TRACKING AVIAN MALARIA PARASITE INFECTION IN SPRING MIGRATORY AND RESIDENT PASSERINE BIRDS IN COASTAL TEXAS, 2014-2019

A Thesis

by

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ABSTRACT

Avian migration can have significant effects on disease dynamics in populations and provides a mechanism for large-scale geographic parasite dispersal. For example, birds can be infected by diverse Haemosporidian blood parasites that can cause avian malaria with impacts on host fitness. To characterize the Haemosporidian parasites transported by migratory birds, and identify the physical, ecological, and life history characteristics of the avian hosts associated with infections, we studied spring migrating passerines arriving to the US coast of the Gulf of Mexico and sympatric resident birds that over winter on the US Gulf Coast. We used mist nets to trap birds and collect blood samples in Matagorda County, Texas, in the springs of 2014-2019. We used PCR and DNA sequencing of the Haemosporidian cytB gene from avian blood samples to determine infection status and characterize the lineages of blood parasites. We found an overall Haemosporidian prevalence of 48.4% in trans-Gulf migrant and resident birds in which some avian families had greater odds of infection than others. Furthermore, birds that over wintered in Central America had significantly greater odds of infection with *Plasmodium* spp. compared to those that over wintered in the US or South America. Additionally, birds foraging on the ground and in the understory had the greatest odds of *Plasmodium* spp. infection compared to canopy foraging birds. Using the MalAvi database of Haemosporidian parasites, we identified 46 different Haemosporidian lineages present in the samples, including 71% in the genus *Plasmodium* and 29% in the genus *Haemoproteus*; this includes 17 novel lineages-never before reported- and six lineages that have not been previously reported in North America. Furthermore, our data

reveal 6 novel geographic associations and 47 novel associations between an established lineage and a bird family. Given recent reports of dramatic declines in North American bird populations over the past five decades, further quantifications of host-parasite relationships in migratory birds and impacts on health are critically important.

DEDICATION

This master's thesis is dedicated to Rose Mary DeBrock, who has always made every effort to be a part of our lives, who has gone to extraordinary lengths to be present for significant life events, and who is always praying for us. There are no words that adequately explain how grateful I am for her. She has been making my world a brighter place for 24 years and I would give anything for her to continue doing so for 24 more.

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Contributors

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CHAPTER I

INTRODUCTION

Species movements, especially avian migration, may be a mechanism for the geographic range expansion and large-scale dispersal of parasites [1, 2]. For example, the emergence of several important zoonotic pathogens across North America, including, West Nile virus and Lyme disease resulted from dispersal by avian migrants [3]. Further, migration is believed to have significant effects on disease dynamics in migratory populations because migration is energetically expensive, as is mounting an immune response [4]. For example, previous studies of waterfowl have recorded a positive relation between number of parasites species per host and migratory distance flown, indicating migratory behavior most likely results in increased parasite exposure [5]. Taken together, studies of parasites in birds during migration are important not only for understanding geographic foci of parasite dispersal, but also potential impacts on avian health.

A large diversity of Haemosporidian species in the genera *Haemoproteus, Plasmodium,* and *Leucocytozoon* infect birds worldwide. These parasites are transmitted by dipteran vectors including mosquitos, biting midges, and simuliid black flies [6]. Many classes of animals can be infected by these blood parasites including birds, reptiles, mammals, and fish. Avian Haemosporidian life cycles follow four main stages with only 1). exo-erythrocytic merogony, and 2). intra-erythrocytic development with production of infectious gametocytes being the two stages relevant to the avian hosts; the other stages apply to the dipteran vectors [7]. In addition to blood pathology, these

parasites can cause organ pathology in birds due to damage by exoerythrocytic stages which can occur systemically including in the brain, eyes, nerves, heart, and skeletal muscles, among others [7].

Infection by Haemosporidians presents with two phases, an acute phase and a chronic phase. The acute stage occurs immediately after infection and is characterized by high parasitemia and clinical signs such as lethargy, reduced weight gain, severe anemia, and sometimes death [6]. The chronic stage occurs days or weeks after acute infection, but the bird may experience seasonal relapses. In the chronic phase, infected animals experience low parasitemia and while this infection can last for years, they only have mild impacts on individual fitness [6]. Although, a recent study showed that these chronic effects, though small, can accumulate, and eventually reduce phenotypic quality and Darwinian fitness [8] through tangible processes. For example, a positive association was documented between Haemosporidian infection prevalence/intensity and migratory timing in younger wood- warblers by Degroote and Rodewald (2010; [9]). Later migratory timing, due to infection prevalence and intensity, compared to conspecifics may result in negative reproductive performances of such individuals. Birds typically captured in studies concerning avian malaria are those experiencing chronic infection since their propensity to fly into a net is not hampered by an infection [8].

Avian migration provides the opportunity for Haemosporidian parasites to be transported to new locations where they may infect new communities of avian hosts. When infected migratory birds reach their breeding grounds, local vectors may transmit the pathogens of migrant birds to naïve resident birds [10] and recent studies indicate

that birds may be more susceptible to allopatric malarial infections than to sympatric malarial infections [11]. Furthermore, it has been shown that *Haemoproteus* infections can be especially lethal in non-adapted bird species due to marked organ damage [7]. Across taxa, there exists a latitudinal gradient of diversity in which species richness is greater in tropical climates; as such, the lineage richness of avian Haemosporidians has been shown to follow the patterns of their hosts; increasing in tropical avian hotspots [12]. Evidence of the exchange of blood parasites between Neotropical and North American individuals has already been provided in breeding and non-breeding Blue-winged Teals (*Spatula discors*) [13], revealing that intercontinental parasite exchange can occur. Furthermore, Clark et al. (2016) found lower Haemosporidian prevalence in birds wintering in higher latitudes, providing evidence to support the 'avoiding the tropics' hypothesis whereby parasite prevalence increases with decreasing wintering latitude [14].

Aspects of bird ecology and life history have been important in predicting infection with Haemosporidians. Given these parasites are transmitted by vectors, life histories which put birds in greater contact with infected vectors are likely to be associated with infection prevalence in the birds. For example, ground foraging Ecuadorian bird species were found to have the highest *Plasmodium* prevalence in a study of Amazonian birds by Svensson- Coelho et al. (2013) [15] and some Haemosporidian vector species have been caught in higher abundance in the canopy layer [16]. Conversely, *Plasmodium* prevalence was highest among mid- to high- level

foragers in Georgia, USA while *Haemoproteus* has been observed to be highest among birds with low- to mid-level foraging and nest heights [17, 18].

Also, aspects of the physiology of migrating birds may influence infection probability. Physiological preparation for migration involves building up an excess of body fat which then provides energy for the trip [19]. Migrating birds deplete their fat stores during flight and the resulting poor body conditions may make them more susceptible infections [20, 21]. Such a result was found in a study of migrating Goldcrests (*Regulus regulus*) (Merila & Svensson, 1995), where the authors found that birds showing signs of infection/inflammatory diseases had significantly lower fat stores than birds that did not show such signs [22]. Relationships between fat deposits and infection status, however, may vary over time and space as well as across species. Garvin, Szell & Moore (2006) found an inverse relationship between fat deposit and infection status in only two of five focal species, Summer Tanagers (*Piranga rubra*) and Scarlet Tanagers (*Piranga olivacea*), in a study involving the same Nearctic-Neotropical spring trans-Gulf migrating species as in our study [23]. This study also found no relationship of gender to prevalence and a great degree of interspecies variability in prevalence.

The objectives of this project were to (i) quantify the Haemosporidian infection prevalence in migrating birds compared to Texas wintering birds (ii) determine what physiological, ecological, and life history factors are associated with infection overall and with specific genera; and (iii) identify relationships between host species and Haemosporidian parasite lineages. I hypothesized that wintering ground, foraging guild,

fat score, and muscle score would significantly predict infection prevalence. Specifically, birds wintering in lower latitudes, foraging in higher forest strata, and with low fat and muscle scores, would have increased Haemosporidian prevalence. Given recent reports of dramatic declines (~30%) in North American bird populations over the past five decades [24], further studies of host-parasite relationships in migratory birds and impacts on avian health are critically important.

