Characterization of the *Plasmodium* and *Haemoproteus* parasite community in temperate-tropical birds during spring migration

Spencer DeBrock [a,b], Emily Cohen [c,d], Sujata Balasubramanian [a,b], Peter P. Marra [c,e], Sarah A. Hamer [a,b,*]

[a] Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA
[b] Schubot Center for Avian Health, Department of Veterinary Pathobiology, Texas A&M University, USA
[c] Smithsonian Migratory Bird Center, Washington D.C, USA
[d] University of Maryland Center for Environmental Science, Appalachian Laboratory, USA
[e] Department of Biology and McCourt School of Public Policy, Georgetown University, 37th and O Streets NW, Washington, DC, 20057, USA

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ABSTRACT

Animal movements, especially avian migration, can be a mechanism for the large-scale dispersal and geographic range expansion of parasites. The host-parasite relationships among birds during migration have yet to be fully explored. We characterized the haemosporidian parasite lineages in passerines during spring migration on the Texas coast of the Gulf of Mexico, and identified associations among wintering origin (US, Central America, South America) and foraging height (canopy, understory, ground) and infection status. We examined 743 samples representing 52 species of 10 families over six years, 2014–2019. We used PCR and DNA sequencing of the haemosporidian cytB gene from avian blood samples to determine infection status with the genera *Plasmodium* and *Haemoproteus* and characterize the lineages of blood parasites. We found an overall haemosporidian infection prevalence of 48.4% among neotropical migrant and Texas wintering birds. Among families, Icterids had the highest prevalence (75%, 24 individuals, 4 species sampled) whereas Parulids had the lowest prevalence (38.4%, 177 individuals, 18 species sampled). Among infected birds, *Plasmodium* spp. infections were more common than *Haemoproteus* spp. infections in species that winter in Central America compared to those that winter in the US or South America. Similarly, among infected birds, *Plasmodium* spp. infections were more common than *Haemoproteus* spp. infections in species that forage on the ground or in the understory compared to those that forage in the canopy. Infected birds harbored 65 different haemosporidian lineages (71% *Plasmodium*; 29% *Haemoproteus*) of which 17 lineages have never previously been reported and six lineages were documented for the first time in North America, having been previously detected only in Central or South America. These data are consistent with the premise that intercontinental parasite dispersal may be facilitated by passerine birds. Future studies focused on surveillance, the probability of establishment of parasite lineages, and the use of individual bird tracking methods to understand infection dispersion over time will allow a more comprehensive understanding of changing avian host-haemosporidian relationships.

1. Introduction

A large diversity of haemosporidian species in the genera *Haemoproteus, Plasmodium,* and *Leucocytozoon* infect birds worldwide. These parasites are transmitted by dipteran vectors including mosquitoes, biting midges, hippoboscid louse flies, and simuliid black flies (Atkinson and van Ripper, 1991). Infection of birds by haemosporidians may result in acute or chronic clinical signs. Immediately after infection, birds can develop high parasitemia which may be associated with systemic organ pathology due to damage by exoerythrocytic parasite stages (Atkinson and van Ripper, 1991; Valkiunas and Iezhova, 2017). The chronic phase occurs days or weeks after infection, when infected birds experience low parasitemia and mild clinical impacts that may last for years, with or without seasonal relapses (Atkinson and van Ripper, 1991). These chronic impacts can accumulate and eventually reduce phenotypic quality and fitness (Asghar et al., 2011).

Migratory birds move rapidly and in high numbers between tropical and temperate latitudes annually and are implicated in the dispersal of

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parasites and spread of disease. For example, genetic data support the dispersal of influenza A viruses, antimicrobial resistant bacteria, and avian haemosporidians, across continental and intercontinental spatial scales by migratory birds (Fourment et al., 2017; Ahlstrom et al., 2018; Ferraguti et al., 2019). Furthermore, because migration is energetically expensive, there can be notable effects of parasite carriage on individual migrants or migratory populations (Altizer et al., 2011). For instance, a meta-analysis by Risely et al. (2018) showed that infection is associated with reduced body condition, delayed migration, and lower survival. An understanding of avian host-parasite relationships in the context of migration is important for both uncovering the geographic origin of parasite dispersal as well as revealing potential impacts on avian health.

