relation between presence of ectoparasites and infections status (estimate = 1.51, p-value = 0.0337).

We used the same predictors for the intensity of infection models. The models using monthly precipitation also did not find significant predictors (Table 3). The model using daily precipitation (Figure 6) was significant for precipitation (estimate = -0.07, p-value = 0.0258) and showed that Site 2 was significantly higher in parasitemia from the rest (estimate = 2.76, p-value = 0.018). This model explained 6.5% of the variance and model selection did not select any variable. A second model for precipitation one day before sampling was performed. For this model precipitation was significant (estimate= -0.101, p-value = 0.0444) and model selection produced a model with precipitation as the only predictor (estimate = -0.105, p-value = 0.0235).

We also separated *Haemoproteus* and *Plasmodium* to test for the variables that affect intensity, independently (*Plasmodium* models were not performed due to low sampling size). The models using monthly precipitation had a positive relation with parasitemia (Table 4), as well as the model using only monthly precipitation (using INAMHI and TRMM monthly precipitation variables). However, they were not significant. The model with daily precipitation (Figure 7) showed significance for precipitation (estimate = -0.09, p-value = 0.0021) and for Site 2 (estimate = 3.53, p-value = 0.0005). However, model selection eliminated all the variables. This model explained 9.4 % of the variance. The model using

precipitation for one day before (Figure 8) was significant for precipitation (estimate = -0.13, p-value = 0.021). This model explained 14.9% of the variance. AIC selection model only retained precipitation (estimate = -0.12, p-value = 0.0202, 8% of the variance). We also tried predicting intensity with precipitation 2 days before capture (estimate = -0.051, p-value = 0.0395) and the monthly precipitation (estimate = 0.005, p-value = 0.378). Accumulated and average precipitation 2 and 3 days before capture also showed to be significant (negative relation).

DISCUSSION

We found a high prevalence in *G. plebejus,* and infection from three lineages of *Haemoproteus* (one of which is a new lineage) and two lineages of *Plasmodium.* Both higher prevalence and parasitemia was found for *Haemoproteus*. We also produced a series of Generalized Linear Models to assess what abiotic or biotic variables were related to the infection status or parasitemia in infected individuals. We found the variables we hypothesized as important at the beginning of the study, were not good predictors in the different models produced.

The high total levels of prevalence in *G. plebejus* (81.3%) could be explained by the high abundance of the host in the study area (second most abundant) since prevalence usually increases alongside local host abundance (Matthews et al., 2016). Further studies of less abundant species in the community is required to assess if this pattern is maintained. Also, *Haemoproteus* showed a higher prevalence than *Plasmodium.* Usually the most prevalent parasites tend to be the ones that are more host-generalist (Galen & Witt, 2014). Even though *Haemoproteus* is regarded as a host-conservative parasite (Clark et al., 2014), cases of host-generalism have been reported (Illera et al., 2017; Clark et al., 2014), and high levels of host generalization and an ample host range for parasite lineages of *Haemoproteus* have been previously found in Ecuador (Möens & Pérez-Tris, 2016). Cadena et al. (2019) also found the same pattern of higher prevalence of *Haemoproteus* infecting *Z. capensis* in the same community.

The most prevalent *Haemoproteus* lineage was JQ988544. This lineage has not been reported in MalAvi, and NCBI BLASTn showed it has only been reported once in *Amazilia viridicauda* (unpublished data, accession code JQ988544), a hummingbird native of the Peruvian Andes (Weller & Boesman, 2019). The capacity to infect Apodiformes and

Passeriformes in different geographical areas may indicate this lineage is host-generalist. *Haemoproteus* lineage MK216030/ZOCAP08 had the lowest prevalence for this population and one of the lowest mean parasitemia values. However, it was reported to be the most prevalent (51.4%) parasite in *Zonotrichia capensis* by Cadena-Ortiz et al. (2019) in the same study area, and is reported to infect *Zonotrichia capensis* in other areas (Fecchio et al., 2019; Jones et al., 2013). The low prevalence of MK216030/ZOCAP08 in *G. plebejus* suggests that it might be host-specialist of *Z. capensis* that spilled over to *G. plebejus*. It could also be the case that it is virulent for *G. plebejus* and not for *Z. capensis.* Another possibility is that *G. plebejus* is not an optimal host for the reproduction of the parasite (mean parasitemia was 3.42 infected erythrocytes).

The new *Haemoproteus* lineage (GEPLE01) presented a low prevalence and the lowest mean parasitemia of the *Haemoproteus* lineages. The low levels of prevalence and parasitemia could point towards a sporadic and uncommon infection of the host, especially since it is believed to mainly infect Parulidae (Gibb et al., 2005; Burry-Caines & Bennett, 1992). *Plasmodium* lineages KF537281/ZOCAP11 and MK077679/ZOCAP15 had the lowest prevalence of all the samples and the second lowest mean parasitemia values (Table 1). Cadena-Ortiz et al. (2019) reported ZOCAP11 as the second most prevalent lineage in *Z. capensis* in BPJ. This lineage has been found also in *Z. capensis* in another study in the Andes of Colombia (González et al., 2015). The low prevalence of this lineage in the present study could mean a higher virulence towards *G. plebejus* or non-competence of the host. Finally, ZOCAP15, was reported by Cadena-Ortiz et al. (2019) as a new lineage. In their study, an individual infected with this lineage had one of the highest parasitemia values, but in this study its mean parasitemia was the lowest of both genera. The capacity of ZOCAP15 to effectively establish an infection may be lower for *G. plebejus*.

