Host migration and environmental temperature influence avian haemosporidians prevalence: a molecular survey in a Brazilian Atlantic rainforest

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ABSTRACT

Avian haemosporidians are parasites with great capacity to spread to new environments and new hosts, being considered a good model to host-parasite interactions studies. Here, we examine avian haemosporidian parasites in a protected area covered by *Restinga* vegetation in northeastern Brazil, to test the hypothesis that haemosporidian prevalence is related to individual-level traits (age and breeding season), species-specific traits (diet, foraging strata, period of activity, species body weight, migratory status, and nest shape), and climate factors (temperature and rainfall). We screened DNA from 1,466 birds of 70 species captured monthly from April 2013 to March 2015. We detected an overall prevalence (*Plasmodium*/*Haemoproteus* infection) of 22% (44 host species) and parasite's lineages were identified by mitochondrial cyt *b* gene. Our results showed that migration can be an important factor predicting the prevalence of *Haemoproteus* (*Parahaemoproteus*), but not *Plasmodium*, in hosts. Besides, the temperature, but not rainfall, seems to predict the prevalence of *Plasmodium* in this bird community. Neither individual-level traits analyzed nor the other species-specific traits tested were related to the probability of a bird becoming infected by haemosporidians. Our results point the importance of conducting local studies in particular environments to understand the degree of generality of factors impacting parasite prevalence in bird communities. Despite our attempts to find patterns of infection in this bird community, we should be aware that an avian haemosporidian community organization is highly complex and this complexity can be attributed to an intricate net of factors, some of which were not observed in this study and should be evaluated in future studies. We evidence the importance of looking to host-parasite relationships in a more close scale, to assure that some effects may not be obfuscated by differences in host life-history.

Subjects Biodiversity, Conservation Biology, Ecology, Parasitology, Zoology
Keywords *Plasmodium*, *Haemoproteus*, Host-parasite interaction, Avian malaria, Parasite diversity

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INTRODUCTION

The avian haemosporidians of the genera *Plasmodium* and *Haemoproteus* are vector-borne parasites that infect a wide range of host species (*Hellgren, Pérez-Triz & Bensch, 2009*; *Ricklefs et al., 2014*) and have frequently switched to new host species and new environments throughout their evolutionary history (*Ricklefs, Fallon & Bermingham, 2004*; *Valkiūnas, 2005*; *Ricklefs et al., 2014*; *Ellis et al., 2019*). These intracellular parasites reproduce sexually in different dipteran vectors: mosquitoes (Culicidae) are vectors of *Plasmodium*, and biting midges (Ceratopogonidae) and hippoboscid flies (Hippoboscidae) are vectors of *Haemoproteus* (*Parahaemoproteus*) and *Haemoproteus* (*Haemoproteus*), respectively (*Valkiūnas, 2005*; *Santiago-Alarcon, Palinauskas & Schaefer, 2012*). Concerning their vertebrate hosts, *Plasmodium* and *Haemoproteus* (*Parahaemoproteus*) infect birds of various orders, while *Haemoproteus* (*Haemoproteus*) is more specific and infects birds of the order Columbiformes and some sea birds (*Work & Rameyer, 1996*; *Valkiūnas, 2005*; *Padilla et al., 2006*; *Levin & Parker, 2012*; *Levin et al., 2012*).

Haemosporidians are closely connected to their hosts in interaction with outcomes ranging from sublethal effects on the host fitness (*Ortego et al., 2008*; *Knowles, Palinauskas & Sheldon, 2010*) to the decline and extinction of populations (*van Riper et al., 1986*; *Atkinson et al., 1995*, *2000*). These parasites can exert selective pressure on host populations through effects on reproductive success, lifetime, and survival (*Hamilton & Zuk, 1982*; *Scott, 1988*; *Spencer et al., 2005*; *Asghar et al., 2015*; *Ricklefs et al., 2016*). Therefore, identifying the geographical distribution, host preferences, and infection prevalence of these parasites may help the development of appropriate management strategies to promote biodiversity conservation efforts worldwide.

Haemosporidian parasite prevalence, distribution, and richness vary widely across host species and can be affected by several factors. Prevalence may increase with age of host, since older individuals tend to have higher infection risk as a result of accumulated exposure to parasites or potentially immunosenescence (*Atkinson et al., 1995*; *Ricklefs et al., 2005*; *Wood et al., 2007*; *Eastwood et al., 2019*). However, this relationship is not consistent among studies and is not always observed, which may be indicative that it depends on the parasite and host species studied (*Wood et al., 2007*; *Antonini et al., 2019*). Shape and height of birds nest may also influence haemosporidian prevalence, nest height must be associated with the spatial feeding preferences of vectors when seeking hosts, while its shape must determine the birds' exposure to vectors (*Cerný, Votýpka & Svobodová, 2011*; *Fecchio et al., 2011*; *González et al., 2014*; *Lutz et al., 2015*; *Matthews et al., 2015*).

A migration strategy is another factor that may also influence haemosporidian prevalence in birds, since migratory species are exposed to different vectors and parasites as a consequence of their habitat change during their annual cycle (*Waldenström et al., 2002*; *Hellgren et al., 2013*; *Ricklefs et al., 2017*; *Slowinski et al., 2018*; *Pulgarín-R et al., 2019*; *Soares, Latta & Ricklefs, 2019*). This relationship does not occur evenly between parasites and host species, as can be seen in studies that have observed that migratory birds can be infected by parasites from their wintering grounds (*Waldenström et al., 2002*) or have

little or no haemosporidian lineage shared with resident birds (*Ricklefs et al., 2017*; *Pulgarín-R et al., 2019*). Likewise, the prevalence may be higher in migratory bird breeding (*Hellgren et al., 2013*) or wintering areas (*Pulgarín-R et al., 2019*).

Environmental factors can also be related to prevalence in a bird community. Seasonality, which involves temperature and rainfall, may influence vector infection dynamics and hence the haemosporidian transmission (*Medeiros et al., 2016*; *Ferreira Junior et al., 2017*; *Hernández-Lara, González-García & Santiago-Alarcon, 2017a*), and was even considered an important driver of host specialization (*Fecchio et al., 2019*). However, seasonality, in the same way as the host life-history traits, is not always linked to haemosporidian prevalence, not even in the tropics (*Ishtiaq, Bowden & Jhala, 2017*). Moreover, as seasonality is also associated with the breeding period of birds, a relationship could be observed between these two variables influencing the haemosporidian prevalence, since during bird's breeding season may be an increase in adult susceptibility to infections, due to changes in its behavior and immunity (*Drobney, Train & Fredrickson, 1983*; *Richner, Christe & Oppliger, 1995*; *Ardia, 2005*). Besides that, juveniles born throughout this time may also be more susceptible to infections (*Cosgrove et al., 2008*; *Møller, 2010*; *Santiago-Alarcon et al., 2011*; *Ferreira Junior et al., 2017*; *Rodrigues et al., 2020*). Therefore, many factors interact to determine the parasite prevalence in birds, revealing distinct results in studies testing the same relationships in different communities. This makes the knowledge of these interactions in different ecosystems valuable, to better understand the various factors that interact and influence the dynamics of parasite-host infection in natural environments.

Here, we tested whether the probability of an individual being infected with haemosporidian parasites is related to (1) its individual-level traits (i.e., age and breeding condition), which may influence the host immune defense mechanisms and individual exposure to vectors; (2) its species-specific traits (i.e., diet, foraging strata, period of activity, species body weight, migratory status, and nest shape), which may be linked to differential vector exposure; and (3) climate factors (i.e., temperature and rainfall), which might influence in vector abundance and richness. These hypotheses were tested in an area of Brazilian Restinga, a poorly sampled phytophiosionomy for hemoparasites, and therefore with a high potential to house new haemosporidian lineages.