CHAPTER II

MATERIALS AND METHODS

We sampled passerine birds at the Clive Runnells Family Mad Island Marsh Preserve, operated by the Nature Conservancy, in Matagorda County, TX, where migrants first make landfall after flying over the Gulf of Mexico in their spring migration (Figure 1; [2]). Each spring from mid-March – mid May, 2014-2019, mist nets were opened daily for eight hours per day, weather permitting (see Cohen et al., 2015 for more information). For each individual, we applied a federal USGS leg band, measured sex, age, tarsus and wing chord length, fat and muscle score, and mass, screened for ectoparasites, and took a blood sample before releasing them. Fat score was determined on a scale of 1-5 with five being the highest and muscle score on a scale of 1-4 with four being the most muscular. Birds were sexed, aged, fat scored and muscle scored in the field according to Pyle (1997) [25]. The amount of blood extracted was never more than one per cent of the bird's body weight, with sampled volume ranging from 30-100uL. Blood was acquired either through brachial or jugular venipuncture using 27-gauge needles (Becton Dickinson and Company, Franklin Lakes, NJ) and capillary tubes, in the former, or 1ml insulin syringes (EXELINT International Co., Los Angeles, CA), in the latter. Blood samples were either stored in dry tubes, stored in ~300ul RNAlater (Ambion inc., Austin, TX) or combined with 225ul TRIzol-LS (Thermo Fisher Scientific, Waltham, MA) then put on ice and stored in a -20°C before storage in a -80°C freezer. If birds were recaptured within three hours of taking data from them, they were

released immediately. Otherwise, they were resampled; however, no bird was blood sampled twice in the same day.

In the lab, we processed blood samples in order to quantify Haemosporidian parasite prevalence using a nested PCR and sequencing of the parasite cytB gene, the locus of choice for the MalAvi database of avian Haemosporidian parasites [26], -using previously described methods [27]. DNA was extracted using the E.Z.N.A tissue DNA kit (Omega Bio-Tek, Norcross, GA). For years 2014-2015, when blood was stored in dry tubes, 200uL of PBS was used to resuspend the frozen blood. A small amount of this resuspended blood sample was extracted from the tube using a modified 1000uL pipet tip (cut with scissors to make it wider). A small aliquot was taken in order to save the blood sample for any potential future projects, instead of utilizing the entire sample. For years 2016- 2019, when blood was stored in a liquid preservative, ~50uL of the homogenized preserved whole blood sample was used in the extraction. A negative control tube was included in every extraction. For PCR, we used Failsafe PCR 2X PreMix E and Failsafe Enzyme Mix (Lucigen, Middleton, WI) with specific primers to make a master mix which targets a 479bp DNA fragment in a two-step nested PCR. These primers were HAEMNFI [5'-CATATATTAAGAGAAITATGGAG-3'], HAEMNR3 [5'-ATAGAAAGATAAGAAATACCATTC-3'], HAEMF [5'-ATGGTGCTTTCGATATATGCATG-3'], and HAEMR2 [5'-GCATTATCTGGATGTGATA ATGGT-3'] (Integrated DNA Technologies, Coralville, IA). In the first reaction, 24uL of master mix was combined with 1uL of a 1:10 diluted DNA sample (diluted with 9uL autoclaved, DI water and 1uL of extracted DNA). The

cycle conditions were: 30 sec at 94° C, 30 sec at 50° C, and 45 sec at 72° C for 20 cycles with an incubation stage before and after at 94° C for three minutes and 72° C for 10 min, respectively. In the second reaction, 24uL of master mix, at the same proportion as above, was combined with 1uL of the initial reaction. The cycle conditions were the same as the initial PCR but for 35 cycles instead of 20. Several negative controls were used in each reaction including an H_2O blank, a sample of the extraction negative, and a no template control. A previously positive sample from the year 2014 served a positive control. Following gel electrophoresis of aliquots from the second PCR reaction, all samples with a 479bp amplified fragment were considered positive for infection with an avian Haemosporidian parasite.

To identify the parasite lineages, PCR amplicons were purified using ExoSAP-IT (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). Sanger sequencing of both the forward and reverse strands was performed by Eton Biosciences (San Diego, CA). Raw sequences were first blasted in the MalAvi database to identify the most similar published lineage, which in many cases was identical. Next, a multiple alignment, using Multalin (http://multalin.toulouse.inra.fr/multalin/), was performed of the forward and reverse sequences from the field sample and the sequence with the most homology from MalAvi in order to identify any mismatches. In the case of 100% sequence homology, the sequence from the blood sample was classified as the named lineage in the MalAvi database. If the reverse and forward sequences were identical to each other but differed from a published lineage by one or more single nucleotide polymorphisms, the sequence was classified as a novel lineage. All novel lineages were then compared to each other

using Mega version X [28] to determine unique novel lineages from repeat novel lineages. All detected lineages were deposited to the MalAvi database and GenBank (GenBank accession numbers MW081078-081139 & MW139686)

Foraging guild and primary wintering grounds of each species of bird was assigned using the Cornell Lab of Ornithology All About Birds [29, 30]. Additional guidance for determining wintering ground came from Cohen et al. (2015; [2]). When the wintering ground range was primarily Central America and did not extend past Colombia or Venezuela, they were categorized as Central American migrants. If the winter range extended past Colombia and Venezuela they were categorized as South American migrants. Two exceptions arose from this rule, Acadian Flycatchers (*Empidonax virescens*) and Black-and-white Warblers (*Mniotilta varia*), who both have very small wintering rangers that straddle the boarder of Panama and Colombia but do not go much further. Because the majority of their wintering range extended into Columbia these were categorized as South American to reflect what may be their primary wintering ground location. Finally, if the wintering range included our field site, or indeed Texas as a whole, the bird was categorized as a 'wintering resident', for the purpose of this study. Common Yellowthroats (*Geothlypis trichas*), Gray Catbirds (*Dumetella carolinensis*), and Lincoln Sparrows (Melospiza lincolnii) were an exception to this rule. All of these species had wintering ranges in Central America and in Texas, meaning that birds caught early in our study period were most likely wintering resident individuals, but individuals of these species caught later were most likely migrating from more southern locations. To account for this, and provide a more accurate representation

of our sampled birds, individuals of these species that were caught within the first week of sampling each year were categorized as wintering resident and individuals caught later were categorized as Central American migrants. Foraging guild was determined using the description of "Behavior" by Cornell Lab [29] in the following categories: ground foraging, understory foraging, canopy foraging (Table 1) [30].

We further performed an analysis based on fallout days across all years where we defined a fallout day as a day on which more than 50 birds were banded. Such days were identified in the overall data set and then identified in the data set of blood sampled birds. When a blood sampled bird was sampled on a fallout day, the fallout variable was designated as a 1 and when a bird was sampled not on a fallout day the variable was designated as a 0. A separate mixed effect logistic regression with year as a random variable was run to determine if fallout days were a significant predictor of infection status.