Avian migration may provide a mechanism for exotic haemosporidian lineages to be transported to new locations where they may infect new communities of avian hosts. Evidence for this phenomenon has been documented in the Galapagos, where migratory Bobolinks (*Dolichonyx oryzivorus*) were implicated in introducing ephemeral *Plasmodium* lineages found in the Galapagos birds (Levin et al., 2013). Further, a genetically distinct suite of haemosporidian parasites was found among the migratory vs. resident birds in Japan (Yoshimura et al., 2014). Recent studies indicate that birds may be more susceptible to allopatric malarial infections than to sympatric malarial infections (Sarquis-Adamson and MacDougall-Shackleton, 2016), and that *Haemoproteus* infections can be especially lethal in non-adapted bird species (Valkiunas and Iezhova, 2017).

Attributes of a species' life history are important in predicting infection probability with haemosporidians. Across taxa, there exists a latitudinal gradient of diversity in which species richness is greater in tropical climates; *Plasmodium* and *Haemoproteus* have been shown to follow this pattern with greater lineage richness in the tropics (Clark et al., 2014). Furthermore, Clark et al. (2016) found lower haemosporidian prevalence in birds wintering in higher latitudes. Accordingly, the wintering origin of birds may be important in predicting infections. Additionally, factors which put individuals in greater contact with vectors are likely to be associated with greater risk of infection. For example, ground foraging Ecuadorian bird species were found to have elevated *Plasmodium* prevalence relative to those that foraged higher in the canopy (Svensson-Coelho et al., 2013), likely attributed to greater

mosquito contact. Conversely, *Culex pipiens*, a vector of *Plasmodium*, have been caught in higher abundance in the canopy layer (Anderson et al., 2006). Therefore, relationships among foraging height, vector contact, and infection probability are likely to vary across space and time.

The objectives of this study were to (i) quantify *Plasmodium* and *Haemoproteus* infection prevalence in migrating and wintering resident birds; (ii) determine how infection varies across avian families, life history traits (e.g., foraging height; wintering origin), and energetic condition; and (iii) identify relationships between host species and *Plasmodium* and *Haemoproteus* parasite lineages. Given the greater diversity of *Plasmodium* and *Haemoproteus* parasites in the tropics (Clark et al., 2016), we predicted that migrants arriving from South America would harbor a greater richness of parasite lineages than those from Central America or Texas wintering residents. Further, given the variation in vector abundance across forest strata, we predicted variation in *Plasmodium* and *Haemoproteus* infection prevalence among birds within different foraging strata.

2. Materials and methods

2.1. Study site and sampling

We sampled passerine birds at the Nature Conservancy's Clive Runnells Family Mad Island Marsh Preserve, in Matagorda County, TX (28°37′36.4″N, 96°06′03.9″W) (Fig. 1; Cohen et al., 2015), where many species of Nearctic-Neotropical migratory birds first make landfall after flying over the Gulf of Mexico in their spring migration (Cohen et al., 2017). We captured birds during the peak of spring migration (March–May) over six years (2014–2019). Mist nets were opened daily for approximately 8 h, weather permitting (see Cohen et al., 2015 for more information). For each captured individual, we applied a federal USGS leg band, took morphological measurements, assessed the amount of subcutaneous fat stores, and took a blood sample before release. Fat score was determined on a scale of 0–5 with five being the highest (Helms and Drury, 1960); due to the low number of birds with high fat scores, this variable was categorized into 4 levels (0, 1, 2, and ≥3) for analysis (Goymann et al., 2010). Mass was standardized by dividing the

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

mass by unflattened wing chord length to create a 'body size' variable (Wang and Moore, 1997).

Blood was acquired either through brachial venipuncture using 27-gauge needles and capillary tubes or jugular venipuncture using 1 ml insulin syringes. The amount of blood extracted was not more than one per cent of the bird's body weight, with sampled volume ranging from 30 to 100μL (Hamer et al., 2012). Blood samples were either stored in dry tubes (years 2014–2015), in ~300μl RNAlater (Ambion inc., Austin, TX) (years 2016–2018), or in 225ul TRIzol-LS (Thermo Fisher Scientific, Waltham, MA) (year 2019); in the field all samples were placed on ice followed by −20 °C and −80 °C storage. If birds were recaptured within 3 h of taking data from them, they were released immediately (Cohen et al., 2015). Otherwise, they were resampled; however, no bird was blood sampled twice in the same day.