MATERIALS & METHODS

Study site

The study was carried out in Barreira do Inferno Rocket Launch Center of the Brazilian Air Force (acronym in Portuguese, Centro de Lançamento Barreira do Inferno–CLBI; 5°55′S 35°9′W), a protected area of ~1,800 ha located in Parnamirim, State of Rio Grande do Norte, northeastern Brazil (Fig. 1). The area is in a tropical coastal vegetation region, named *Restinga*, which is a type of vegetation associated with the Atlantic Rainforest Domain (for more details about the study site, see *Rodrigues et al. (2020)*). Following the local climatic data (Fig. S1), we determined as rainy season the period that goes from March to August and, as dry season the period from September to February.

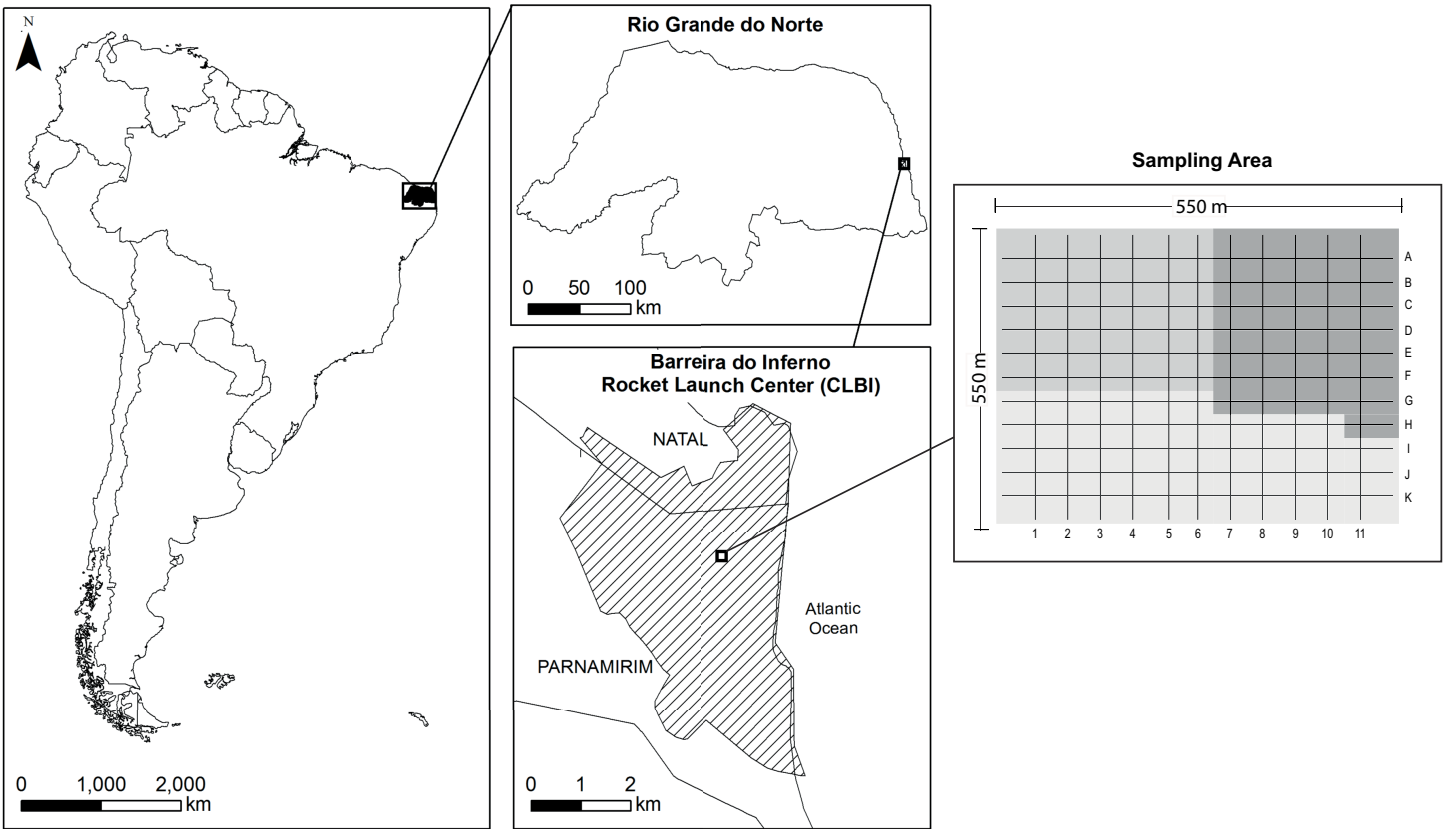

Figure 1 Map of the study site sampled for a bird community infected by Plasmodium and Haemoproteus, Barreira do Inferno Rocket Launch Center of the Brazilian Air Force, Parnamirim, State of Rio Grande do Norte, Brazil. We sampled 36 to 49 points per month in a cyclical way so that all points were sampled one time at every three month. These three sample areas are indicated by the different shades of gray in the image—two areas with 36 sample points and one area with 49 sample points. Full-size DOI: 10.7717/peerj.11555/fig-1

Bird sampling

We monitored monthly the haemosporidian infection in a bird community within a 30 ha plot (550 m × 550 m) from April 2013 to March 2015. The birds were captured following the protocol described in *Rodrigues et al. (2020)*. All captured birds were identified and banded with individual aluminum bands provided by CEMAVE/ICMBio (permission 3239). Recaptured birds were not used in this study. We evaluated birds for age (adult or young) based on their plumage, labial commissure, and cranial ossification; and the presence of brood patch, by visual examination of the birds. We obtained blood samples through brachial venipuncture with a sterile needle (13 × 4.5 mm) and stored the blood in filter paper kept at 4 °C until DNA extraction.

Our use of mist-nets and banding was approved by the Brazilian biodiversity monitoring agency (Institute Chico Mendes for Biodiversity Conservation—ICMBio, Brazilian National Center for Bird Conservation—CEMAVE, permit 3239). We followed standard ethical protocols for wildlife animals. Time handling the birds was kept to the minimum, and all birds were released after banding, data, and sample collection. This study was approved by the Ethics Committee in Animal Experimentation (CETEA), Universidade Federal de Minas Gerais, Brazil (Protocol #254/2011).

Parasite detection

The parasite detection followed the protocols described in *Rodrigues et al. (2020)*. Lineages without previous records in the database were considered new lineages and deposited in GenBank under accession numbers MH260577, MK291501, MK291502, MK291503, MK291504, MK291505, MK291506, and MK291507. New occurrences of sequences previously described were also deposited in GenBank under accession numbers MK264392, MK264393, MK264394, MK264395, MK264396, MK264397, MK264398, MK264399, MK264400, MK264401, MK264402, MK264403, MK264404, MK264405, MK264406, MK264407, MK264408, MK264409, MK264410, and MK264411.

Modeling the factors predicting avian malaria prevalence

To test which factors influence the haemosporidian prevalence in the bird community, we modeled the individual probability of infection separately for each parasite genus (*Plasmodium* and *Haemoproteus*), as a function of individual and species-level traits of hosts, as well as the climatic conditions. We chose to model the individual probability of infection rather than the prevalence in host populations (see *Fecchio et al., 2013*) since we have predictor variables at the individual level, which included age of host (adult or young) and breeding condition (breeding or non-breeding). The breeding condition was determined by the presence of a brood patch at the time of the capture. Species-specific traits included average body mass (g), migratory status (migratory or resident), nest shape (open-cup vs closed), diet (frugivore, nectarivore, granivore, insectivorous, omnivore, or combinations of two or three diets), foraging stratum (ground, understory, midheight, canopy, and combinations of two or three foraging strata), and period of activity (day or night). We obtained the species-specific traits data from Handbook of the Birds of the World Alive (*Hoyo, Elliott & Christie, 2011*) and, from *Wilman et al. (2014)*.
The classification of the diet was made considering the food item or the combination of food items that covered at least 80% of the total diet, based on the data from *Wilman et al. (2014)*, using at most three main food items per species of bird. We considered as migratory the species of birds with some type of seasonal displacement in the area, following the classification used by *Somenzari et al. (2018)*. Finally, we used mean monthly temperature and total monthly precipitation for the climatic variables (through the 24 months study), which were centered and scaled before analysis. We obtained the climate data from a Brazilian Meteorological Database for Education and Research (*INMET, 2017*). Because in some cases we were unable to obtain all the individual-level traits in the field, we removed from the dataset individuals with missing information to run the analysis.