According to the phylogeny JQ988544 and MK216030/ ZOCAP08 grouped with *H. coatneyi* and *Haemoproteus* (P.) sp. 1 (ZC1) respectively, and the new lineage (GEPLE01) grouped with *H. paruli*. *Haemoproteus coatneyi* can infect a large range of hosts from different families (Thraupidae, Trochilidae, Tyrannidae, Icteridae) (Svensson-Coelho, 2013), whereas *H. paruli* is known to infect the family Parulidae, and share the same living range of the parulids in the Americas (Burry-Caines & Bennett, 1992). On the other hand, the *Plasmodium* lineage KF537281/ZOCAP11 is placed within *P. homopolare* and lineage MK077679/ZOCAP15 is placed within *P. cathemerium*. *Plasmodium homopolare* is known to infect a wide range of species (Emberizidae, Parulidae, Thraupidae) (Walther et al., 2014). It belongs to the subgenus *Novyella*, and not much is known about its virulence, life cycle, or ecology other than that it is more prevalent in the tropics (Valkiūnas & Iezhova, 2018), common in passerines, and rarely virulent (Schoener et al., 2014). *Plasmodium cathemerium,* in turn is a generalist species and is believed to be more than mildly virulent (Vanstreels et al., 2015; Atkinson & Van Riper, 1991). Further molecular studies of multiple infections of *P. cathemerium* are of special interests given the partial immunity that it has been reported to provide to the host towards other *Plasmodium* species (Redmond, 1939).

Our GLM results showed that age and sex of the host did not predict prevalence or parasitemia. This pattern may be caused by the high number of infected individuals and the great variance of the parasitemia in those infected (Figures 4–7). However, some authors argue that while male sex may drive higher prevalence, the level of sexual dimorphism is a better predictor (Svensson-Coelho et al., 2013), because higher levels of testosterone are invested in maintaining higher levels of dimorphism (Mougeot et al., 2005). Since our chosen host displays a low level of dimorphism (Ridgely & Greenfield, 2001), sex may not be able to predict infection status in this species. Other studies have also found no relation between

sex or age, and infection (Ribeiro et al., 2005). We did find significant differences in sampling area, since Site 2 was predominantly higher in mean parasitemia. This result may reflect a relationship between microclimates, landscape features, and parasitemia. Nevertheless, Site 2 was characterized by having non-native vegetation, this is opposite to what was found in *Z. capensis* (Cadena-Ortiz et al., 2019), suggesting mosquitoes biting one species might avoid the other.

The negative relation between precipitation and infection status and intensity was intriguing and unexpected. These trends are opposite to those described by Cadena-Ortiz et al. (2019) in *Z. capensis* in BPJ. However, similar trends have been described before in dry mountain systems for *Haemoproteus* and *Leucocytozoon* (Illera et al., 2017). If these relations are real, they could reflect climatic adaptations of the lineages to their particular habitat, such as moderate precipitation values (Illera et al., 2017). Nevertheless, some of the lineages infecting *G. plebejus* were also infecting *Z. capensis.* Monthly precipitation estimates also produced models with non-significant positive relation. They were obtained with both INAMHI and TRMM precipitation values. TRMM precipitation values are regarded as reliable estimates although they have been noted to underestimate precipitation in the Andes and during El Niño periods (Erazo et al., 2018), and INAMHI precipitation was the variable used by Cadena-Ortiz et al. (2019). These findings could suggest different reactions to precipitation depending on the scale of the measurement. However, we suggest that future studies increase sampling effort in order to provide a higher number of replicates to the models and include local measures of precipitation.

Furthermore, we suggest that future studies consider other variables that might result in better fit models and predictors of status and intensity of infection, as well as patterns of infection and parasitemia in the community as a whole (given the seemingly contradictory

results between the two most prevalent species in the study area). It has been suggested that variables like daily temperature variability, mean temperature during the vector maturation process, mean temperature during the vector extrinsic incubation period, and water persistence could be more relevant than the usually used variables (i.e.: absolute total precipitation and temperature) when predicting infection (Stresman, 2010). Also, *Plasmodium* infections are reported to vary between dietary guilds, being more prevalent in insectivorous hosts (Ribeiro et al., 2005). Other variables that could be considered are: vegetation type and other local landscape features, abundance of vectors, proximity to ponds, vector seasonality, type of nest, roosting microhabitat and foraging height (for different species). An assessment of the whole community including the abovementioned variables could better reveal the factors that drive infection status and parasitemia in this dry Andean forest.

CONCLUSIONS

We found that the common trend of higher prevalence and parasitemia of *Plasmodium* in the Neotropics does not apply to every ecosystem. We found three lineages of *Haemoproteus* infecting *G. plebejus* (one of those a new lineage), and two lineages of *Plasmodium.* Both lineages of *Plasmodium* are believed to be virulent, however, their prevalence and parasitemia in the population was minimal. Three lineages, ZOCAP08 (*Haemoproteus*), ZOCAP11 (*Plasmodium*), and ZOCAP15 (*Plasmodium*) were also found infecting *Z. capensis,* in the same community, which suggests that they are not host-specific parasites. Their low prevalence and parasitemia also suggests that *G. plebejus* is not their optimal host. We also found high levels of haemosporidian prevalence that could be driven by the abundance of the host.

Sex, age, and physical condition did not predict neither parasitemia nor infection status. However, we found a negative relationship with some variables of precipitation (sampling day, 1 and 2 days before sampling). We found that the model for parasitemia using monthly estimates provided a positive relation that was not statistically significant.