2.2. Molecular testing of blood samples

We processed blood samples in order to quantify *Plasmodium* and *Haemoproteus* 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 (Bensch et al., 2009), using previously described methods (Hellgren et al., 2004). 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. Next, 50-100μL of this resuspended blood sample was used for DNA extraction. For years 2016–2019, when blood was stored in a liquid preservative, approximately 50uL of the homogenized preserved whole blood sample was used in the extraction. For PCR, we used Failsafe PCR 2X PreMix E and Enzyme Mix (Lucigen, Middleton, WI) to amplify a 479bp DNA fragment in a two-step nested PCR (Hellgren et al., 2004). The template for the nested reaction was a 1:10 dilution of the product from the initial PCR (1uL of extracted DNA diluted with 9uL water). A field-collected, sequence-confirmed positive sample from 2014 served as a positive control. Following gel electrophoresis, all samples with a 479bp amplified fragment were considered positive for infection with *Plasmodium* or *Haemoproteus*.

2.3. Parasite lineage determination

PCR amplicons were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) following manufacturer instructions. Sanger sequencing of the forward and reverse strands was performed by Eton Biosciences (San Diego, CA). Raw sequence reads, untrimmed, were first scrutinized using the web based MalAvi BLAST tool (http://130.235.244.92/Malavi/) to identify parasite genus and the most similar published lineage(s). Next, a multiple alignment, using Multalin (http://multalin.toulouse.inra.fr/multalin/), was performed of the forward and reverse sequences to generate a consensus sequence, and align with the sequence/s with the most homology from MalAvi to identify mismatches. Sequences were trimmed to the length of the MalAvi region of analysis. 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 (previously unreported/unpublished) lineage. All novel lineages were then compared to each other using Mega version X (Kumar et al., 2018). Samples with evidence of more than one lineage (mixed lineages, or co-infections) were detected by visually examining sequencing chromatographs for double-nucleotide peaks (Perez-Tris and Bensch, 2005). We designated a sample as having a mixed infection if the same double-base call was present in the forward and reverse sequence within the trimmed region of the sequence. All detected lineages were deposited to the MalAvi database and GenBank (GenBank accession numbers MW081078-139 & MW139686).

2.4. Assignment of life history traits

Wintering grounds of each species was categorized as US, Central America, or South America (Cornell Lab of Ornithology, 2019). Species with wintering ranges primarily in Mexico and Central America that did not extend south beyond Colombia or Venezuela were categorized as Central American migrants. Species with wintering ranges exclusively in South America were categorized as South American migrants. Two exceptions included the Acadian Flycatchers (*Empidonax virescens*) and Black-and-white Warblers (*Mniotilta varia*) which were categorized as South American to reflect their primary wintering areas, although both have ranges that potentially extend into southern Panama. Finally, if the wintering range included anywhere in the state of Texas, the bird was categorized as a 'wintering resident' with the following exceptions. Common Yellowthroats (*Geothlypis trichas*), Gray Catbirds (*Dumetella carolinensis*), and Lincoln Sparrows (*Melospiza lincolnii*) have primarily Central American wintering grounds that extend into Texas, such that birds caught early in our study period (mid-March) may have been wintering resident birds while individuals caught later in the spring were most likely migrating from more southern locations (see Cohen et al., 2015b). To account for this, 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 species accounts and previous work on these species during stopover in the following categories: ground foraging, understory foraging, canopy foraging (Cornell Lab of Ornithology, 2019; Barrow et al., 2000) (Table 1).

2.5. Statistical analysis

A bivariate analysis was run on each of the following variables individually using Chi-squared or Fisher's exact tests to determine factors significantly associated with the dependent variables of (i) infection status (positive or negative for a haemosporidian parasite) or (ii) specific parasite genus (*Plasmodium* or *Haemoproteus*). Factors for both included wintering ground; foraging guild; avian family; fat score; and body size. We included avian family to address taxonomic relationships, and body size and fat score to control for potential influences of body surface area on infection risk (Atkinson and van Ripper, 1991). In these bivariate analyses, a liberal p value of <0.25 was used to assess significance, a common approach when bivariate analysis is followed by post-hoc regression analysis (Dohoo et al., 2014).