We accounted for different types of pseudoreplication (*Hurlbert, 1984*) in our dataset. Phylogenetically related bird species, for instance, would have similar infection probabilities (*Ricklefs & Fallon, 2002*; *Waldenström et al., 2002*), as well as individuals captured at the same occasion (same month and same seasonality; *Kim & Tsuda, 2010*; *Ferreira Junior et al., 2017*). Therefore, to control for such potential dependences, we used the R package *lme4* (*Bates et al., 2015*; *R Core Team, 2016*; *Harrison et al., 2018*) to fit Generalized Linear Mixed Models (GLMM; *Bolker et al., 2009*). The infection status was

recorded as a binary response variable (0: uninfected; 1: infected) and modeled as a Bernoulli trial with a binomial distribution of errors and the logit link function. The hosts individual- and species-level traits, and the climatic conditions entered as fixed factors, without any interactions; and the temporal (season and month) and phylogenetic (order, family, gender, species) factors entered as random factors–nested within each dimension (temporal: month nested in season; phylogenetic: species nested in the genus, and genus nested in the family) and crossed among dimensions. Here we used data referring to the taxonomic classification of birds, considering that this is similar to the currently known phylogenetic classification. The random effects were modeled affecting only the intercept, but not the slope of the model.

The final model was obtained by backward selection of the fixed factors only–the random structure was maintained complete in all models (*Barr et al., 2013*). Starting from the full model, we used the likelihood ratio test to remove the fixed factors that do not contribute significantly to the model fit (*Crawley, 2013*). The likelihood ratio test compares the data likelihood under the full model against the data likelihood under a model with fewer factors and was performed using an analysis of variance (ANOVA) performed by the anova function. In each step, we removed the fixed factors that explained the small part of the deviance. We used the *r.squaredGLMM* function implemented in the R package *MuMIn* (*Barton, 2018*) to compute both the marginal and conditional R^2 for the final model; and the *icc* function implemented in the R package *sjstats* to compute the adjusted intraclass-correlation of the random factors. The marginal-R^2 gives the percentage of variance explained by the fixed factors, while the conditional-R^2 gives the total percentage of variance explained by the full model, including the fixed and the random factors (*Nakagawa & Schielzeth, 2013*). Finally, the adjusted intraclass correlation gives the percentage of the residual variance explained by each random factor (*Nakagawa, Johnson & Schielzeth, 2017*). The overdispersion test was not necessary, because an overdispersion test does not make sense with a binary response variable (*Crawley, 2013*). All these analyses were made separately for each parasite genus (i.e., *Plasmodium* and *Haemoproteus*), but we included in the dataset only host species with at least one individual infected by the parasite genus that was being analyzed on each occasion.

RESULTS

Overall malaria prevalence

We captured 1,466 individual birds of 25 families and 70 bird species, of which 322 (22%, 44 species) were infected by *Plasmodium*/*Haemoproteus*. All samples that screened positive were subjected to the cytochrome *b* PCR, which successfully amplified infections from 145 individuals. We obtained high-quality sequences from 117 samples. This is a well-established methodology for detecting haemosporidians that has been successfully applied in many other studies (e.g. *Lacorte et al., 2013*; *Fecchio et al., 2017a*; *Ferreira Junior et al., 2017*; *Ricklefs et al., 2017*; *Ferreira et al., 2020*; *Lopes et al., 2020*; *Rodrigues et al., 2020*; *Soares, Young & Ricklefs, 2020*), allowing us to observe the parasite prevalence and richness in a host community and compare the identified lineages with other haemosporidian studies around the world. Unfortunately, we were not able to collect and analyze blood

smears from the captured birds, which would greatly enrich our findings and allow us to assess the discrepancy between the number of positive samples and the number of successfully sequenced lineages. We detected *Plasmodium* in 35 individuals of 18 species (Table 1) and *Haemoproteus* infections were detected in 67 individuals of 15 species (Table 2). Among more highly-captured species ($n \geq 7$), the highest prevalence of infection were detected in *Cyclarhis gujanensis* (n = 9/14, 64.3%), *Tachyphonus rufus* (n = 69/108, 64%), and *Columbina passerina* (n = 11/21, 52.4%). The majority of bird species caught is resident in the region, but we have captured 15 migratory species of which nine were infected (*Elaenia* spp. [3 species; n = 69/431], *Myiarchus tyrannulus* [1/4], *Turdus amaurochalinus* [23/164], *Schistochlamys ruficapillus* [4/13], *Turdus flavipes* [3/13], *Cyanerpes cyaneus* [1/1] and *Vireo chivi* [5/10]; Table S1).

Haemosporidian diversity

We recovered 27 cyt *b* lineages from 117 individuals, of which 18 were *Plasmodium* lineages, detected in 35 birds (18 species), and 9 were *Haemoproteus* lineages, detected in 67 birds. Fifteen of the 117 high-quality sequences exhibited multiple infections, based on double peaks in the chromatograms, and were removed from the dataset. Among the *Haemoproteus* lineages, 3 were *Haemoproteus* (*Haemoproteus*) lineages detected in 9 birds (2 species), and 6 were *Haemoproteus* (*Parahaemoproteus*) lineages detected in 58 birds (13 species), as shown in Table 2. A total of eight lineages were detected here for the first time (five *Plasmodium* and three *H.* (*Parahaemoproteus*)). Of the 27 lineages, 14 (52%) were detected only once. Of the 13 lineages detected at least twice, eight (30%) were found in more than one host species (Table 1 and Table 2). Most of the lineages detected in only one host species were found in only one individual (14 lineages), and the remaining lineages were found in two (2 lineages), three (1 lineage), or four (2 lineages) individuals.

Despite the higher richness of *Plasmodium* lineages detected in birds, there was a higher number of birds infected by *Haemoproteus* (35 and 67 birds, respectively), mainly by *H.* (*Parahaemoproteus*). The most prevalent *Plasmodium* lineage was BAFLA04, detected in 7 birds, being four captured at the rainy season and three at the dry season. Among the *Parahaemoproteus* lineages, we highlight the lineage TARUF02, which was detected here for the first time in 44 birds (21 at the rainy season and 23 at the dry season) and have an apparent preference in infecting birds of the species *Tachyphonus rufus*. Of the total 44 birds infected by this lineage, 37 were *T. rufus* species, and the other seven occurrences of this lineage were detected in seven different bird species (Table 1 and Table 2). Besides, we recorded only two infections by other lineages in *T. rufus* species (PADOM11 and BAFLA04).

Factors predicting haemosporidian prevalence

Our final dataset for prevalence analysis, after excluding individuals with missing data, included 1,187 individual birds. Although the subgenus *Haemoproteus* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) are classified within the same genus, they are very different concerning their vertebrate hosts and vectors (*Valkiūnas, 2005*), which made us consider it important to treat these two groups differently in our study. However, as the number of

Table 1 Distribution of *Plasmodium* lineages across bird species captured in Barreira do Inferno Rocket Launch Center of the Brazilian Air Force, Parnamirim, State of Rio Grande do Norte, Brazil.