Our models explained very little variance of the system. We suggest that future studies include other variables that could produce better fit models to predict prevalence and parasitemia such as daily temperature variability, vegetation type, water persistence, foraging height, among others mentioned in the Discussion section. Future studies would also benefit from a bigger sample effort and an analysis of the whole community.

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ANEXO A: TABLES

Table 1. Prevalence and mean parasitemia with their corresponding confidence intervals[a].

Species/Lineages[b]	Prevalence[c]	Prevalence 95% CI	Prevalence 99% CI	Mean parasitemia[d]	Mean parasitemia 95% CI	Mean parasitemia 99% CI
Total samples	0.813	0.6965–0.8929	0.6597–0.9164	62.38	45.17–87.56	41.57–95.53
Haemoproteus sp.	0.578	0.4529–0.6964	0.4122–0.7317	82.14	59.49–111.57	55.49–122.19
JQ988544/-	0.438	0.3194–0.5628	0.2846–0.6035	41.3	26.86–62.55	23.52–73.63
MK216030/ZOCAP08	0.016	0.0009–0.0833	0.0002–0.1087	3.42	0.00–10.27	0.00–17.11
New lineage	0.125	0.0587–0.2325	0.0470–0.2682	2.77	0.80–7.52	0.48–8.19
Plasmodium sp.	0.125	0.0587–0.2325	0.0470–0.2682	22	7.75–55.25	5.63–57.63
KF537281/ZOCAP11	0.094	0.0417–0.1933	0.0287–0.2282	2.73	0.64–8.16	0.42–9.61
MK077679/ ZOCAP15	0.031	0.0056–0.1070	0.0024–0.1398	0.02	0.00–0.05	0.0–0.08

a. Prevalence, mean parasitemia and the confidence intervals calculated with Quantitative Parasitology 3.0.
b. Lineages are named after the NCBI accession code/MalAvi lineage code.
c. Prevalence was calculated using positive PCR results. Confidence intervals were calculated using Sterne's method.
d. Mean parasitemia was calculated using positive PCR results. Confidence intervals were calculated using The Bias-Corrected and accelerated (BCa) bootstrap interval method. In red appear uncertain confidence intervals due to small number of replicates.

Table 2. Estimate and p-values for the variables of the logistic model for infection status using monthly precipitation from INAMHI.

Variable	Estimate	p-value
Intercept	2.49321	0.00932
Sex (male)	-0.01821	0.98184
Age (Juvenile)	-0.66951	0.58651
Site (3)	-1.72650	0.11513
Physical condition	-0.12107	0.69881
Monthly precipitation	-0.01199	0.28167

Table 3. Estimate and p-values for the variables of the negative binomial model for parasitemia using monthly precipitation from INAMHI.

Variable	Estimate	p-value
Intercept	4.419787	<2e-16
Sex (male)	-0.133609	0.772
Age (juvenile)	-0.909909	0.237
Site (3)	-0.785233	0.22
Physical condition	-0.050049	0.76
Monthly precipitation	-0.002044	0.757

Table 4. Estimate and p-values for the variables of the negative binomial model for parasitemia in *Haemoproteus* using monthly precipitation from INAMHI.

Variable	Estimate	p-value
Intercept	4.104114	<2e-16
Sex (male)	0.033626	0.947
Age (juvenile)	-0.132881	0.882
Site (2)	0.715725	0.308
Physical condition	-0.061366	0.688
Monthly precipitation	0.002801	0.689