Mixed effect logistic regression models were made with random intercepts and wintering ground, foraging guild, body size, fat score, and family as fixed effects 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 significant predictors from the bivariate analysis were included in the model together and stepwise regression was utilized to determine the model with the best fit based on model comparisons. 'Year' was run in a separate fixed effect model to characterize annual variation and then included as a random effect in all other models to control for annual variation. Statistics were conducted using the program STATA (StataCorp, 2019).

3. Results

Seven-hundred and forty-three samples were processed, including birds migrating from the tropics (n = 654, 88%) and birds assumed to be wintering in Texas (n = 89, 12%), representing 52 species and 10 passerine families. The most commonly sampled avian families were Parulidae (n = 177, 23.8%), followed by Cardinalidae (n = 172, 23.1%) and Turdidae (n = 135, 18.2%). We captured birds presumably wintering in the Texas (n = 89, 12%), Central America (n = 459, 61.8%), and South America (n = 195, 26.2%) and generally characterized as

Table 1
Number of birds sampled and percent infected with *Plasmodium* or *Haemoproteus* for each avian family. Species belonging to each avian family are indicated, with indication of life history traits used in the analysis as follows: First superscript denotes[a] Texas 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 for which individual bird wintering range was assigned based on time of capture.

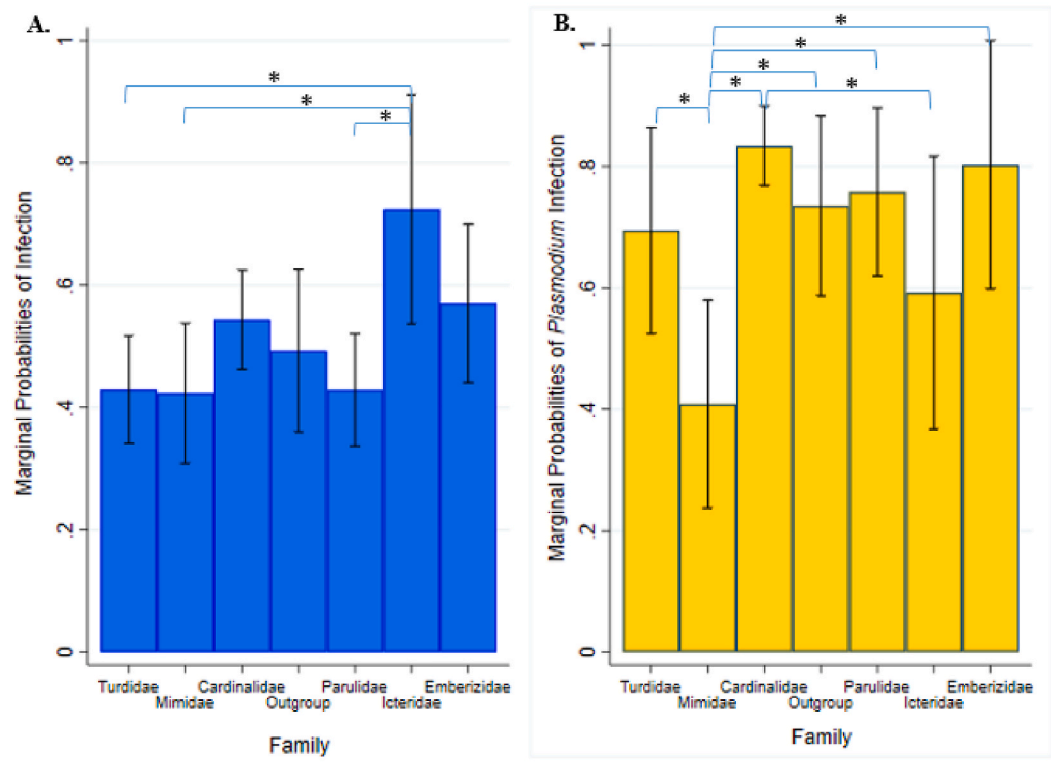| Family	Species included	Sampled (n)	Infected (%)
Turdidae	*Catharus minimus*[c,a], *Catharus guttatus*[a,a], *Catharus ustulatus*[c,a], *Catharus fuscescens*[c,a], *Hylocichila mustelina*[b,a]	135	60 (44.4)
Mimidae	*Toxostoma rufum*[a,a], *Dumetella carolinensis*[a/b,b], *Mimus polyglottos*[a,a]	113	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]	172	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]	61	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]	177	68 (38.4)
Icteridae	*Molothrus ater*[a,a], *Icterus spurius*[b,c], *Icterus galbula*[b,c], *Icteria virens*[b,b]	24	18 (75)
Emberizidae	*Melospiza lincolnii*[a/b,a], *Passerculus sandwichensis*[a/b,a], *Melospiza georgiana*[a/b,a], *Zonotrichia albicollis*[a,a]	61	35 (57.4)