Bird species	*Plasmodium* lineages									
	BAFLA03	BAFLA04	CALON01	CPCT57	DENPET03	FOGRI01	FOMEL04	H012	HYAMA01	
Cantorchilus longirostris (25)			2							
Coereba flaveola (114)		3			3					
Cyanocorax cyanopogon (2)								1		
Cyclarhis gujanensis (14)										
Elaenia chilensis (244)	1	1								
Elaenia spectabilis (9)		1								
Formicivora grisea (10)						1				
Formicivora melanogaster (9)							1			
Herpsilochmus pectoralis (24)										
Herpsilochmus sellowi (27)										
Hylophilus amaurocephalus (29)									1	
Leptotila verreauxi (9)		1								
Piaya cayana (8)				1						
Polioptila plúmbea (15)										
Tachyphonus rufus (108)		1								
Turdus amaurochalinus (156)					1					
Turdus flavipes (11)					1					
Turdus leucomelas (98)										

Bird species	*Plasmodium* lineages									Total
	LECOR02	PADOM09	PADOM11	PADOM17	PAMIT01	POPLU01	TUAMA01	TURNUD02	U12	
Cantorchilus longirostris (25)										2
Coereba flaveola (114)		1								7
Cyanocorax cyanopogon (2)					1					1
Cyclarhis gujanensis (14)					1					1
Elaenia chilensis (244)		1		1				1		5
Elaenia spectabilis (9)										1
Formicivora grisea (10)										1
Formicivora melanogaster (9)										1
Herpsilochmus pectoralis (24)	1									1
Herpsilochmus sellowi (27)	1									1
Hylophilus amaurocephalus (29)										1
Leptotila verreauxi (9)										1
Piaya cayana (8)										1
Polioptila plúmbea (15)		1				1				2
Tachyphonus rufus (108)			1							2
Turdus amaurochalinus (156)							1		1	3
Turdus flavipes (11)		1								2
Turdus leucomelas (98)					1					1

Note:
The number of individuals captured for each species is denoted in parentheses.

Table 2 Distribution of *Haemoproteus* lineages across bird species captured in Barreira do Inferno Rocket Launch Center of the Brazilian Air Force, Parnamirim, State of Rio Grande do Norte, Brazil.

		Haemoproteus lineages								
Bird species	**Família**	**COTAL01**	**ELALB01**	**NYMAC01**	**PAPOL03**	**SocH3**	**SocH4**	**TARUF02**	**UN203**	**VIREO02**	**Total**
Coereba flaveola (114)	Thraupidae							1			1
Columbina passerina (21)	Columbidae					4	1				5
Columbina talpacoti (14)	Columbidae	4									4
Coryphospingus pileatus (5)	Thraupidae							1			1
Cyclarhis gujanensis (14)	Vireonidae							1	5		6
Elaenia chilensis (244)	Tyrannidae		1						1		2
Formicivora grisea (10)	Thamnophilidae							1			1
Myiarchus tyrannulus (4)	Tyrannidae		1								1
Neopelma pallescens (27)	Pipridae							1			1
Nystalus maculatus (10)	Bucconidae			3							3
Pachyramphus polychopterus (7)	Tityridae				2						2
Tachyphonus rufus (108)	Thraupidae							37			37
Tangara cayana (67)	Thraupidae							1			1
Turdus flavipes (11)	Turdidae							1			1
Vireo chivi (10)	Vireonidae									1	1

Note:
The number of individuals captured for each species is denoted in parentheses. SocH3, SocH4 and COTAL01 are *Haemoproteus* (*Haemoproteus*) lineages. ELALB01, NYMAC01, PAPOL03, TARUF02, UN203 and VIREO02 are *Haemoproteus* (*Parahaemoproteus*) lineages.

Table 3 The parameters of the minimal models (binomial GLMMs) explaining the probability of infection by *Plasmodium* sp. and *Parahaemoproteus* sp.

Parasite genus	Main effects	Estimate	Std. Error	Odds ratio	Z value	*P* value	$R2_m$	$R2_c$
Plasmodium	Intercept	−2.85	0.32	0.05	−8.69	2e−16*	0.08	0.24
	Temperature	0.59	0.21	1.80	2.73	0.0060*		
Parahaemoproteus	Intercept	−0.70	0.19	0.49	−3.60	0.0003*	0.43	0.43
	Migratory	−3.37	0.54	0.03	−6.42	4.32e−10*		

Note:
Both models have the same random structure (see Tables S1–S3).

birds infected by *H.* (*Haemoproteus*) was too low (n = 9), we only included in these analyses birds infected by *H.* (*Parahaemoproteus*).

The GLMM analysis indicated that the temperature influences the probability of infection by *Plasmodium* (Table S2), with the increase of one standard deviation on temperature (0.99 °C) resulting in an increase of 1.8 in the odds of infection by *Plasmodium* (the odds ratio, Table 3). However, the temperature is not an important factor influencing the probability of infection by *Parahaemoproteus* and neither *Plasmodium* nor *Parahaemoproteus* were influenced by rainfall.

The probability of infection was not influenced by any of the individual host traits tested, such as age and breeding condition for both parasite genus. Besides, when considering species-specific traits, only the probability of infection by *Parahaemoproteus*

was affected by the species migratory status (Table S3), with the odds of be infected by *Parahaemoproteus* in migratory birds being 0.03 of that in non-migratory birds. However, there was no influence on the probability of infection by any haemosporidian when considering diet, foraging stratum, period of activity, species body mass, and nest shape.

For *Parahaemoproteus* GLMM the ICC_{adj} was equal to zero for all random factors (Table S4), and, consequently, marginal and conditional R^2 were identical ($R^2_m = R^2_c = 0.43$, Table 3). This means that the random factors do not explain anything about the dispersion of model residuals. In this case, the GLMM collapse to a simple GLM. For *Plasmodium* GLMM, otherwise, the ICC_{adj} was also equal to zero for almost all random factors, except for random factor 'species' which was equal to 0.17 (Table S4), indicating that 17% of the residual variance is correlated within species. Thus, marginal and conditional R^2 were not identical ($R^2_m = 0.08$, and $R^2_c = 0.24$, Table 3) and, therefore, the species of the birds had a small influence on the probability of *Plasmodium* infection. This indicates that, apart from the other factors, birds of different species will have different probabilities of becoming infected by *Plasmodium*.

DISCUSSION

What does influence avian haemosporidian prevalence? Here, we found that migratory birds were less likely to be infected with *Haemoproteus* (*Parahaemoproteus*) when compared to resident birds and that the probability of infection by *Plasmodium* was positively influenced by temperature. By observing avian haemosporidians in a diverse region, and exploring how ecological variables are related to parasite infection probability in wild birds, we can compare these interactions we have found here with patterns of interactions already observed in avian communities in different contexts, to add knowledge that allows us to better understand infectious diseases in wild birds.

Migratory behavior of birds from CLBI had a significant and inverse association with the probability of infection by *Haemoproteus* (*Parahaemoproteus*), but there was no effect of migration on the probability of infection by *Plasmodium*. The current knowledge of the host specificity of the parasite lineages predicts that *Haemoproteus* parasites tend to be more host-specific than *Plasmodium* parasites (*Ishtiaq et al., 2007*, *2010*; *Dimitrov, Zehtindjiev & Bensch, 2010*). Therefore, it is possible that migratory birds are not suitable hosts for *Parahaemoproteus* lineages with which they geographically overlap during their annual cycle. This agrees with the study presented by *Hellgren et al. (2007)* showing that *Haemoproteus* and *Leucocytozoon* had a significant affiliation to a single resident bird fauna, while *Plasmodium* lineages showed a higher degree of infecting both resident and migratory bird species. If that is true in the studied bird community, migrants may be less likely to become infected by *Haemoproteus* in CLBI when compared to resident birds, due to the greater specificity this parasite presents concerning its local hosts. Because *Plasmodium* lineages are usually more generalist, they can probably infect migrants as well as resident birds, which would explain why we did not find the same influence of migratory behavior on the probability of infection by these parasites. If, on the one hand, the migration has the potential to increase the exposure of birds to parasites by concentrating individuals at breeding, overwintering or, migratory stopover sites (*Waldenström et al.,*

2002), on the other hand, migration could make possible the escape of birds from habitats where parasite transmission stages have accumulated, or selective removal of infected hosts during movements (Hall, Altizer & Bartel, 2014).

Alternatively, our results might be related to the hypothesis that the selection experienced by migratory birds in their breeding and wintering areas resulted in greater investment in immune defense (Møller & Erritzøe, 1998). If a bird individual is negative for haemosporidian infections, it might be because either the host individual is not infected, the parasite is dormant in tissues and not found in the bloodstream (Valkiūnas, 2005), or that it occurs in such low intensities in the avian blood that it is not detected by PCR screening. This former situation could be a cue that the bird was able to fight the infection, reducing its parasitemia. In that case, migrants having greater investment in immune defense could also have low parasitemia when infected.