ANEXO B: FIGURES

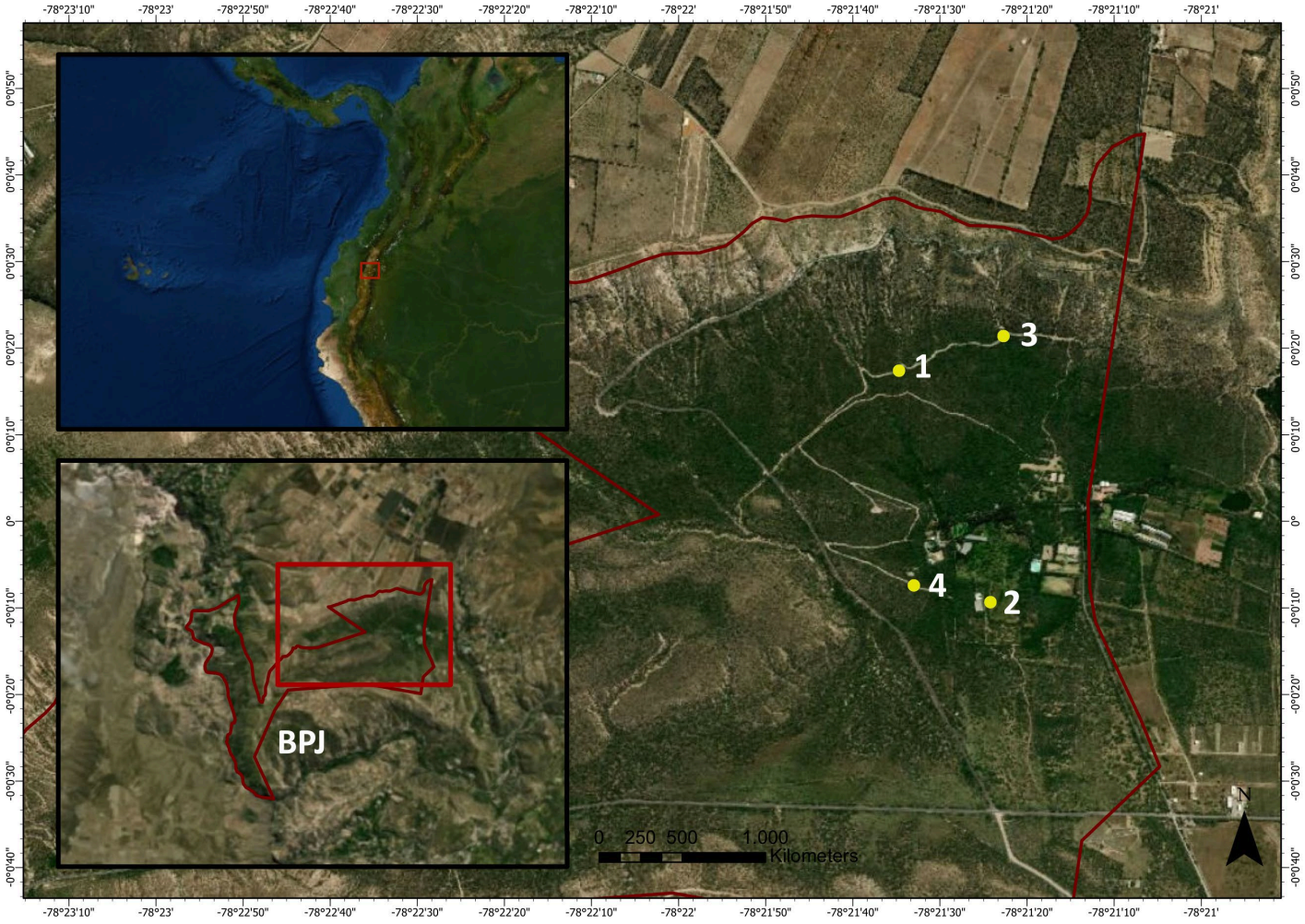

Figure 1: Map of the study area showing the sampling sites (yellow dots) within BPJ (dark red contour).

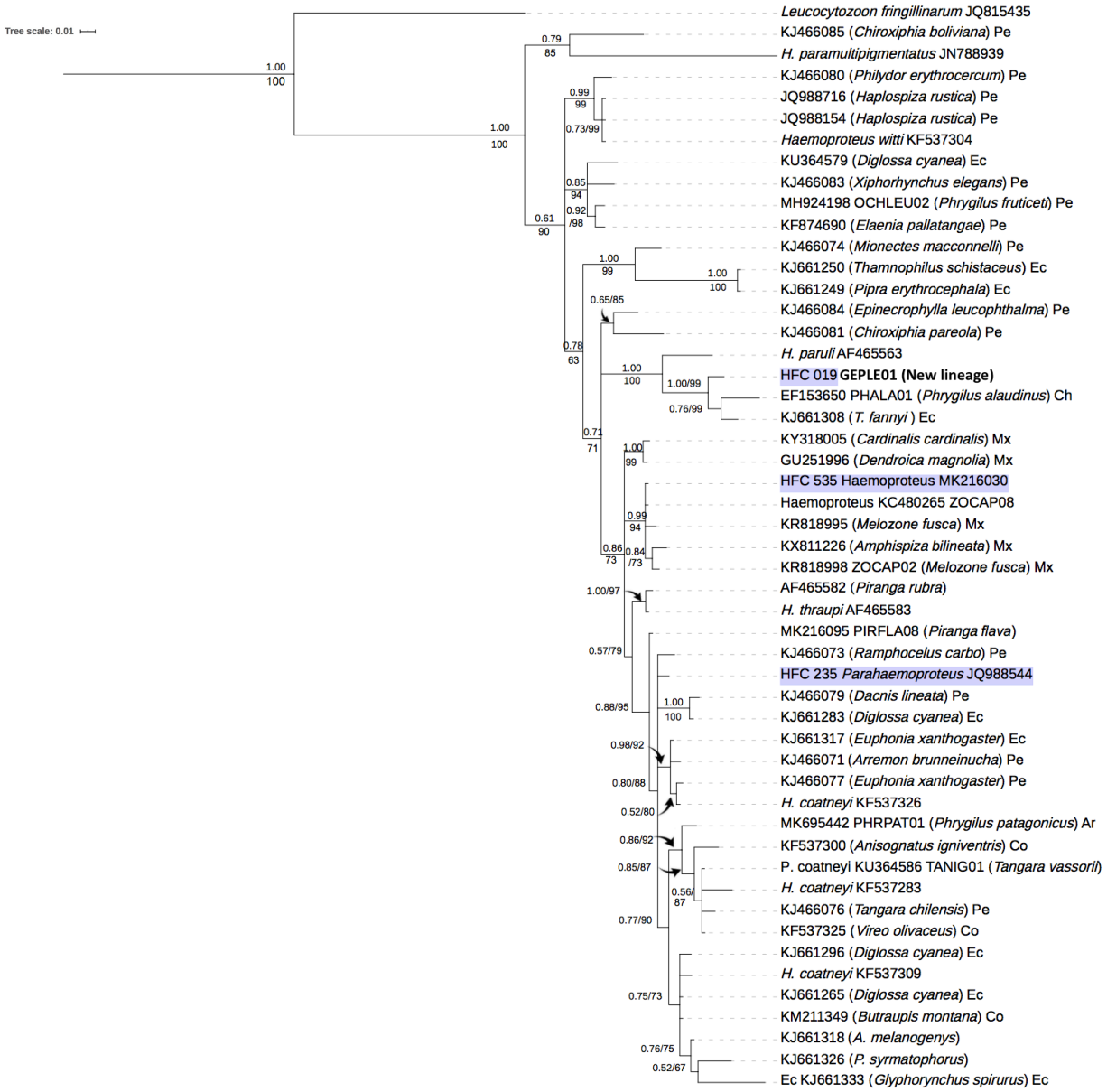

Figure 2. Bayesian inference phylogenetic tree from mtDNA cytochrome b gene for *Haemoproteus*. sequences. Numbers on branches represent nodal support values (Bayesian posterior probabilities/maximum likelihood bootstrap support). Highlighted labels are the lineages sampled. Labels appear in the following order: *parasite species*, NCBI accession code, MalAvi lineage code, (*Host species*), country. Samples from the study are preceded by the sampling code of the sample used.