Table 2
Bivariate analysis of variables for *Plasmodium* or *Haemoproteus* infection status using chi-squared and Fisher's exact tests.

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)	
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)	

1.3–9.7; Fig. 2). In terms of annual variation, 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).

Of the positive samples, 297 (82.3%) were successfully identified to genus and 295 to lineage. *Plasmodium* was the most common (n = 210, 71%) while *Haemoproteus* comprised 29% of infections (n = 85). Overall, we found 48 previously described lineages comprising 28 *Plasmodium* lineages and 20 *Haemoproteus* lineages (Table 3). In addition, we found 17 novel lineages (previously unreported/unpublished) that occurred in 34 samples. Of these, 11 (64.7%) were novel *Haemoproteus* lineages, and six (35.3%) were novel *Plasmodium* lineages (Table 3). The most common *Plasmodium* lineage, and the most common lineage overall, was PADOM11, representing 20.7% of all lineages and 29.05% of *Plasmodium* infections (Table 4). The most common *Haemoproteus* lineages were MAFUS02 and the novel lineage CARCAR02, each representing 12.9% of infections within that genus. Among the infections found in the birds include six lineages not before detected in North America including CYCYA01, DIGLAF01, MYCAME03, & RAMCAR01, which were all previously reported from only South America, and TOXRUF01 and VIGRI02, which were both previously reported from only Central America (Table 4). Similarly, among the lineages found in the birds include 47 new associations between previously reported lineages and host families (Table 4). Among samples that were sequenced, we observed a mixed lineage infection prevalence of at least 13.5% (n = 40 mixed infections).

Of the 297 infected birds for which the parasite genus was determined, a bivariate analysis showed wintering grounds, foraging guild, family, and fat score to be significantly associated with genus of parasite (Table 5), although fat was not a significant predictor in the final model. Among infected birds, the majority of migrants were infected with *Plasmodium* (n = 198, 76.1%) while the majority of wintering residents were infected with *Haemoproteus* (n = 24, 64.9%). The final mixed effect logistic regression model of infected birds showed that Texas wintering birds and South American migrants had significantly lower 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) (Fig. 3). Infected birds that foraged in the canopy had significantly lower infection with *Plasmodium* than infected 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) (Fig. 4). Infected birds in Mimidae and Icteridae had

foraging on the ground (n = 347, 46.7%), in the understory (n = 193, 26%), and in the canopy (n = 203, 27.3%).

Of the 743 samples, 360 tested positive for *Plasmodium* or *Haemoproteus* infection (48.4%) including 47.7% of migrating (n = 312) and 53.9% of birds assumed to be wintering in the US (n = 48). In a bivariate analysis, we found that foraging guild and family were significantly associated with infection status, while wintering ground and fat were not (Table 2). However, in the final mixed effect model with year as a random effect, only family remained as a significant predictor of infection status. The variable of body mass was not retained because the estimate was unstable (estimate >>1000, p = 0.02), and foraging guild was dropped to maximize model fit. Icterids had 3.6 times the odds of infection compared to Mimidae and Parulidae (p = 0.014, 95% C.I. = 1.3–10.3; p = 0.015, 95% C.I. = 1.3–9.97, respectively), and 3.5 times the odds of infection compared to Turdidae (p = 0.013, 95% C.I. =

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

Table 3

Novel (previously unreported/unpublished) *Plasmodium* and *Haemoproteus* lineages detected in blood samples collected during spring migration on the Texas Gulf coast, organized by genus.