Despite the absence of data about mosquitoes in CLBI to allow us to correctly evaluate the vector-parasite-host relationship, the positive association observed between temperature and *Plasmodium* prevalence may be related to the effects of temperature on the vectors of this parasite. For avian haemosporidians, the temperature is commonly described as an important abiotic factor influencing the parasite development and vector breeding opportunities (Beier, 1998; Santiago-Alarcon, Palinauskas & Schaefer, 2012; Medeiros et al., 2016; Mordecai et al., 2019). It has been demonstrated that the development of different malaria parasites in vectors can be influenced by the climate and is generally hampered by low increments in temperature (LaPointe, Goff & Atkinson, 2010; Zamora-Vilchis, Williams & Johnson, 2012). Temperature also determines the rate at which mosquitoes develop into adults, the frequency of their blood-feeding, and the rate at which parasites are acquired (Patz et al., 2000). Garamszegi (2011) has shown that a 1 °C increase in global temperature led to a two- to three-fold increase in the average prevalence of *Plasmodium* in birds. It is also demonstrated that studies made with samples from years and localities where temperature anomalies were strongly expressed generally detected higher *Plasmodium* prevalence than surveys based on samples that were less affected by temperature anomalies (Garamszegi, 2011). Sehgal et al. (2011), in a study conducted on Olive Sunbirds (*Cyanomitra olivacea*) in West and Central Africa, also showed an association between higher temperatures and elevated *Plasmodium* prevalence, with data indicating that the maximum temperature of the warmest month was the most important indicator for elevated malaria prevalence. In contrast, Zamora-Vilchis, Williams & Johnson (2012), in a study conducted in Australia, demonstrated that in areas with high temperatures the birds had a higher prevalence of *Haemoproteus*, and relationships for *Leucocytozoon* and *Plasmodium* were also positive but not statistically significant. Given that, in a warmer climate, the vector abundance may increase, and the transmission of vector-borne diseases must be higher. On the other hand, in our study the infection probability by *Parahaemoproteus* lineages was not influenced by temperature and, it is possible that biting midges, which act as their vectors, do not have their reproduction and development as closely related to climatic factors as *Plasmodium* vectors. Immature biting midges require a certain amount of free water or moisture, being able to develop in a wide range of habitats that meet that criterion like pools, streams, marshes, bogs, beaches,

swamps, tree holes, irrigation pipe leaks, saturated soil, animal dung, and even rotting fruit and other vegetation (*Mellor, Boorman & Baylis, 2000*). With so many possible breeding sites, *Parahaemoproteus* vectors are probably present in the community during the whole year and, even if there is some reduction in their abundance due to changes in temperature and rainfall (*Mellor, Boorman & Baylis, 2000*), it may be less evident for biting midges than for mosquitoes.

Neither age nor breeding condition explained the probability of infection by haemosporidians. Although many studies have evidenced that birds age and/or breeding condition may influence haemosporidian prevalence (*Wood et al., 2007*; *Ferreira Junior et al., 2017*; *Hernández-Lara, González-García & Santiago-Alarcon, 2017b*; *Eastwood et al., 2019*), others have failed to detect such an association (*Ricklefs et al., 2005*; *Matthews et al., 2015*). There was also no association between prevalence and rainfall or, except for migratory behavior, any tested species-specific traits (diet, foraging strata, period of activity, species body weight, and nest shape). It is true that many studies have shown that different host-traits and abiotic factors are important determinants in a host-parasite interaction (*Ricklefs et al., 2005*; *Wood et al., 2007*; *Medeiros et al., 2016*; *Ferreira Junior et al., 2017*; *Hernández-Lara, González-García & Santiago-Alarcon, 2017a*; *Ishtiaq, Bowden & Jhala, 2017*; *Fecchio et al., 2017b*; *Eastwood et al., 2019*). However, there are many variations in these studies' results, and some of them fail to detect these interactions. Based on the mixed results found in these studies, it is possible that the relationship between species-specific traits as well as individual-level traits and the risk of infection by haemosporidian parasites might be location-dependent. It is important to highlight that several factors might be working together to determine such variations we see in all these different studies, including the host species that, as we observed, had a small influence on the infection probability by *Plasmodium*. That small influence of the species on *Plasmodium* prevalence could be related to factors that were not tested in our studies, like phylogeny or co-infection with other hemoparasites. Studying haemosporidian infections only at the community level reduces our ability to detect if some species are more susceptible to the most common *Plasmodium* lineages in CLBI, for example. That is why species-specific studies are also important and allow us to identify some relationships that may be overshadowed in a bird community (*Rodrigues et al., 2020*).

The CLBI harbors a diverse community of avian haematozoan lineages distributed among 63% (44/70) of the bird species sampled in this study and we estimated an overall parasite prevalence of ~22%. Estimates for the prevalence of haemosporidian parasites in bird communities from Brazil indicate a great variation both among different ecosystems and between different sites in the same ecosystems. The estimated prevalence in *Cerrado* varied from 21% to 42% (*Belo et al., 2011*; *Fecchio et al., 2013*; *Lacorte et al., 2013*). In other Brazilian habitats it has also been observed a great variation in prevalence estimates, e.g., 17.4% (*Fecchio et al., 2017c*) to 21.7% (*Svensson-Coelho et al., 2013*) in Amazonian Region; 38.5% (*Lacorte et al., 2013*) to 42% (*Ferreira Junior et al., 2017*) in Seasonally Dry Tropical Forest; and 12.4% to 39.6% in Atlantic Forest (*Ribeiro et al., 2005*; *Sebaio et al., 2010*; *Lacorte et al., 2013*). This considerable variation in prevalence among studies is evidence that we still have many aspects of this complex, spatially variable

parasite–host system to understand. The avian haemosporidian parasite–host community in northeast Brazil adds to our understanding of the distribution and diversity of avian haemosporidian parasites and examines the ecological factors that influence host susceptibility. However, we acknowledge that much remains to be investigated in the parasite-host relationship in Restinga and suggest that future studies use information from blood smears and mixed infections to extend the ability to detect and identify haemosporidians in this bird community.

CONCLUSIONS

In this first exploration of avian haemosporidian parasites in a largely unexplored region of Brazil, we could demonstrate that this environment harbors a high diverse community of *Plasmodium* and *Haemoproteus* parasites. We established that migration can be an important factor predicting the prevalence of *Haemoproteus*, but not *Plasmodium*, in hosts. Thus, in CLBI, *Haemoproteus* lineages infect preferably resident birds and should be more difficult to disperse into new environments. The other individual- and species-level traits were not important in determining the probability of infection by *Plasmodium* or *Haemoproteus* in our study, which indicates a great variation of the influence of these factors on haemosporidian prevalence in different communities. The temperature, but not the rainfall, seems to predict the *Plasmodium* prevalence in this bird community. This result raises the possibility that ongoing climate change will impact the dynamics of *Plasmodium* transmission, a subject that should be explored in future studies. The higher number of birds infected by *Haemoproteus* lineages than by *Plasmodium* is an uncommon finding in Brazil and led us to suggest that northeast Brazil must have a different haemosporidian infection dynamics when compared to other studied regions of the country. Further investigations in northeast biomes and sampling of the haemosporidian vectors are needed to better understand the transmission dynamics and to elucidate the factors promoting higher levels of *Haemoproteus* infection in birds of this region.

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Competing Interests

Érika M. Braga is an Academic Editor for PeerJ.

Author Contributions

- Raquel A. Rodrigues conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Gabriel M.F. Felix analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Mauro Pichorim performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Patricia A. Moreira conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Erika M. Braga conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study was approved by the Ethics Committee in Animal Experimentation (CETEA), Universidade Federal de Minas Gerais, Brazil (Protocol #254/2011).