In order to increase power, bird species were grouped by family according to the phylogeny by the American Ornithological Society check-list of North American Birds (Table 1) [31]. In order to unmask any species level effects, we chose to analyze effects of infection on fat score, muscle score and standardized mass on the Baltimore Oriole (*Icterus galbula*), Rose-breasted Grosbeak (*Pheucticus ludovicianus*), Red-eyed Vireo (*Vireo olivaceus*), Scarlet Tanager, and Summer Tanager. Focal species were chosen based on Garvin et al. (2006; [23]) for purposes of comparison. Fat scores three & four were combined into one category since very few depleted migrants had a fat score of four; no blood sampled bird had a fat score of five. Muscle scores 0-1 and, in a separate

category, 2-3, were also combined since very few birds had a muscle score of zero or three [32]. Mass was standardized by dividing mass by unflattened wing chord length according to Wang & Moore (1997; [33]). A bivariate analysis was run on each factor individually with infection status or genus infection using a Chi-squared or Fischer's exact test, where appropriate, to determine factors significantly associated with the dependent variables. Mixed effect logistic regression models with year as a random effect and wintering ground, foraging guild, sex, standardized body mass, fat score, muscle score, and family as fixed effects were used to further analyze any factors that were (i) significantly associated with infection; and, for infected birds, (ii) significantly associated with the *Plasmodium* vs. the *Haemoproteus* genus. All predictors were included in the model together and stepwise regression was utilized to determine the model with the best fit based on model comparisons. Statistics were conducted using the program STATA (College Station, TX).

Figure 1: Location of field site in Clive Runnells Family Mad Island Marsh Preserve in Texas, USA (Image credit: Google Earth).

CHAPTER III

RESULTS

Seven-hundred and forty-three bird samples, consisting of spring migrants (n=654, 88%) and Texas wintering residents (n=89, 12%), representing 52 different species and 10 different avian families were collected and processed in this study. Of the 743 blood samples, 360 tested positive for Haemosporidian infection (48.4%) with the minority of migrant birds being infected (n=312, 47.7%) and the majority of Texas winterers being infected (n=48, 53.9%). Of those positive samples, 297 (82.3%) were successfully sequenced with a genus determined and 295 had a lineage determined, including novel lineages. *Plasmodium* was the most common genus infecting the birds in general (n= 211, 71%) while *Haemoproteus* comprised 29% of infections (n=86). Overall, we found 46 previously described lineages comprised of 27 *Plasmodium* lineages and 19 *Haemoproteus* lineages. In addition, we found 35 samples with previously uncharacterized lineages representing 17 novel lineages. Of these, 11 (64.7%) were novel lineages most closely related to a *Haemoproteus* lineage, and 6 (35.3%) were novel lineages most closely related to a *Plasmodium* lineage (Table 2). The most common *Plasmodium* lineage, and the most common lineage overall was PADOM11, representing 23.3% of *Plasmodium* infections (Table 3). The most common *Haemoproteus* lineage was MAFUS02 representing 4.2% of infections within that genus. Among the infections found in the birds include six lineages not detected in North America before including CYCYA01, DIGLAF01, MYCAME03, & RAMCAR01, which were all previously reported from South America, and TOXRUF01 and VIGRI02,

which were both previously reported from Central America (Table 3). Similarly, among the lineages found in the birds include 47 new associations between an established lineage and a host family (Table 3). Although quantification is ongoing, we are already observing a mixed lineage infection prevalence of at least 10.3% (n=282).

A total of five US over wintering birds were recaptured and resampled. A Northern Cardinal (*Cardinalis cardinalis*) initially negative when captured in 2015, tested positive in 2018. A Lincoln's Sparrow (*Melospiza lincolnii*) was negative in 2015 on initial capture and three days later on recapture. Of three Lincoln's Sparrows recaptured in 2016, one was initially positive and remained positive five days later with the lineage remaining consistent in that time interval: BT7, a *Plasmodium* lineage. Another Lincoln's Sparrow was positive upon initial capture on March 18 but was negative upon recapture on April 6. The third Lincoln's Sparrow was positive upon initial capture on March 18, but negative upon recapture three days later.

In a bivariate analysis we found that foraging guild and family were significantly associated with infection status, while wintering ground and the measures of individual condition, fat and muscle score, were not influential (Table 4). In a separate fixed effect logistic regression model with year as the sole predictor, birds in the years 2015 and 2019 were significantly less likely to be infected than birds in 2016 (OR= 0.47, p=0.005, 95% CI = 0.27 – 0.79; OR= 0.57, p=0.042, 95% CI= 0.34 – 0.98, respectively). In the final mixed effect model with year as a random effect, avian family and standardized mass were significant predictors of infection status. The most commonly represented avian family in the sample set was Parulidae (n=177, 23.8%), followed by Cardinalidae

(n= 172, 23.1%) and Turdidae (n= 135, 18.2%). Icterids had 3.6 times the odds of infection compared to Mimidae, and 3.6 times the odds of infection compared to Parulidae (p=0.014, 94% = 1.3 – 10.3; p=0.015, 1.3 – 9.97, respectively) (Figure 2). Although standardized mass as significant (p=0.03), the estimate was unstable and should not be considered; the variable was retained to maximize fit.

Within species, most individuals could not be identified to sex (n=421, 59.3%) and we captured more males (n=200, 65.8%) than females (n=104, 34.2%) with no difference in prevalence between males and females when excluding unknown sexed birds in a separate mixed effect logistic regression (p=0.23). We sampled a greater number of birds on fallout days (n=422) than not on fallout days (n=321) and birds on fallout days were more often not infected (n=224, 53.5%) while birds not on fallout days were about equal in prevalence of infection (n=162, Infected: 50.5%, Uninfected: 49.5%). In a separate mixed effect model, birds that were sampled on fallout days had no differential odds of infection with Haemosporidian blood parasites (p=0.3).

We wished to explore family further by investigating a few focal species and how their body condition was impacted by *Haemoproteus* infection. Among Baltimore Orioles, six out of eight individuals sampled across all years were infected with *Haemoproteus* (75%). In a Fischer's exact test, it was determined that infection did not have a significant effect on fat score (p=0.68), muscle score (p=0.75), or standardized mass (p=1.0). Among Rose-breasted Grosbeaks, two out of nine individuals were infected with *Haemoproteus* (18.2%) and a Fischer's exact showed that infection had no effect on fat score (p=1.0), muscle score (p=0.49), or standardized mass (p=1.0). Among

Red-eyed Vireos, 11 of 18 were infection with *Haemoproteus* (73.3%) and infection had a significant effect on fat score (p=0.029), with infected birds having greater fat stores, but no effect on muscle score (0.73) or standardized mass (p=1.0). Among Scarlet Tanagers, three out of four were infected with *Haemoproteus* (75%) and infection did not have an effect on fat score (p=0.5), muscle score (p=0.75), or standardized mass (p=1.0). Finally, among Summer Tanagers eight out of 15 were infected with *Haemoproteus* (83.3%) and infection had a weak effect on fat score (p=0.12) and an effect on muscle score (p=0.03) – where infected birds had lower fat and muscle scores - but no effect on standardized mass (p=1.0).

In order to explore physiological, ecological, or life history differences in the birds infected with *Plasmodium* vs. those infected with *Haemoproteus* (n= 297 birds), a bivariate analysis showed that wintering grounds, foraging guild, family, and fat score to be significantly associated with genus of parasite (Table 5). In the end, fat was kept in the model to maximize fit but was not a significant predictor of genus infection.

We captured birds in each foraging guild, over wintering in the US (n=89, 12%) and Central (n=459, 61.8%) and South America (n= 195, 26.2%) that were mainly ground foraging (n= 347, 46.7%) and canopy foraging (n= 203, 27.3%) and less so understory foraging (n=193, 26%). A subset of this data- those birds that were infection positive and had a determined genus- was used to analyze parasite genus. The majority of migrants were infected with *Plasmodium* (n=211, 71.04%) while the majority of wintering residents were infected with *Haemoproteus* (n=86, 28.96%). In fact, the final mixed effect logistic regression model showed that birds wintering in the US or South

America had significantly lower odds of infection with *Plasmodium* compared to birds who winter in Central America (OR= 0.09, p=0.000, 95% CI= 0.03 – 0.26; OR= 0.2, p=0.003, 95% CI = 0.08 – 0.6, respectively) (Figure 3). Birds that foraged in the canopy had significantly lower odds of infection with *Plasmodium* than birds that foraged in the understory (OR= 0.14, p=0.042, 95% CI= 0.02 – 0.93) or on the ground (OR= 0.08, p=0.007, 95% CI= 0.014 – 0.51) (Figure 4). Compared to Turdidae, birds in the family Mimidae had 0.15 times the odds of infection with *Plasmodium* (p=0.04, 95% CI= 0.026 – 0.92). Birds in Mimidae and Icteridae had significantly lower odds of infection with *Plasmodium* compared to Cardinalidae (OR= 0.04, p=0.002, 95% CI = 0.01 – 0.31; OR= 0.1, p=0.003, 95% CI = 0.03 – 0.51, respectively). In fact, birds in Mimidae also had 0.1 times the odds of *Plasmodium* infection compared to Turdidae, Parulidae, and Emberizidae (p=0.035, 95%, CI = 0.03 – 0.88; p=0.000, 95%, CIs=0.02-0.33; p=0.009, 95%=.01- 0.5, respectively) (Figure 2).