Lineage name	Number detected	Families (species) infected	GenBank accession
Plasmodium			
KEWA01	1	Parulidae (*Geothlypis formosa*)	MW081130
SEIAUR04	2	Parulidae (*Seiurus aurocapilla, Geothlypis trichas*)	MW091126
DUMCAR09	1	Mimidae (*Dumetella carolinensis*)	MW081134
GEOTRI12	1	Parulidae (*Geothlypis trichas*)	MW081139
CATFUS21	1	Turdidae (*Catharus fuscescens*)	MW081133
ICTGAL04	1	Icteridae (*Icterus galbula*)	MW139686
Haemoproteus			
CARCAR02	11	Cardinalidae (*Cardinalis cardinalis*)	MW081128
MIMPOL03	4	Mimidae (*Mimus polyglottos*)	MW081124
ICTSPU01	3	Icteridae (*Icterus spurius*)	MW081125
CATUST43	2	Icteridae, Turdidae (*Icterus galbula, Catharus ustulatus*)	MW081127
VIOLI17	1	Vireonidae (*Vireo olivaceus*)	MW081129
DUMCAR08	1	Mimidae (*Dumetella carolinensis*)	MW081131
PIOLI04	1	Cardinalidae (*Piranga olivacea*)	MW081132
LISP01	1	Emberizidae (*Melospiza lincolnii*)	MW081135
CARCAR30	1	Cardinalidae (*Cardinalis cardinalis*)	MW081138
DUMCAR10	1	Mimidae (*Dumetella carolinensis*)	MW081137
VIOLI18	1	Vireonidae (*Vireo olivaceus*)	MW081136

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). Infected birds in Mimidae also had 0.1 times the odds of *Plasmodium* infection compared to infected birds of 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) (Fig. 2).

A total of five US wintering birds were recaptured and resampled during the course of the study. A Northern Cardinal (*Cardinalis cardinalis*) was initially negative when captured in 2015 and tested positive in 2018 for *Haemoproteus*. A Lincoln's Sparrow (*Melospiza lincolnii*) was negative in 2015 on initial capture as well as at recapture three days later. Of three Lincoln's Sparrows captured and 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. The other two were positive upon initial capture but negative upon recapture three days later.

4. Discussion

We found 48.4% of 743 samples from birds captured along the US coast of the Gulf Coast during spring migration over a six-year period were infected with *Plasmodium* or *Haemoproteus* parasites, in which *Plasmodium* infections were more common (71% of infected birds) than *Haemoproteus* infections (29% of infected birds). In contrast, Soares et al. (2020) conducted a study of Neotropical migratory birds in the Dominican Republic during spring migration and winter and found a *Haemoproteus* and *Plasmodium* combined infection prevalence of only 31% (n = 419). Further, in another spring migration study on the coast of the Gulf of Mexico, Garvin et al. (2006) found less than half of the level of infection as in the current study, with 11.7% of birds infected with *Haemoproteus* and 6.7% of birds infected with *Plasmodium* (n = 1, 705). Infection prevalence in this study is closer to what has been observed during the breeding season. For example, Matthews et al. (2016) reported a combined *Haemoproteus* and *Plasmodium* infection prevalence of 44% (n = 329) in Eastern Tennessee, and Ricklefs et al. (2005) found a combined *Haemoproteus* and *Plasmodium* prevalence of 38.6% (n = 757) in Southern Missouri. DeGroote and Rodewald (2010), however, detected a *Haemoproteus* infection prevalence of 63.8% and a

Table 4

Previously documented *Plasmodium* and *Haemoproteus* lineages detected in blood samples collected during spring migration on the Texas Gulf coast, organized by genus.