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Our use of mist-nets and banding was approved by the Brazilian Biodiversity Monitoring Agency (Institute Chico Mendes for Biodiversity Conservation—ICMBio, Brazilian National Center for Bird Conservation—CEMAVE, permission 3239).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Lineages without previous records in the database were considered new lineages and are available in GenBank under accession numbers MH260577, MK291501, MK291502, MK291503, MK291504, MK291505, MK291506 and MK291507.

New occurrences of sequences previously described are also available in GenBank: MK264392, MK264393, MK264394, MK264395, MK264396, MK264397, MK264398, MK264399, MK264400, MK264401, MK264402, MK264403, MK264404, MK264405, MK264406, MK264407, MK264408, MK264409, MK264410 and MK264411.

Data Availability

The following information was supplied regarding data availability:

The raw data for host age, breeding condition, and presence of ectoparasites of birds are available as Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.11555#supplemental-information.

REFERENCES

Antonini Y, Lobato DNC, Ramos JA, de Moreira PA, Braga EM. 2019. Patterns of avian malaria in tropical and temperate environments: testing the "The enemy release hypothesis". *Biota Neotropica* **19(4)**:1–6 DOI 10.1590/1676-0611-bn-2018-0716.

Ardia DR. 2005. Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. *Journal of Animal Ecology* **74(3)**:517–524 DOI 10.1111/j.1365-2656.2005.00950.x.

Asghar M, Hasselquist D, Hansson B, Zehtindjiev P, Westerdahl H, Bensch S. 2015. Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* **347(6220)**:436–438 DOI 10.1126/science.1261121.

Atkinson CT, Dusek RJ, Woods KL, Iko WM. 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. *Journal of Wildlife Diseases* **36(2)**:197–204 DOI 10.7589/0090-3558-36.2.197.

Atkinson CT, Woods KL, Dusek RJ, Sileo LS, Iko WM. 1995. Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). *Parasitology* **111(S1)**:S59–S69 DOI 10.1017/S003118200007582X.

Barr DJ, Levy R, Scheepers C, Tily HJ. 2013. Random effects structure for confirmatory hypothesis testing: keep it maximal. *Journal of Memory and Language* **68(3)**:1–43 DOI 10.1016/j.jml.2012.11.001.

Barton K. 2018. Package "MuMIn." cran.r-project.org. *Available at https://cran.r-project.org/web/packages/*.

Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67(1)**:1–48 DOI 10.18637/jss.v067.i01.

Beier JC. 1998. Malaria parasite development in mosquitoes. *Annual Review of Entomology* **43(1)**:519–543 DOI 10.1146/annurev.ento.43.1.519.

Belo NO, Pinheiro RT, Nia E, Reis S, Ricklefs RE, Braga M. 2011. Prevalence and lineage diversity of avian haemosporidians from three distinct cerrado habitats in Brazil. *PLOS ONE* **6(3)**:1–8 DOI 10.1371/journal.pone.0017654.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24(3)**:127–135 DOI 10.1016/j.tree.2008.10.008.

Cerný O, Votýpka J, Svobodová M. 2011. Spatial feeding preferences of ornithophilic mosquitoes, blackflies and biting midges. *Medical and Veterinary Entomology* **25(1)**:104–108 DOI 10.1111/j.1365-2915.2010.00875.x.

Cosgrove CL, Wood MJ, Day KP, Sheldon BC. 2008. Seasonal variation in *Plasmodium* prevalence in a population of blue tits *Cyanistes caeruleus*. *Journal of Animal Ecology* **77(3)**:540–548 DOI 10.1111/j.1365-2656.2008.01370.x.

Crawley MJ. 2013. *The R book*. London: John Wiley & Sons Ltd.

Dimitrov D, Zehtindjiev P, Bensch S. 2010. Genetic diversity of avian blood parasites in SE Europe: Cytochrome b lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from Bulgaria. *Acta Parasitologica* **55(3)**:201–209 DOI 10.2478/s11686-010-0029-z.

Drobney RD, Train CT, Fredrickson LH. 1983. Dynamics of the platyhelminth fauna of wood ducks in relation to food habits and reproductive state. *The Journal of Parasitology* **69(2)**:375–380 DOI 10.2307/3281239.

Eastwood JR, Peacock L, Hall ML, Roast M, Murphy SA, Silva AG, Peters A. 2019. Persistent low avian malaria in a tropical species despite high community prevalence. *IJP: Parasites and Wildlife* **8(1733)**:88–93 DOI 10.1016/j.ijppaw.2019.01.001.

Ellis VA, Sari EHR, Rubenstein DR, Dickerson RC, Bensch S, Ricklefs RE. 2019. The global biogeography of avian haemosporidian parasites is characterized by local diversification and intercontinental dispersal. *Parasitology* **146(2)**:213–219 DOI 10.1017/S0031182018001130.

Fecchio A, Ellis VA, Bell JA, Andretti CB, D'horta FM, Silva AM, Tkach VV, Weckstein JD. 2017b. Avian malaria, ecological host traits and mosquito abundance in southeastern Amazonia. *Parasitology* **144(8)**:1117–1132 DOI 10.1017/S003118201700035X.

Fecchio A, Wells K, Bell JA, Tkach VV, Lutz HL, Weckstein JD, Clegg SM, Clark NJ. 2019. Climate variation influences host specificity in avian malaria parasites. *Ecology Letters* **22(3)**:547–557 DOI 10.1111/ele.13215.

Fecchio A, Lima MR, Silveira P, Braga ÉM, Marini MÂ. 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. *Emu* **111(2)**:132–138 DOI 10.1071/MU10063.

Fecchio A, Svensson-Coelho M, Bell J, Ellis VA, Medeiros MC, Trisos CH, Blake JG, Loiselle BA, Tobias JA, Fanti R, Coffey ED, De Faria IP, Pinho JB, Felix G, Braga EM, Anciães M, Tkach V, Bates J, Witt C, Weckstein JD, Ricklefs RE, Farias IP. 2017a. Host associations and turnover of haemosporidian parasites in manakins (Aves: Pipridae). *Parasitology* **144(7)**:984–993 DOI 10.1017/S0031182017000208.

Fecchio A, Pinheiro R, Felix G, Faria IP, Pinho JB, Lacorte GA, Braga EM, Farias IP, Aleixo A, Tkach VV, Collins MD, Bell JA, Weckstein JD. 2017c. Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds. *Ecography* **0(3)**:1–10 DOI 10.1111/ecog.03058.

Fecchio A, Lima MR, Svensson-Coelho M, Marini MÂ, Ricklefs RE. 2013. Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. *Parasitology* **140(2)**:181–192 DOI 10.1017/S0031182012001412.

Ferreira Junior FC, Rodrigues RA, Ellis VA, Leite LO, Borges MAZ, Braga ÉM. 2017. Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. *PLOS ONE* **12**:1–18.

Ferreira FC, Alves LGM, Jager GB, Franzini LD, Mesquita DO, Díaz-Delgado J, Catão-Dias JL, Braga ÉM. 2020. Molecular and pathological investigations of Plasmodium parasites infecting striped forest whiptail lizards (*Kentropyx calcarata*) in Brazil. *Parasitology Research* **119(8)**:2631–2640 DOI 10.1007/s00436-020-06756-7.

Garamszegi LZ. 2011. Climate change increases the risk of malaria in birds. *Global Change Biology* **17(5)**:1751–1759 DOI 10.1111/j.1365-2486.2010.02346.x.

González AD, Matta NE, Ellis VA, Miller ET, Ricklefs RE, Gutiérrez HR. 2014. Mixed species flock, nest height, and elevation partially explain avian haemoparasite prevalence in Colombia. *PLOS ONE* **9(6)**:e100695 DOI 10.1371/journal.pone.0100695.

Hall RJ, Altizer S, Bartel RA. 2014. Greater migratory propensity in hosts lowers pathogen transmission and impacts. *Journal of Animal Ecology* **83(5)**:1068–1077 DOI 10.1111/1365-2656.12204.

Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* **218(4570)**:384–387 DOI 10.1126/science.7123238.