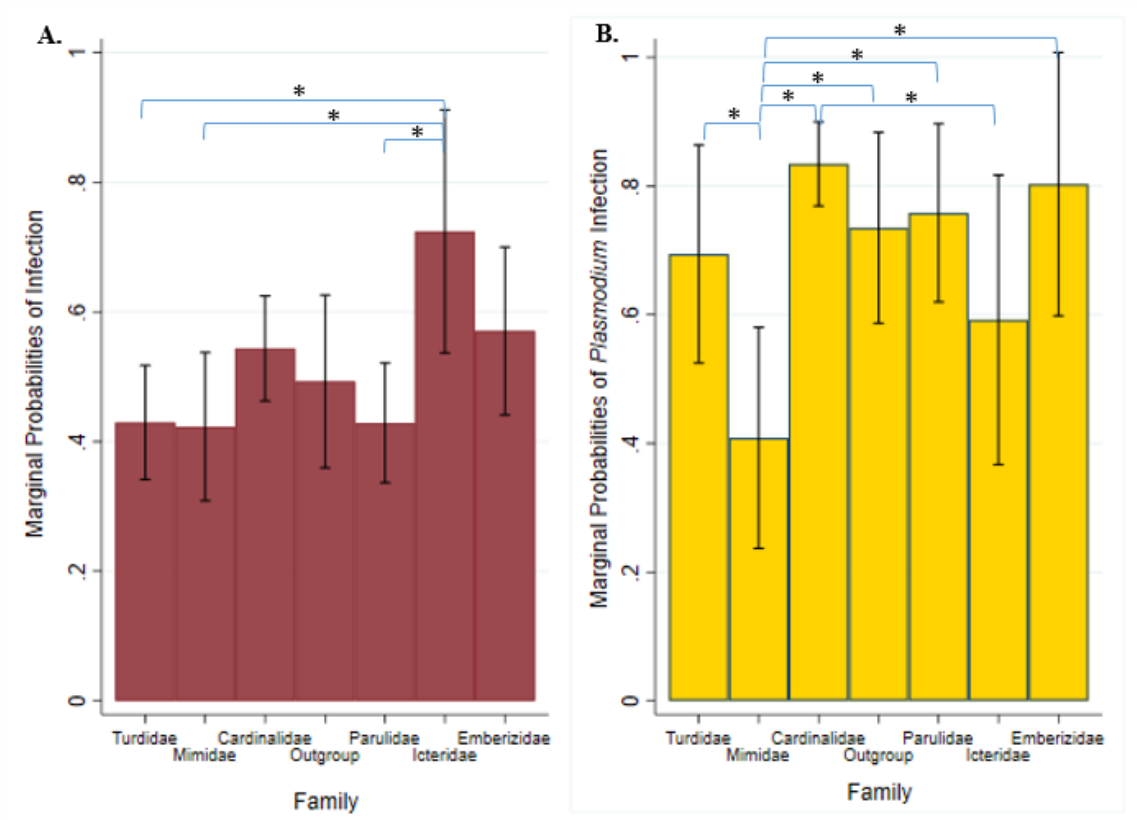

Figure 2: Differences by avian family in the probabilities of a) infection versus non-infection with a Haemosporidian parasite and b) *Haemoproteus* versus *Plasmodium* infection adjusted for the significant predictors in the respective models. Single asterisks with brackets beneath denote significant differences between families.

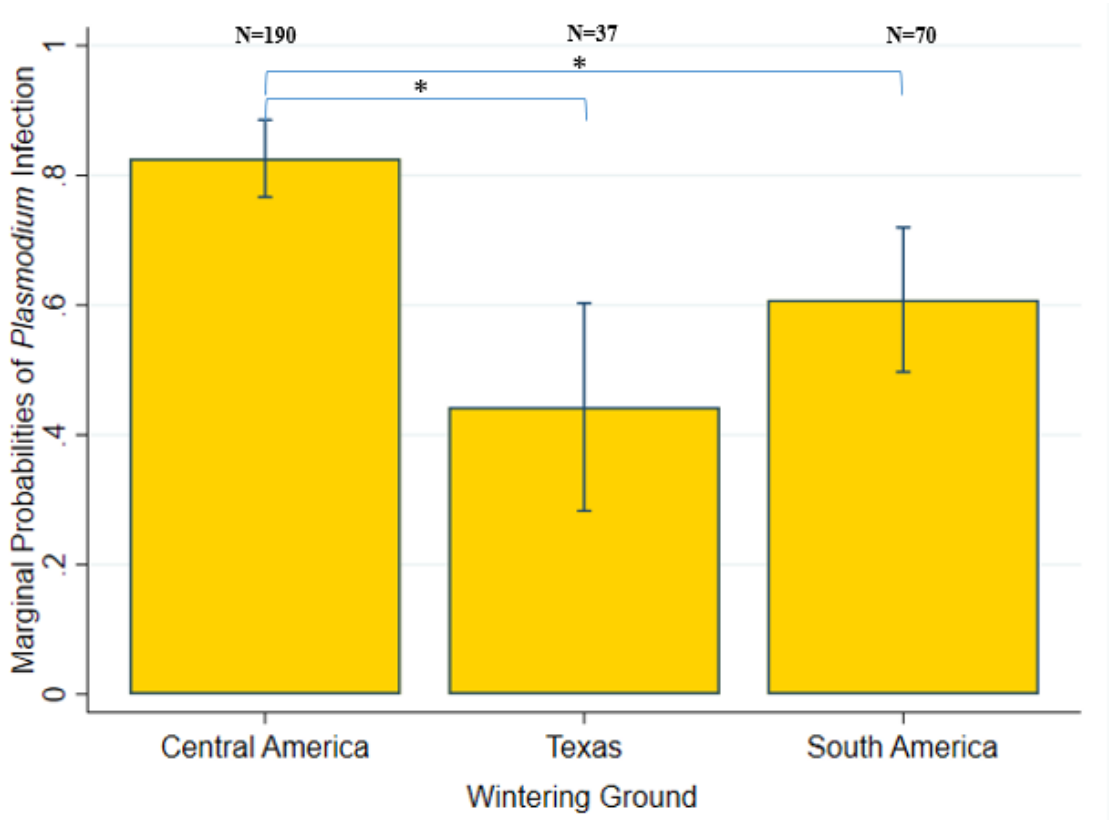

Figure 3: Differences by wintering ground in the probability of *Haemoproteus* versus *Plasmodium* infection adjusted for the significant predictors in the model. Single asterisks with brackets beneath denote significant differences between categories.