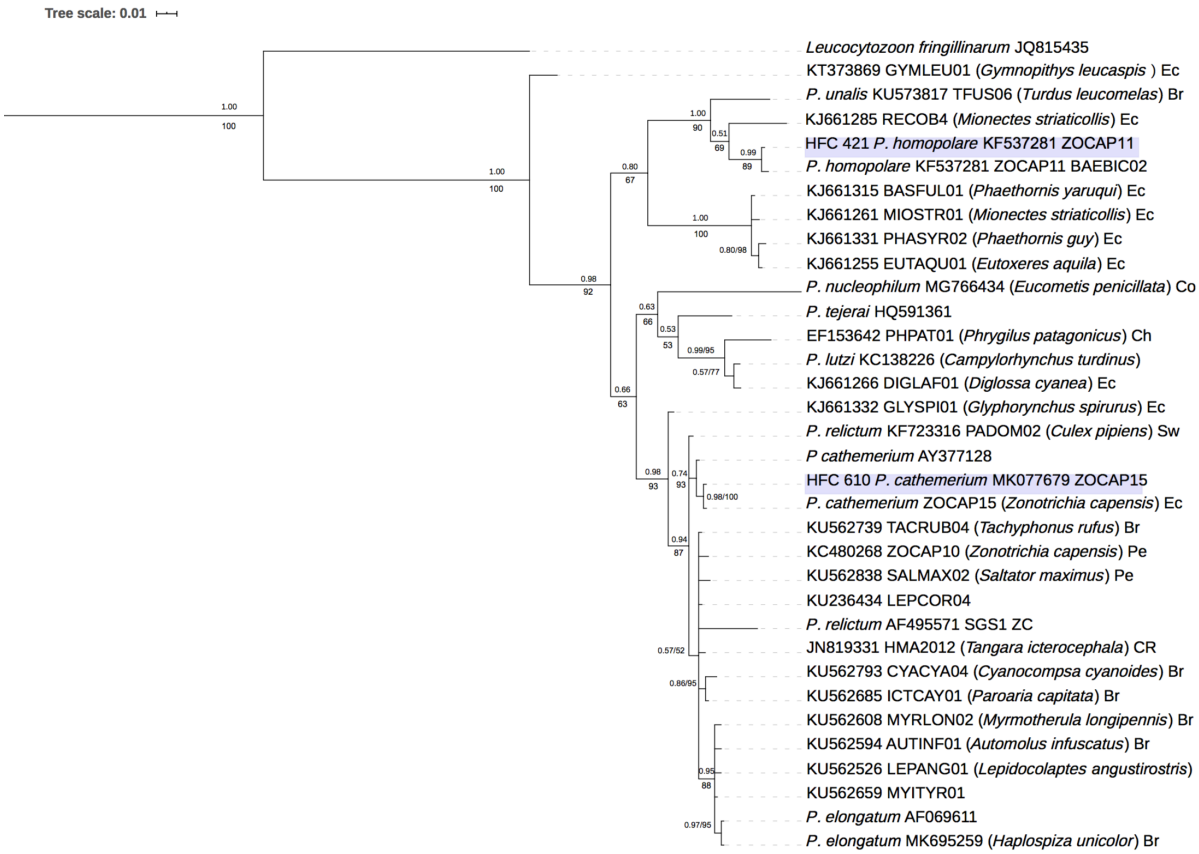

Figure 3. Bayesian inference phylogenetic tree from mtDNA cytochrome b gene for *Plasmodium* sequences. Numbers on branches represent nodal support values (Bayesian posterior probabilities/maximum likelihood bootstrap support). Highlighted labels are the lineages sampled. Labels appear in the following order: *parasite species*, NCBI accession code, MalAvi lineage code, (*host species*), country. Samples from the study are preceded by the sampling code of the sample used.