Harrison XA, Donaldson L, Correa-Cano ME, Evans J, Fisher DN, Goodwin CED, Robinson BS, Hodgson DJ, Inger R. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* **6(1)**:e4794 DOI 10.7717/peerj.4794.

Hellgren O, Pérez-Triz J, Bensch S. 2009. A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. *Ecology* **90(10)**:2840–2849 DOI 10.1890/08-1059.1.

Hellgren O, Wood MJ, Waldenström J, Hasselquist D, Ottosson U, Stervander M, Bensch S. 2013. Circannual variation in blood parasitism in a sub-Saharan migrant passerine bird, the garden warbler. *Journal of Evolutionary Biology* **26(5)**:1047–1059 DOI 10.1111/jeb.12129.

Hellgren O, Waldenström J, Peréz-Tris J, Szöll Ösi E, Hasselquist D, Krizanauskiene A, Ottosson U, Bensch S. 2007. Detecting shifts of transmission areas in avian blood parasites—a phylogenetic approach. *Molecular Ecology* **16(6)**:1281–1290 DOI 10.1111/j.1365-294X.2007.03227.x.

Hernández-Lara C, González-García F, Santiago-Alarcon D. 2017a. Landscape and urban planning spatial and seasonal variation of avian malaria infections in five different land use types within a Neotropical montane forest matrix. *Landscape and Urban Planning* **157**:151–160 DOI 10.1016/j.landurbplan.2016.05.025.

Hernández-Lara C, González-García F, Santiago-Alarcon D. 2017b. Spatial and seasonal variation of avian malaria infections in five different land use types within a Neotropical montane forest matrix. *Landscape and Urban Planning* **157**:151–160 DOI 10.1016/j.landurbplan.2016.05.025.

Hoyo JD, Elliott A, Christie DA. 2011. *Handbook of the birds of the world alive*. Barcelona: Lynx Edicions.

Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54(2)**:187–211 DOI 10.2307/1942661.

INMET. 2017. Instituto Nacional de Meteorologia. Banco de Dados Meteorológicos para Ensino e Pesquisa—BDMEP. Brasília, DF, Brasil. *Available at http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep* (accessed 11 December 2017).

Ishtiaq F, Bowden CGR, Jhala YV. 2017. Seasonal dynamics in mosquito abundance and temperature do not influence avian malaria prevalence in the Himalayan foothills. *Ecology and Evolution* **7(19)**:8040–8057 DOI 10.1002/ece3.3319.

Ishtiaq F, Clegg SM, Phillimore AB, Black RA, Owens IPF, Sheldon BC. 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. *Journal of Biogeography* **37(1)**:120–132 DOI 10.1111/j.1365-2699.2009.02189.x.

Ishtiaq F, Gering E, Rappole JH, Rahmani AR, Jhala YV, Dove CJ, Milensky C, Olson SL, Peirce MA, Fleischer RC. 2007. Prevalence and diversity of avian Hematozoan parasites in Asia: a regional survey. *Journal of Wildlife Diseases* **43(3)**:382–398 DOI 10.7589/0090-3558-43.3.382.

Kim KS, Tsuda Y. 2010. Seasonal changes in the feeding pattern of *Culex pipiens pallens* govern the transmission dynamics of multiple lineages of avian malaria parasites in Japanese wild bird community. *Molecular Ecology* **19(24)**:5545–5554 DOI 10.1111/j.1365-294X.2010.04897.x.

Knowles SCL, Palinauskas V, Sheldon BC. 2010. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology* **23(3)**:557–569 DOI 10.1111/j.1420-9101.2009.01920.x.

Lacorte GA, Flix GMF, Pinheiro RRB, Chaves AV, Almeida-Neto G, Neves FS, Leite LO, Santos FR, Braga ÉM. 2013. Exploring the diversity and distribution of neotropical avian malaria parasites—a molecular survey from Southeast Brazil. *PLOS ONE* **8(3)**:1–9 DOI 10.1371/journal.pone.0057770.

LaPointe DA, Goff ML, Atkinson CT. 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *plasmodium relictum* in Hawai'i. *The Journal of Parasitology* **96(2)**:318–324 DOI 10.1645/GE-2290.1.

Levin II, Parker PG. 2012. Prevalence of *Hemoproteus iwa* in Galapagos Great Frigatebirds (*Fregata minor*) and their obligate fly ectoparasite (*Olfersia spinifera*). *Journal of Parasitology* **98(5)**:924–929 DOI 10.1645/GE-3027.1.

Levin II, Valkiūnas G, Iezhova TA, O'Brien SL, Parker PG. 2012. Novel *Haemoproteus* species (Haemosporida: Haemoproteidae) from the Swallow-Tailed Gull (Lariidae), with remarks on the host range of hippoboscid-transmitted Avian Hemoproteids. *Journal of Parasitology* **98(4)**:847–854 DOI 10.1645/GE-3007.1.

Lopes VL, Costa FV, Rodrigues RA, Braga M, Pichorim M, Moreira PA. 2020. High fidelity defines the temporal consistency of host-parasite interactions in a tropical coastal ecosystem. *Scientific Reports* **10(1)**:1–10 DOI 10.1038/s41598-019-56847-4.

Lutz HL, Hochachka WM, Engel JI, Bell JA, Tkach VV, Bates JM, Hackett SJ, Weckstein JD. 2015. Parasite prevalence corresponds to host life history in a diverse assemblage of afrotropical birds and haemosporidian parasites. *PLOS ONE* **10(4)**:1–24 DOI 10.1371/journal.pone.0121254.

Matthews AE, Ellis VA, Hanson AA, Roberts JR, Ricklefs RE, Collins MD. 2015. Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. *Journal of Ornithology* **157(2)**:1–16 DOI 10.1007/s10336-015-1298-y.

Medeiros MCI, Ricklefs RE, Brawn JD, Ruiz MO, Goldberg TL, Hamer GL. 2016. Overlap in the seasonal infection patterns of avian malaria parasites and West Nile Virus in vectors and hosts. *The American Journal of Tropical Medicine and Hygiene* **95(5)**:1121–1129 DOI 10.4269/ajtmh.16-0236.

Mellor PS, Boorman J, Baylis M. 2000. Culicoides biting midges: their role as arbovirus vectors. *Annual Review Entomology* **45(1)**:307–340 DOI 10.1146/annurev.ento.45.1.307.

Møller AP. 2010. Host-parasite interactions and vectors in the barn swallow in relation to climate change. *Global Change Biology* **16(4)**:1158–1170 DOI 10.1111/j.1365-2486.2009.02035.x.

Møller AP, Erritzøe J. 1998. Host immune defence and migration in birds. *Evolutionary Ecology* **12(8)**:945–953 DOI 10.1023/A:1006516222343.

Mordecai EA, Caldwell JM, Grossman MK, Lippi CA, Johnson LR, Neira M, Rohr JR, Ryan SJ, Savage V, Shocket MS, Sippy R, Stewart Ibarra AM, Thomas MB, Villena O. 2019. Thermal biology of mosquito-borne disease. *Ecology Letters* **22(10)**:1690–1708 DOI 10.1111/ele.13335.

Nakagawa S, Johnson PCD, Schielzeth H. 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface* **14(134)**:1–11 DOI 10.1098/rsif.2017.0213.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4(2)**:133–142 DOI 10.1111/j.2041-210x.2012.00261.x.

Ortego J, Cordero PJ, Aparicio JM, Calabuig G. 2008. Consequences of chronic infections with three different avian malaria lineages on reproductive performance of Lesser Kestrels (*Falco naumanni*). *Journal of Ornithology* **149(3)**:337–343 DOI 10.1007/s10336-008-0287-9.

Padilla LR, Whiteman NK, Merkel J, Huyvaert KP, Parker G, Padilla LR, Whiteman NK, Merkel J, Huyvaert KP, Parker PG. 2006. Health assesment of seabirds on Isla Genovesa, Galapagos Islands. *Ornithological Monographs* **60**:86–97.