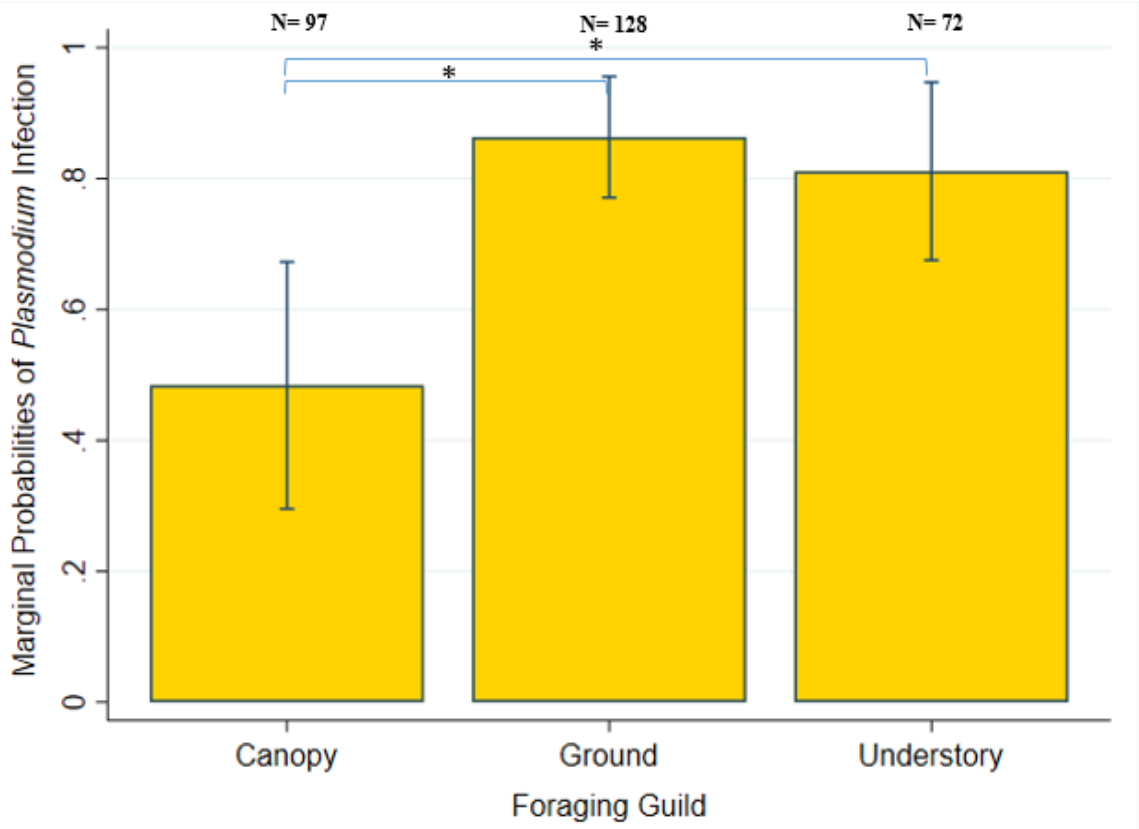

Figure 4: Differences by foraging guild in the probability of *Haemoproteus* versus *Plasmodium* infection adjusted for the significant predictors in the model. Single asterisks with brackets beneath denote significant differences between categories.

CHAPTER IV

DISCUSSION & CONCLUSIONS

Overall, from 743 samples, we found 48.4% of birds captured along the US Gulf Coast during the spring migration of 2014-2019 were infected with a Haemosporidian parasite. The detected infection prevalence is generally higher than previous community prevalence studies of North American birds. For example, Soares et al. (2020) conducted a study of Neotropical migratory birds in the Dominican Republic and found an overall infection prevalence of 18.8% (n=2,681) while Garvin et al. (2006), in a study of migrant birds in the Gulf of Mexico, found an overall infection prevalence of 21.1% (n=1,705) [23, 34]. Indeed, the prevalence we detected in migratory birds during a six-year period is closer to the prevalence reported in breeding season communities. For instance, Matthews et al. (2016) sampled 329 birds in Eastern Tennessee in late May and reported an infection prevalence of 44% [35]. Furthermore, in Southern Missouri, Ricklefs et al. (2005) collected 757 blood samples in early July and found an overall Haemosporidian prevalence of 38.6% [36]. Indeed, Haemosporidian infection prevalence seems to be higher in the Northern hemisphere, in general, where suitable vector habitats may exist in abundance and where birds will experience elevated levels of breeding hormones making them more susceptible to relapse infections [37-39].

The majority of infections in the birds of this Texas study were *Plasmodium* infections (71%). This is in contrast to a study on the Gulf coast of Louisiana, where birds were found to be most often infected with *Haemoproteus* (33.1%) followed by *Plasmodium* infections (31.6%), with the remaining 35.3% split between *Leucocytozoon*

and *Trypanosoma* spp. [23]. This might well be the pattern in the wintering grounds of these birds as well since Fecchio et al. (2011) found, among birds in Brazil, the most frequent infection was also by *Haemoproteus* (66.3%) followed by *Plasmodium* (33.7%) [40]. Perhaps our findings are the result of differences in vector abundance between *Culicoides* midges, the vectors of *Haemoproteus*, and *Culex* mosquitos, the vectors of *Plasmodium*, perhaps *Culicoides* midges are the most abundant at the wintering grounds, where infection is most likely occurring. The birds of this study overwinter in diverse areas, such as Panama and Trinidad for the American Redstart (*Setophaga ruticilla*; [41]), as well as the Yucatan, Honduras, and Venezuela for many wood warblers and thrushes [42, 43]. Studies quantifying avian malaria vectors in these specific countries would add much to our results and be necessary to make definitive conclusions. It has been suggested, however, that *Plasmodium* is less host specific than *Haemoproteus* [6] and such a finding would lend context to our results in that if *Plasmodium* tends to be a generalist parasite, we would expect to observe a greater proportion of *Plasmodium* infections. This is because generalist species will be better competitors and thus be found in greater abundance and infecting more birds.

There was some variation in infection prevalence between years in our study, ranging from a low of 39.7% in 2015 to a high of 58.5% in 2016. Prior long-term studies in other regions have also observed a temporal variation in prevalence. For example, a nine-year study of Amazonian birds by Svensson-Coelho et al. (2013) showed an overall infection prevalence of 21.7%, but with annual variation as low as 14.5% and as high as 33.2% [15]. Similarly, a study of migrant and resident birds in the Dominican Republic,

spanning a total of 5 years, by Soares et al. (2020) reported an overall infection prevalence of 18.8% but with an annual low of 13% and high of 38% [34]. A strong correlation between mosquito abundance and temperature is well documented [44-47] and such variations in vector abundance could translate to yearly variations in parasite prevalence. In a study of the temporal dynamics of a primary avian *Plasmodium* mosquito vector *Culex pipiens*, Lalubin et al. (2013) found relative abundance of the vector populations varied seasonally as well as annually in association with temperature fluctuations [48]. Additionally, *Culex* mosquito density has been reported to vary seasonally and be most abundant during warm-rainy periods [49]. Further long-term studies of bird populations and Haemosporidian infections in relation to vector population dynamics may be useful in explaining the ecological mechanisms that drive variation in avian infection over time.

We found no significant difference in infection status between males and females, although most of the birds in our study were of unknown sex. Across many disease systems, males may experience higher susceptibility to parasites possibly due to the immunosuppressant effects of testosterone or the energetic tradeoffs between immunocompetence and reproductive effort [35, 50, 51], such a relationship is occasionally observed in the avian malaria disease system and the opposite is rare [52]. However, studies that report male-biased parasitism in the Haemosporidian literature are often manipulative studies [53-55], many studies of naturally infected avian communities suggest no such effect of sex on infection probability [35, 56, 57]. Therefore, while previous literature often indicates a theoretical relationship between

parasite infection and sex [50, 53] recent evidence, this study included, appears to show no such relationship between sex and avian Haemosporidians, specifically.