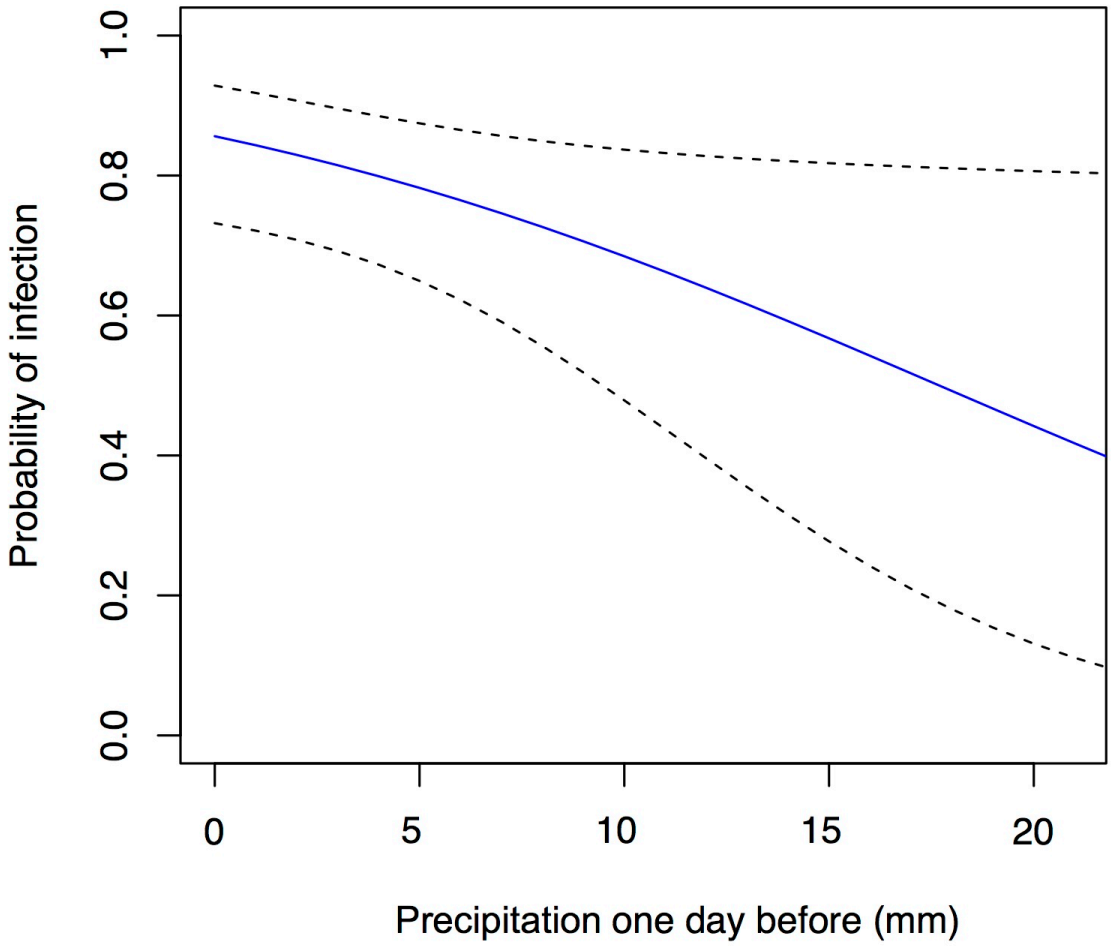

Figure 4. Logistic regression of prevalence predicted by precipitation the day before sampling

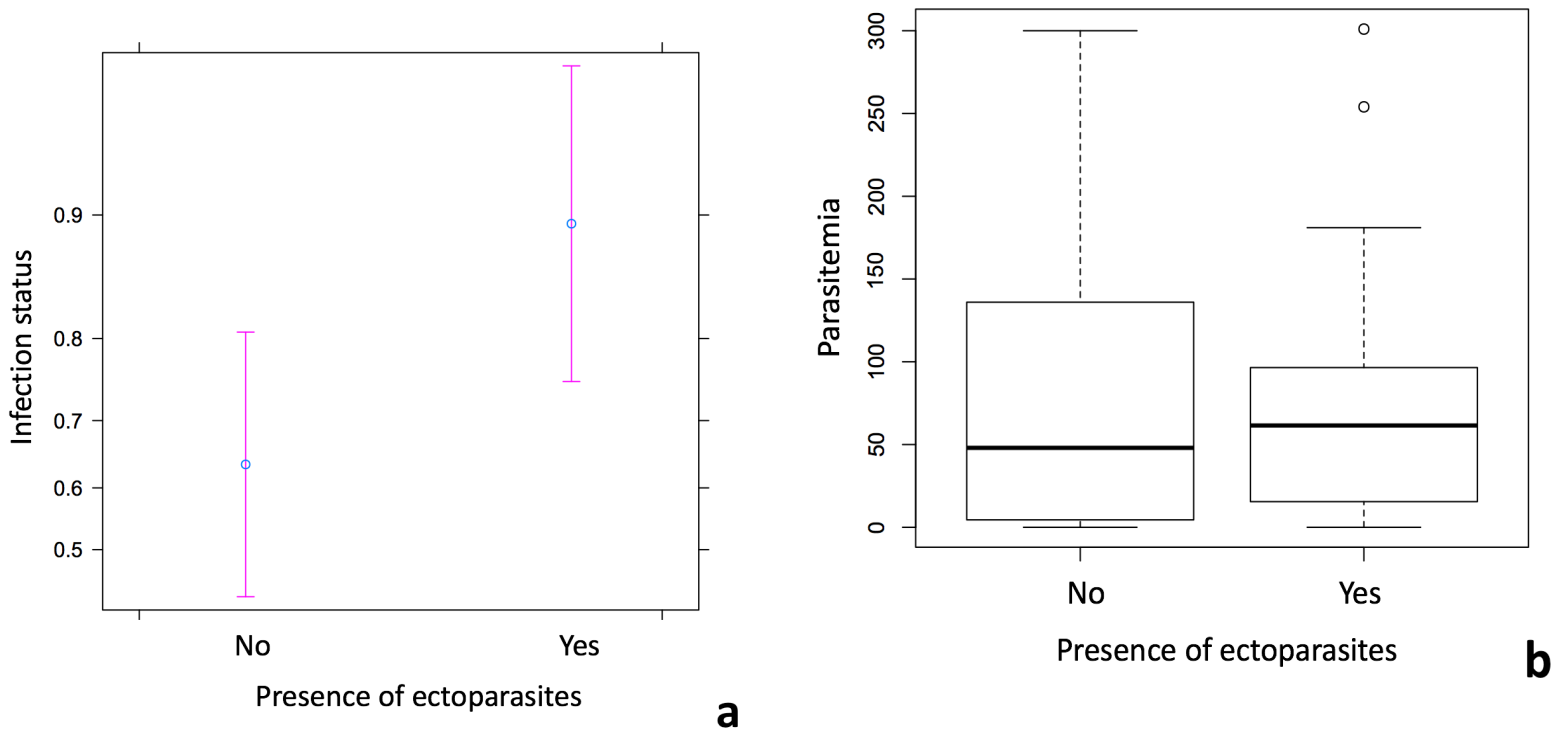

Figure 5. Effect plots of the presence of ectoparasites variable from the models for (a) infection status, and (b) parasitemia.