Patz JA, Graczyk TK, Geller N, Vittor AY. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* **30(12–13)**:1395–1405 DOI 10.1016/S0020-7519(00)00141-7.

Pulgarín-R PC, Gómez C, Bayly NJ, Bensch S, Fitzgerald AM, Starkloff N, Kirchman JJ, González-Prieto AM, Hobson KA, Ungvari-Martin J, Skeen H, Castaño MI, Cadena CD. 2019. Migratory birds as vehicles for parasite dispersal? Infection by avian haemosporidians over the year and throughout the range of a long—distance migrant. *Journal of Biogeography* **46(1)**:83–96 DOI 10.1111/jbi.13453.

R Core Team. 2016. *R: a language and environment for statistical computing*. Vienna: The R Foundation for Statistical Computing.. *Available at http://www.R-project.org/*.

Ribeiro SF, Sebaio F, Branquinho FCS, Marini MÂ, Vago AR, Braga ÉM. 2005. Avian malaria in Brazilian passerine birds: parasitism detected by nested PCR using DNA from stained blood smears. *Parasitology* **130(3)**:261–267 DOI 10.1017/S0031182004006596.

Richner H, Christe P, Oppliger A. 1995. Paternal investment affects prevalence of malaria. *Proceedings of the National Academy of Sciences of the United States of America* **92(4)**:1192–1194 DOI 10.1073/pnas.92.4.1192.

Ricklefs RE, Fallon SM. 2002. Diversification and host switching in avian malaria parasites. *Proceedings of the Royal Society London B* **269(1494)**:885–892 DOI 10.1098/rspb.2001.1940.

Ricklefs RE, Fallon SM, Bermingham E. 2004. Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology* **53**:111119 DOI 10.1080/10635150490264987.

Ricklefs RE, Medeiros M, Ellis VA, Svensson-Coelho M, Blake JG, Loiselle BA, Soares L, Fecchio A, Outlaw D, Marra PP, Latta SC, Valkiūnas G, Hellgren O, Bensch S. 2017. Avian migration and the distribution of malaria parasites in New World passerine birds. *Journal of Biogeography* **44(5)**:1113–1123 DOI 10.1111/jbi.12928.

Ricklefs RE, Outlaw DC, Svensson-Coelho M, Medeiros MCI, Ellis VA, Latta S. 2014. Species formation by host shifting in avian malaria parasites. *Proceedings of the National Academy of Sciences of the United States of America* **111(41)**:14816–14821 DOI 10.1073/pnas.1416356111.

Ricklefs RE, Soares L, Ellis VA, Latta SC. 2016. Haemosporidian parasites and avian host population abundance in the Lesser Antilles. *Journal of Biogeography* **43(7)**:1277–1286 DOI 10.1111/jbi.12730.

Ricklefs RE, Swanson BL, Fallon SM, Martinez-Abrain A, Scheuerlein A, Gray J, Latta S. 2005. Community relationships of avian malaria parasites in southern Missouri. *Ecological Monographs* **75(4)**:543–559 DOI 10.1890/04-1820.

Rodrigues RA, Massara RL, Bailey LL, Pichorim M, Moreira PA, Braga ÉM. 2020. Using a multistate occupancy approach to determine molecular diagnostic accuracy and factors affecting avian haemosporidian infections. *Scientific Reports* **10(1)**:1–10 DOI 10.1038/s41598-019-56847-4.

Santiago-Alarcon D, Bloch R, Rolshausen G, Schaefer HM, Segelbacher G. 2011. Prevalence, diversity, and interaction patterns of avian haemosporidians in a four year study of blackcaps in a migratory divide. *Parasitology* **138(7)**:824–835 DOI 10.1017/S0031182011000515.

Santiago-Alarcon D, Palinauskas V, Schaefer HM. 2012. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews* **87(4)**:928–964 DOI 10.1111/j.1469-185X.2012.00234.x.

Scott ME. 1988. The impact of infection and disease on animal populations: implications for conservation biology. *Conservation Biology* **2(1)**:40–56 DOI 10.1111/j.1523-1739.1988.tb00334.x.

Sebaio F, Braga ÉM, Branquinho F, Manica LT, Marini MÂ. 2010. Blood parasites in Brazilian Atlantic Forest birds: effects of fragment size and habitat dependency. *Bird Conservation International* **20(4)**:432–439 DOI 10.1017/S0959270910000110.

Sehgal RNM, Buermann W, Harrigan RJ, Bonneaud C, Loiseau C, Chasar A, Sepil I, Valkiūnas G, Iezhova T, Saatchi S, Smith TB. 2011. Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. *Proceedings of the Royal Society B: Biological Sciences* **278(1708)**:1025–1033 DOI 10.1098/rspb.2010.1720.

Slowinski SP, Fudickar AM, Hughes AM, Mettler RD, Gorbatenko OV, Spellman GM, Ketterson ED, Atwell JW. 2018. Sedentary songbirds maintain higher prevalence of haemosporidian parasite infections than migratory conspecifics during seasonal sympatry. *PLOS ONE* **13**:1–18.

Soares L, Latta SC, Ricklefs RE. 2019. Neotropical migratory and resident birds occurring in sympatry during winter have distinct haemosporidian parasite assemblages. *Journal of Biogeography* **47(3)**:1–12 DOI 10.1111/jbi.13760.

Soares L, Young EI, Ricklefs RE. 2020. Haemosporidian parasites of resident and wintering migratory birds in The Bahamas. *Parasitology Research* **119(5)**:1563–1572 DOI 10.1007/s00436-020-06646-y.

Somenzari M, Amaral PP, Cueto VR, Guaraldo AC, Jahn AE, Lima DM, Lima PC, Lugarini C, Machado CG, Martinez J, Nascimento JLX, Pacheco JF, Paludo D, Prestes NP, Serafini PP, Silveira LF, Sousa AEBA, Sousa NA, Souza MA, Telino-Júnior WR, Whitney BM. 2018. An overview of migratory birds in Brazil. *Papéis Avulsos de Zoologia* **58**:3 DOI 10.11606/1807-0205/2018.58.03.

Spencer KA, Buchanan KL, Leitner S, Goldsmith AR, Catchpole CK. 2005. Parasites affect song complexity and neural development in a songbird. *Proceedings of the Royal Society B: Biological Sciences* **272(1576)**:2037–2043 DOI 10.1098/rspb.2005.3188.

Svensson-Coelho M, Blake JG, Loiselle BA, Penrose AS, Parker PG, Ricklefs RE. 2013. Diversity, prevalence, and host specificity of avian *Plasmodium* and *Haemoproteus* in a western Amazon assemblage. *Ornithological Monographs* **76(1)**:1–47 DOI 10.1525/om.2013.76.1.1.

Valkiūnas G. 2005. *Avian malaria parasites and other Haemosporidia*. Boca Raton, Florida: CRC Press.

van Riper C III, van Riper SG, Goff ML, Laird M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56(4)**:327–344 DOI 10.2307/1942550.

Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U. 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology* **11(8)**:1545–1554 DOI 10.1046/j.1365-294X.2002.01523.x.

Wilman H, Belmaker J, Simpson J, de la Rosa C, Rivadeneira MM, Jetz W. 2014. EltonTraits 1.0: species-level foraging attributes of the world's birds and mammals. *Ecology* **95(7)**:2027 DOI 10.1890/13-1917.1.

Wood MJ, Cosgrove CL, Wilkin TA, Knowles SCL, Day KP, Sheldon BC. 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology* **16(15)**:3263–3273 DOI 10.1111/j.1365-294X.2007.03362.x.

Work TM, Rameyer RA. 1996. *Haemoproteus iwa* n. sp. in Great Frigatebirds (*Fregata minor* [Gmelin]) from Hawaii: Parasite morphology and prevalence. *The Journal of Parasitology* **82(3)**:489–491 DOI 10.2307/3284091.

Zamora-Vilchis I, Williams SE, Johnson CN. 2012. Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: Implications for disease in a warming climate. *PLOS ONE* **7(6)**:1–8 DOI 10.1371/journal.pone.0039208.