In our study population, wintering grounds was not a significant predictor of Haemosporidian infection. These results are contrary to expectations that migrant birds would have greater Haemosporidian infection prevalence due to the environmental sampling hypothesis, which states that migratory animals may be exposed to more pathogens because they are exposed to several different environment during their journey [58]. Further, given that parasite biodiversity and species richness has been shown to be greatest in the tropics [59], it may be expected that birds that spend time in tropical regions would have a higher prevalence. Central American migrants in this study also had greater odds of infection with *Plasmodium* compared to birds wintering in other areas. This is especially interesting given that *Plasmodium* has been cited as the more pathogenic and severe of the two genera [6]. Overall, our results are suggestive of the migratory escape and migratory culling hypotheses wherein migratory animals seasonally reduce parasitism by escaping exposure and infected migrants succumb to mortality along the migration route [60]. Indeed, prior studies of resident vs. migratory birds have reported that residents can have higher levels of infection. For example, Slowinski et al., (2018) investigated the migratory culling and escape hypotheses by determining Haemosporidian parasite infection in two populations of Dark-eyed Juncos (*Junco hyemalis*) that experience seasonal sympatry during the non-breeding season. They found that the resident population maintained significantly higher parasite prevalence than the migratory population, thereby providing support for the

aforementioned hypotheses [61]. Such a phenomenon may bias our study since acutely infected migrants will not survive the journey and therefore not progress to chronic infection and not be sampled. Additionally, it has also been shown through isotope analysis that areas with higher incidence of avian malaria are generally drier, and further north than areas of lower prevalence [62]. However, though our field site is more northern and therefore drier than the tropical wintering grounds, our study does not support these results.

Ground and understory foragers were more likely to be infected with *Plasmodium* compared to canopy foragers. Similarly, where we found a suggestive trend of Haemoproteus and foraging height, Fecchio et al. (2011) found a significant positive correlation between nest height and *Haemoproteus* prevalence [40]. Corroborating our study, Svensson-Coelho et al. (2013) found decreased *Plasmodium* parasite prevalence with increasing foraging height in Ecuador [15] and Gupta et al., (2020; Preprint) reported foraging strata to be a significant predictor of *Plasmodium* infection in birds in India [63]. In contrast to our study, however, Astudillo et al. (2013) found greater *Haemoproteus* prevalence in birds foraging in lower forest strata while greater *Plasmodium* prevalence was found in birds foraging in the upper strata in Georgia, USA [17]. These findings likely related to variation in the frequency of encounters with infected vectors in different habitats. Because particular vectors species will partition vertical strata, some avian hosts may be more susceptible or more frequently exposed than others [64]. For example, greater numbers of blood-fed biting *Culicoides* midges, the vector of the genus *Haemoproteus*, have been found in canopy traps than in ground

traps in Eastern Tennessee by McGregor et al. (2018; [65]). More supportive of our study, in the Czech Republic, *Culex pipiens* were found in greater abundance on the ground stratum, using bird-baited traps, and biting *Culicoides* midges were the most abundant in the canopy [66]; birds in those forest strata, therefore, are at an increased risk. In Hispaniola, Haemosporidian prevalence may be determined more so by host susceptibility than by habitat, according to data by Latta and Ricklefs (2014; [67]), but our results suggest otherwise, further emphasizing the high degree of site variability in avian Haemosporidian infection dynamics.

Some avian families were associated with a higher infection prevalence than others. For example, birds in the family Icteridae had greater odds of infection than birds in Turdidae, Mimidae, and Parulidae. Prior studies suggest it is not unusual to observe interspecies variation, specifically, low prevalence in some species when community prevalence is high. For example, Purple-crowned Fairy Wrens (*Malurus coronatus*) were found to have a consistent annual infection prevalence of only 5% across a 13-year study, despite the overall avian community prevalence being very high, with some species having as much as 75% prevalence [68]. Garvin et al. (2006) suggest that such results may point to species-specific abilities to cope with the stress of migration and the cost of infection [23]. Additionally, however, some avian families likely have ecological or behavioral traits that increase their exposure to vectors and therefore prevalence [69]. For example, mixed species flocking has been suggested to reduce parasitism by diluting the numbers of conspecifics, thereby circumventing the increased parasitism associated with single species flocks [70, 71] and some species/families of birds at our study site

are known to flock such as the Black and White Warbler (*Mniotilta varia*) and birds in Icteridae [72, 73]. Differences in prevalence between species that forage in similar strata and winter in the same regions may be explained by individual-level factors such as host immunity to infection, host behaviors that influence vector contact (i.e., anti-vector behavior) [74], or even host size whereby larger hosts would have greater surface area which would, theoretically, create more opportunities for a vector to feed.

In order to reveal underlying species level effects, we performed an analysis of a few select focal species in order to contrast and compare with Garvin et al., (2006). The previous paper, investigating Haemosporidian infection in migratory birds on the Gulf coast of Louisiana, found infected birds to have lower fat stores than uninfected birds, but only in Scarlet and Summer Tanagers, but no such effect in any other focal species. Interestingly, we also found infected Summer Tanagers to have lower fat stores, but infected Red-eyed Vireos had higher fat stores. Our results in this analysis were no doubt impacted by our limited sample sizes for infected birds in each focal species, but the effect must be particularly pronounced in Scarlet Tanagers who only had a sample size of four in this analysis. Had the sample size been greater, perhaps we would have detected a similar relationship as that in Garvin et al. The study out of Louisiana further found infected Rose-breasted Grosbeaks and Baltimore Orioles to have lower mean body masses, whereas we found no association between infection and standardized body mass in any focal species.

We detected 46 lineages of Haemosporidians previously reported in MalAvi. Of these, 40 (72.7%) have been detected in North America before, most often in addition to

other geographic regions including Central America, South America, South Sahara, North Africa, Asia, and Europe. We report six (13%) lineages detected in our birds that, while previously published in MalAvi, have not before been detected in North America. Specifically, we observed three birds infected with CYCYA01, one infected with DIGLAF01, two infected with RAMCAR01, and one infected with MYCAME03- all previously reported only in South America. There was also one bird infected with TOXRUF01 and three birds infected with VIGRI02- both previously reported only in Central America. Previous reports detect novel geographic associations with similar frequency. For example, Svensson- Coelho et al. (2013) detected 14 lineages in birds at their field site that had not been reported outside of Ecuador. Furthermore, they detected a lineage closely related to *Haemoproteus enucleator* which, they noted, had not previously been documented outside of South America [15]. Such results are suggestive of intercontinental exchange of parasites between the Americas, evidence for which has been documented in Blue-winged Teals (*Anas discors*) migrating between the American continents [13]. Therefore, studies investigating the propensity of parasite exchange between Passeriformes in the Americas may be useful to elucidate the potential ramifications of transcontinental parasite dispersal.

The present study has its limitations in that birds captured in mist nets may not be representative of the broader population of those species. For example, any bird that may be severely affected by infection may not be capable of flying into our mist nets and therefore would not be represented in this study. Indeed, our study is likely biased toward birds that are chronically infected with avian Haemosporidian parasites and not

those that were clinically impacted by the acute infection. Additionally, our study design does not allow for determination of where a bird was infected, which could include on the breeding, migratory, or wintering grounds. Further, our sample set was comprised of birds captured only during the daytime hours on fair weather days during the spring migration and we have no data on birds that migrate at night, when most migrating passerines are on the move [75]. Also, when determining the 'wintering ground' and 'foraging guild' variables for every bird, although consistent rules were used to determine the category for each bird, the wintering range of most species can contain both Central and South America, representative of the fact that certain populations may winter in different locations from other populations. Finally, due to time constraints, we did not test for the third genus of avian Haemosporidians- *Leucocytozoon* spp. parasites.

This study presents the complex host-parasite relationship of avian Haemosporidian parasites in spring trans-Gulf migrant birds, highlighting some factors including foraging guild and wintering grounds that are predictive of *Plasmodium* infection. In the wake of North America's recent marked loss in bird abundance over the last 50 years [24], we emphasize the importance of building upon this and related studies in order to answer further questions in avian blood parasite ecology, such as how host-parasite interactions in this system may be impacted by global climate change and what impacts this phenomenon could have on migratory and resident avifauna.