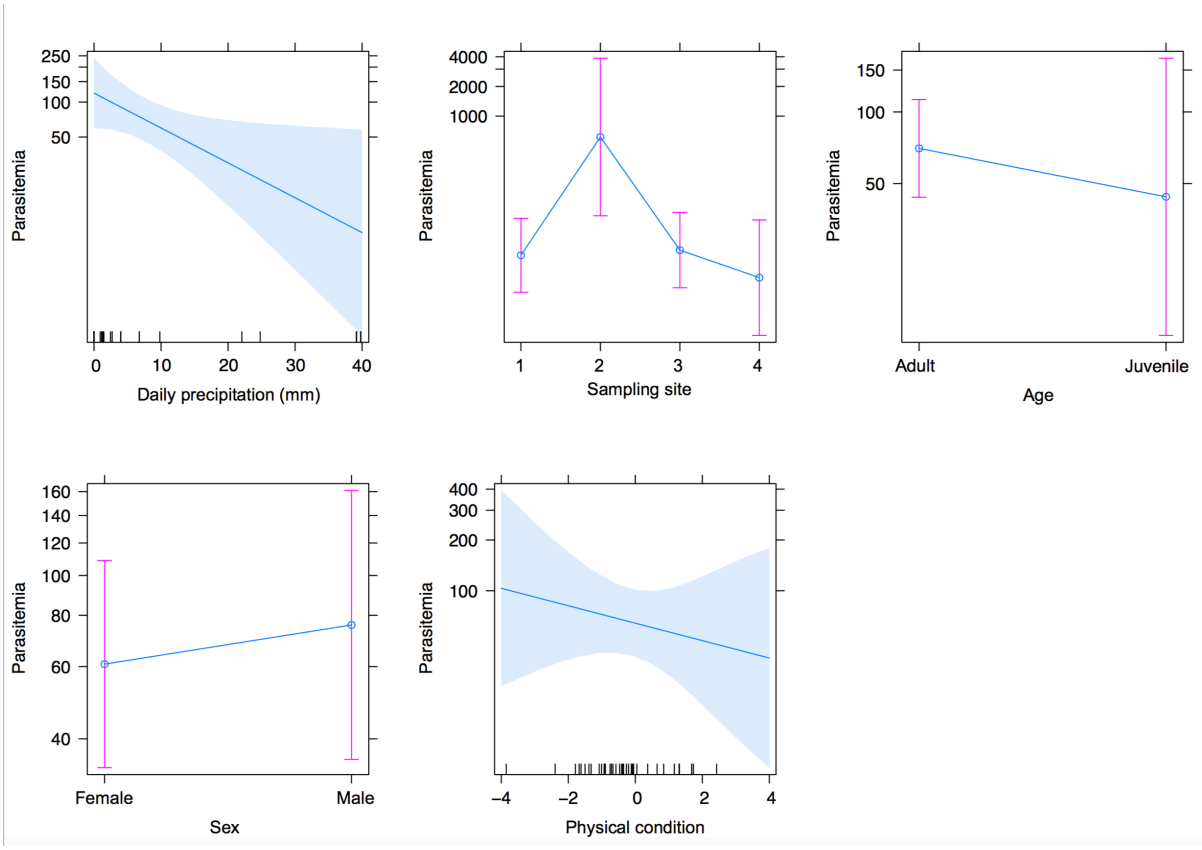

Figure 6. Effect plots from the negative binomial from the model explaining parasitemia with daily precipitation.