Table 1: Species that were categorized into each avian family for the purposes of this analysis with number and percent infected with in each family. Note: first superscript denotes [a] Wintering resident, [b] Central American migrant, [c] South American migrant. Following the comma, the second superscript denotes [a] Ground forager, [b] Understory forager, [c] Canopy forager. Some species have two first superscripts separated by a forward slash, these were the species that had wintering ranges in both Texas and Central America and whose status was determined based on time of capture.

Family	Species included	Infected (%)
Turdidae	*Catharus minimus*[c,a], *Catharus guttatus*[a,a], *Catharus ustulatus*[c,a], *Catharus fuscescens*[c,a], *Hylocichila mustelina*[b,a]	60 (44.4)
Mimidae	*Toxostoma rufum*[a,a], *Dumetella carolinensis*[a/b,b], *Mimus polyglottos*[a,a]	56 (49.6)
Cardinalidae	*Passerina caerulea*[b,c], *Passerina cyanea*[b,a], *Cardinalis cardinalis*[a,c], *Passerina ciris*[b,c], *Pheucticus ludovicianus*[b,c], *Piranga olivacea*[c,c], *Piranga rubra*[c,c]	94 (54.6)
Outgroups (Vireonidae/ Troglodytidae/ Tyrannidae/Cuculidae)	*Empidonax virescens*[c,c], *Thryothorus ludocivianus*[a/b,a], *Tyrannus tyrannus*[c,c], *Contopus virens*[c,c], *Troglodytes aedon*[a,b], *Vireo philadelphicus*[b,c], *Vireo olivaceus*[c,c], *Vireo gilvus*[b,c], *Vireo griseus*[a,c], *Coccyzus americanus*[c,c], *Vireo flavifrons*[b,c]	29 (47.5)
Parulidae	*Mniotilta varia*[b,c], *Setophaga castanea*[c,c], *Setophaga virens*[b,c], *Cardellina canadensis*[c,b], *Geothlypis trichas*[a/b,b], *Setophaga pensylvanica*[b,c], *Setophaga citrina*[b,b], *Geothlypis formosa*[b,b], *Parkesia motocilla*[b,a], *Parkesia noveboracensis*[c,a], *Setophaga magnolia*[b,c], *Setophaga coronata*[a,b], *Seiurus aurocapilla*[b,a], *Protonotaria citrea*[c,c], *Leiothlypis peregrina*[b,c], *Helmitheros vermivorum*[b,b], *Setophaga petechia*[c,c], *Setophaga dominica*[a,c]	68 (38.4)
Icteridae	*Molothrus ater*[a,a], *Icterus spurius*[b,c], *Icterus galbula*[b,c], *Icteria virens*[b,b]	18 (75)
Emberizidae	*Melospiza lincolnii*[a/b,a], *Passerculus sandwichensis*[a/b,a], *Melospiza georgiana*[a/b,a], *Zonotrichia albicollis*[a,a]	35 (57.4)

Table 2: Previously undocumented lineages detected in blood samples from spring migratory birds on the Texas Gulf coast, organized by major genus.

Lineage name	Number of infections in TX study	Families (species) infected in TX study	GenBank accession number
***Plasmodium* genus**			
nov_KEWA01	1	Parulidae (*Geothlypis formosa*)	MW081130
nov_SEIAUR04	2	Parullidae(*Seiurus aurocapilla, Geothlypis trichas*)	MW091126
nov_DUMCAR09	1	Mimidae (*Dumetella carolinensis*)	MW081134
nov_GEOTRI12	1	Parulidae (*Geothlypis trichas*)	MW081139
nov_CATFUS21	1	Turdidae (*Catharus fuscescens*)	MW081133
nov_ICTGAL04	1	Icteridae (I*cterus galbula*)	MW139686
***Haemoproteus* genus**			
nov_CARCAR02	11	Cardinalidae (*Cardinalis cardinalis*)	MW081128
nov_MIMPOL02	4	Mimidae (*Mimus polyglottos*)	MW081124
nov_ICTSPU01	3	Icteridae (*Icterus spurius*)	MW081125
nov_CATUST40	2	Icteridae, Turdidae (I*cterus galbula*, *Catharus ustulatus*)	MW081127
nov_VIOLI17	1	Vireonidae (*Vireo olivaceus*)	MW081129
nov_DUMCAR08	1	Mimidae (*Dumetella carolinensis*)	MW081131
nov_PIOLI04	1	Cardinalidae (*Piranga olivacea*)	MW081132
nov_LISP01	1	Emberizidae (*Melospiza lincolnii*)	MW081135
nov_CARCAR10	1	Cardinalidae (*Cardinalis cardinalis*)	MW081138
nov_DUMCAR10	1	Mimidae (*Dumetella carolinensis*)	MW081137
nov_VIOLI18	1	Vireonidae (*Vireo olivaceus*)	MW081136

Table 3: Lineages detected in blood samples from spring migratory birds on the Texas Gulf coast, organized by major genus: *Plasmodium* & *Haemoproteus*. Note: [a] Novel association between this lineage and this host species. [b] Novel association between this lineage and geographic region; not previously reported in North America.

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
***Plasmodium* genus**				
BAEBIC02 (*Plasmodium homopolare*)	5	Parulidae	Certhidae, Fringillidae, Icteridae, Paridae, Parulidae, Sittidae, Turdidae	N. America, S. America
BT7	30	Cardinalidae[a], Emberizidae[a], Parulidae, Turdidae	Accipitridae, Anatidae, Certhiidae, Charadriidae, Corvidae, Fringillidae, Hirundinidae, Muscicapidae, Paridae, Parulidae, Passeridae, Scolopacidae, Sylviidae, Turdidae	Europe, N. America, Hawaii, C. America, S. America, Asia
CATUST05	4	Emberizidae[a], Turdidae	Anatidae, Certhiidae, Gaviidae, Hirundinidae, Laridae, Paridae, Parulidae, Thamnophilidae, Turdidae	N. America, S. America
CATUST06	5	Cardinalidae[a], Turdidae	Formicariidae, Turdidae	N. America, C. America, S. America

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
COLL4 (*Plasmodium homocircumflexum)*	1	Icteridae	Fringillidae, Icteridae, Laniidae, Mimidae, Muscicapidae, Ploceidae, Pycnonotidae, Sturnidae, Vireonidae	Europe, S. Sahara, N. America, S. America
CYCYA01	3	Parulidae[a], Turdidae[a]	Certhiidae, Columbidae, Fringillidae, Furnariidae, Icteridae, Pipridae, Thamnophilidae	S. America[b]
DENPET03 (*Plasmodium nucleophilum*)	4	Mimidae[a], Parulidae	Anatidae, Certhiidae, Cracidae, Dendrocolaptidae, Fringillidae, Furnariidae, Hirundinidae, Icteridae, Laridae, Muscicapidae, Parulidae, Passeridae, Phoenicopteridae, Pipridae, Psittacidae, Ramphastidae, Spheniscidae, Thamnophilidae, Turdidae, Tyrannidae, Vireonidae	N. America, S. America
DIGLAF01 (*Plasmodium lutzi*)	1	Turdidae[a]	Dendrocolaptidae, Fringillidae	S. America[b]

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
GEOTRI01	20	Cardinalidae[a], Emberizidae[a], Parulidae, Turdidae, Vireonidae[a]	Fringillidae, Parulidae, Turdidae	N. America, S. America
GEOTRI02	4	Cardinalidae[a], Parulidae	Fringillidae, Icteridae, Parulidae, Tyrannidae	N. America, S. America
GEOTRI09	14	Cardinalidae, Icteridae[a], Mimidae, Parulidae	Fringillidae, Hirundinidae, Mimidae, Parulidae, Turdidae	N. America, C. America
GRW06 (*Plasmodium elongatum*)	1	Troglodytidae[a]	Acanthizidae, Anatidae, Apterygidae, Ardeidae, Bucconidae, Callaeatidae, Columbidae, Corvidae, Cracticidae, Dendrocolaptidae, Fringillidae, Furnariidae, Hirundinidae, Meliphagidae, Mimidae, Motacillidae, Nectariniidae, Paridae, Parulidae, Passeridae	Europe, S. Sahara, N. Africa, N. America, S. America, Asia, Australia, Oceania

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
GRW06 (*Plasmodium elongatum*)			Petroicidae, Phasianidae, Ploceidae, Psittacidae, Ptilonorhynchidae, Pycnonotidae, Rallidae, Spheniscidae, Strigidae, Sylviidae, Timaliidae, Turdidae, Zosteropidae	
ICTVIR01	1	Icteridae[a]	Parulidae	N/A
LINN1 (*Plasmodium matutinum*)	1	Mimidae[a]	Apterygidae, Corvidae, Fringillidae, Meliphagidae, Paridae, Passeridae, Rallidae, Spheniscidae, Strigidae, Turdidae	Europe, N. America, Asia, Australia
MELMEL05	3	Cardinalidae[a]	Fringillidae	N. America
MYCAME03	1	Cuculidaea[a]	Ciconiidae	S. America[b]
PADOM09	1	Mimidae	Anatidae, Certhiidae, Dendrocolaptidae, Fringillidae, Furnariidae, Hirundinidae, Icteridae, Laridae, Mimidae, Muscicapidae, Parulidae, Passeridae, Spheniscidae, Turdidae, Tyrannidae	N. America, South America

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
PADOM11	61	Cardinalidae[a], Icteridae, Mimidae, Parulidae	Anatidae, Certhiidae, Dendrocolaptidae, Fringillidae, Gaviidae, Hirundinidae, Icteridae, Mimidae, Muscicapidae, Paridae, Parulidae, Passeridae, Picidae, Spheniscidae, Strigidae, Turdidae, Tyrannidae, Vireonidae	N. America, South America
RAMCAR01	2	Icteridae[a], Mimidae[a]	Fringillidae	S. America[b]
RWB01	4	Cardinalidae[a], Emberizidae[a]	Fringillidae, Icteridae, Parulidae, Tyrannidae, Strigidae	N. America
SEIAUR01 (*Plasmodium cathemerium*)	4	Cardinalidae[a], Parulidae	Anatidae, Certhiidae, Corvidae, Fringillidae, Hirundinidae, Icteridae, Parulidae, Passeridae, Strigidae, Turdidae, Tytonidae	N. America, C. America, S. America
SETCOR03	2	Cardinalidae[a], Vireonidae[a]	Parulidae	N. America
TACTHA01	5	Cardinalidae[a]	Fringillidae, Hirundinidae	N. America
TUMIG03 (*Plasmodium unalis*)	13	Cardinalidae[a], Mimidae,	Certhiidae, Fringillidae, Icteridae, Laridae	N. America

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
TUMIG03		Parulidae, Turdidae	Mimidae, Parulidae, Spheniscidae, Sturnidae, Sylviidae, Turdidae, Tyrannidae	S. America
TUMIG23	1	Turdidae[a]	N/A	N/A
VIOLI03	3	Vireonidae	Certhiidae, Vireonidae	N. America, S. America
WW3	8	Cardinalidae[a], Emberizidae[a], Parulidae, Vireonidae[a]	Corvidae, Estrildidae, Fringillidae, Hirundinidae, Icteridae, Laridae, Motacillidae, Muscicapidae, Nectariniidae, Parulidae, Passeridae, Ploceidae, Pycnonotidae, Sylviidae, Turdidae	Europe, S. Sahara, N. America, S. America
***Haemoproteus* genus**				
CARCAR29	1	Cardinalidae[a]	N/A	N/A
CHIPAR01	3	Vireonidae[a]	N/A	N/A
COLL2 (*Haemoproteus pallidus*)	5	Turdidae	Muscicapidae, Ptilonorhynchidae, Sylviidae, Turdidae, Tyrannidae	Europe, S. Sahara, N. America, Asia, Australia
ICTGAL01	2	Icteridae[a]	N/A	N/A
ICTGAL02	3	Icteridae[a]	N/A	N/A

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
JUHYE02	1	Emberizidae[a]	Fringillidae	N. America
MAFUS02	11	Mimidae	Mimidae	N. America
MAFUS02				C. America
MIMGIL01	2	Mimidae	Mimidae	N/A
PACPEC02	3	Cardinalidae[a]	Dicruridae, Fringillidae, Tyrannidae	N. America
PACPEC02				C. America, S. America, Oceania
PHEMEL02	3	Cardinalidae[a], Tyrannidae[a]	Fringillidae, Icteridae	N. America
PIRLUD02	7	Cardinalidae[a], Parulidae[a], Turdidae[a]	Fringillidae	N. America
PIRUB01	1	Cardinalidae[a]	Fringillidae	N. America
SETAUD05	3	Parulidae[a]	Parulidae	N. America
TOXRUF01	1	Mimidae	Mimidae	C. America[b]
VIGIL07	1	Vireonidae	Vireonidae	N. America

Table 3 continued

Lineage name	Number found in TX study	Host families in TX study	Host families previously identified	Previous regions identified
VIGIL09	1	Vireonidae	Certhiidae, ColumbidaeFringillidae, Thamnophilidae, Turdidae, Tyrannidae, Vireonidae	N. America, S. America
VIGRI02	3	Vireonidae	Vireonidae	C. America[b]
VIOLI06 (*Haemoproteus vireonis*)	4	Vireonidae	Dendrocolaptidae, Fringillidae, Vireonidae	N. America, S. America
VIOLI11	3	Parulidae[a], Vireonidae[a]	N/A	N/A

Table 4: Bivariate analysis of variables for infection status. Chi-squared and Fischer's exact tests were used, as well as a liberal p-value cutoff of 0.25.

Variable	Categories	Sample size (N)	Number positive (%)	p value
Wintering ground	North Am.	89	48 (53.9)	0.32
	Central Am.	459	225 (49)	
	South Am.	195	87 (44.6)	
Foraging guild	Ground	347	160 (46.1)	**0.053***
	Understory	193	87 (45.1)	
	Canopy	203	113 (55.7)	
Family	Turdidae	135	60 (44.4)	**0.003***
	Mimidae	113	56 (49.6)	
	Cardinalidae	172	94 (54.6)	
	Outgroup	61	29 (47.5)	
	Parulidae	177	68 (38.4)	
	Icteridae	24	18 (75)	
	Emberizidae	61	35 (57.4)	

Table 4 continued

Variable	Categories	Sample size (N)	Number positive (%)	p value
Fat Score	0	222	112 (50.4)	0.437
	1	265	128 (48.3)	
	2	168	73 (43.4)	
	3 & 4	87	46 (52.9)	
Muscle Score	1 & 2	218	99 (45.4)	0.285
	3 & 4	525	261 (49.7)	

Table 5: Bivariate analysis of variables for infection with *Plasmodium* (as opposed to *Haemoproteus*). Chi-squared and Fischer's exact tests were used, as well as a liberal p-value cutoff of 0.25.

Variable	Categories	Sample size (N)	*Plasmodium* (%)	p value
Wintering Grounds	North Am.	37	13 (35.1t)	**0.000***
	Central Am.	190	157 (82.6)	
	South Am.	70	41 (58.6)	
Foraging guild	Ground	128	111 (86.7)	**0.000***
	Understory	72	55 (76.4)	
	Canopy	97	45 (46.4)	
Family	Turdidae	44	37 (84.1)	**0.000***
	Mimidae	42	22 (52.4)	
	Cardinalidae	85	59 (69.4)	
Family	Outgroup	25	8 (32)	
	Parulidae	61	56 (91.8)	
Family	Icteridae	16	7 (43.7)	
	Emberizidae	24	22 (91.7)	
Fat Score	0	95	67 (70.5)	**0.149***
	1	103	76 (73.8)	
	2	65	49 (75.4)	
	3 & 4	33	18 (54.5)	
Muscle Score	0 & 1	84	60 (71.4)	0.927
	2 & 3	213	151 (70.9)	

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