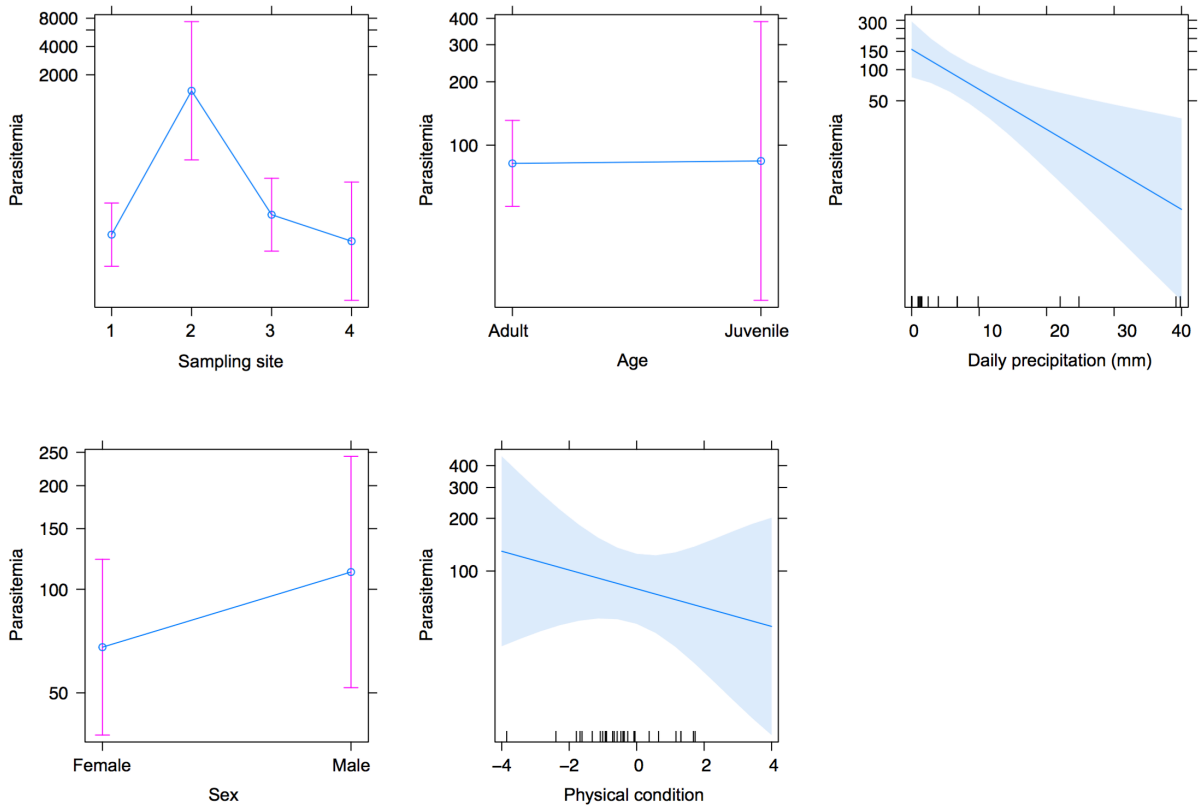

Figure 7. Effect plots from the negative binomial model explaining parasitemia for *Haemoproteus* lineages with daily precipitation.

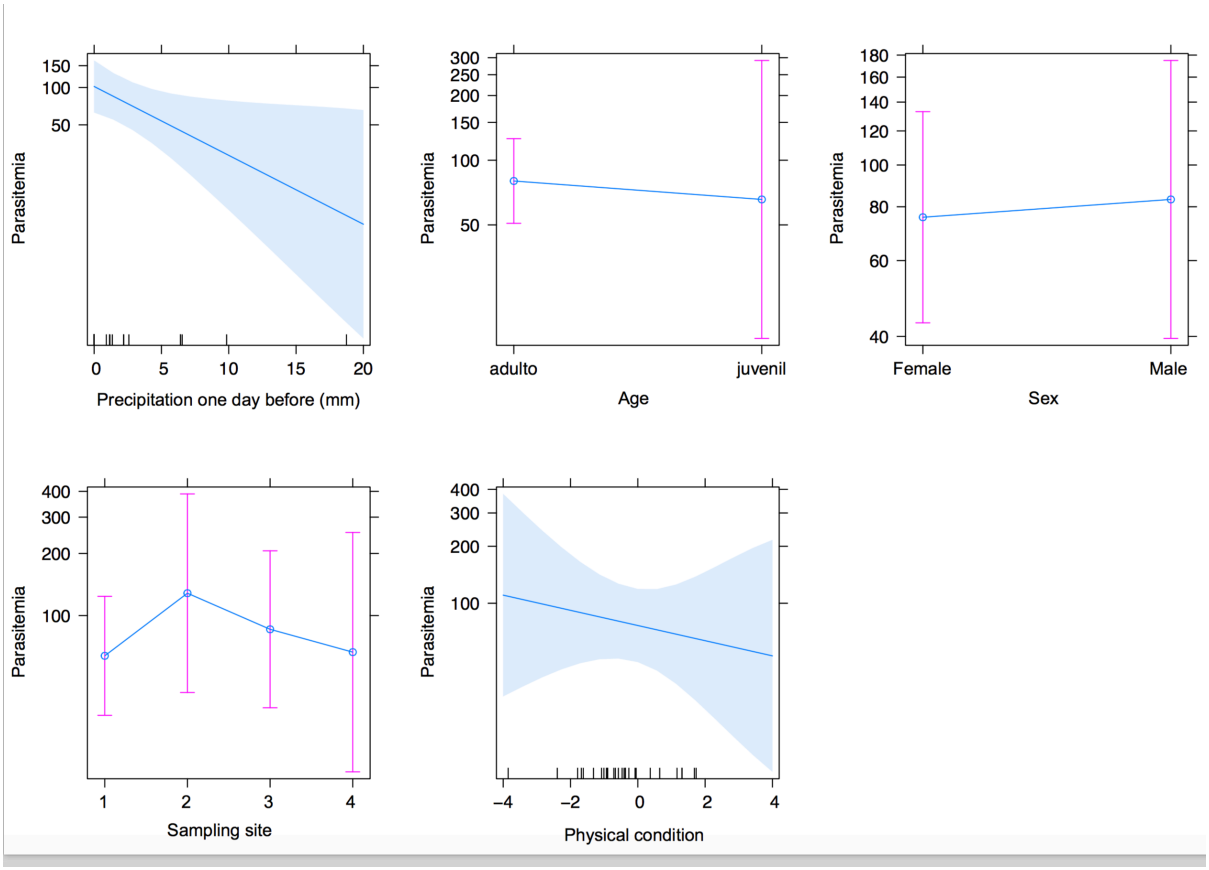

Figure 8. Effect plots from the negative model explaining parasitemia for *Haemoproteus* lineages with precipitation one day before the sampling day.