Lineage name	No. detected	Host families in TX study	Host families previously identified	Previous regions identified
Plasmodium				
BAEBIC02 (*Plasmodium homopolare*)	5	Parulidae	Certhidae, Fringillidae, Icteridae, Paridae, Parulidae, Sittidae, Turdidae	N. America, S. America
BT7 (*Plasmodium*)	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 (*Plasmodium*)	4	Emberizidae[a], Turdidae	Anatidae, Certhiidae, Gaviidae, Hirundinidae, Laridae, Paridae, Parulidae, Thamnophilidae, Turdidae	N. America, S. America
CATUST06 (*Plasmodium*)	5	Cardinalidae[a], Turdidae	Formicariidae, Turdidae	N. America, C. America, S. America
COLL4 (*Plasmodium homocircumflexum*)	1	Icteridae	Fringillidae, Icteridae, Laniidae, Mimidae, Muscicapidae, Ploceidae, Pycnonotidae, Sturnidae, Vireonidae	Europe, S. Sahara, N. America, S. America
CYCYA01 (*Plasmodium*)	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, Sphenscidae, Thamnophilidae, Turdidae, Tyrannidae, Vireonidae	N. America, S. America
DIGLAF01 (*Plasmodium lutzi*)	1	Turdidae[a]	Dendrocolaptidae, Fringillidae	S. America[b]
GEOTRI01 (*Plasmodium*)	20	Cardinalidae[a], Emberizidae[a], Parulidae, Turdidae, Vireonidae[a]	Fringillidae, Parulidae, Turdidae	N. America, S. America
GEOTRI02 (*Plasmodium*)	4	Cardinalidae[a], Parulidae	Fringillidae, Icteridae, Parulidae, Tyrannidae	N. America, S. America
GEOTRI09 (*Plasmodium*)	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, Petroicidae, Phasianidae, Ploceidae, Psittacidae, Ptilonorhynchidae, Pycnonotidae, Rallidae, Spheniscidae, Strigidae, Sylviidae, Timaliidae, Turdidae, Zosteropidae	Europe, S. Sahara, N. Africa, N. America, S. America, Asia, Australia, Oceania
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 (*Plasmodium*)	3	Cardinalidae[a]	Fringillidae	N. America
MYCAME03 (*Plasmodium*)	1	Cuculidaea[a]	Ciconiidae	S. America[b]
PADOM09 (*Plasmodium*)	1	Mimidae	Anatidae, Certhiidae, Dendrocolaptidae, Fringillidae, Furnariidae, Hirundinidae, Icteridae, Laridae, Mimidae, Muscicapidae, Parulidae, Passeridae, Spheniscidae, Turdidae, Tyrannidae	N. America, South America
PADOM11 (*Plasmodium*)	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 (*Plasmodium*)	2	Icteridae[a], Mimidae[a]	Fringillidae	S. America[b]
RWB01 (*Plasmodium*)	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 (*Plasmodium*)	2	Cardinalidae[a], Vireonidae[a]	Parulidae	N. America
TACTHA01 (*Plasmodium*)	5	Cardinalidae[a]	Fringillidae, Hirundinidae	N. America
TUMIG03 (*Plasmodium unalis*)	13	Cardinalidae[a], Mimidae,	Certhiidae, Fringillidae, Icteridae, Laridae	N. America
TUMIG03 (*Plasmodium*)	13	Parulidae, Turdidae	Mimidae, Parulidae, Spheniscidae, Sturnidae, Sylviidae, Turdidae, Tyrannidae	S. America
TUMIG23 (*Plasmodium*)	1	Turdidae[a]	N/A	N/A
VIOLI03 (*Plasmodium*)	3	Vireonidae	Certhiidae, Vireonidae	N. America, S. America
WW3 (*Plasmodium*)	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				
CARCAR29 (*Haemoproteus*)	1	Cardinalidae[a]	N/A	N/A
CHIPAR01 (*Haemoproteus*)	3	Vireonidae[a]	N/A	N/A
COLL2 (*Haemoproteus pallidus*)	5	Turdidae	Muscicapidae, Ptilonorhynchidae, Sylviidae, Turdidae, Tyrannidae	Europe, S. Sahara, N. America, Asia, Australia
	2	Icteridae[a]	N/A	N/A

Table 4 (*continued*)

Lineage name	No. detected	Host families in TX study	Host families previously identified	Previous regions identified
ICTGAL01 (*Haemoproteus*)				
ICTGAL02 (*Haemoproteus*)	3	Icteridae[a]	N/A	N/A
JUHYE02 (*Haemoproteus*)	1	Emberizidae[a]	Fringillidae	N. America
MAFUS02 (*Haemoproteus*)	11	Mimidae	Mimidae	N. America
MIMGIL01 (*Haemoproteus*)	2	Mimidae	Mimidae	N/A
PACPEC02 (*Haemoproteus*)	3	Cardinalidae[a]	Dicruridae, Fringillidae, Tyrannidae	N. America
PHEMEL02 (*Haemoproteus*)	3	Cardinalidae[a], Tyrannidae[a]	Fringillidae, Icteridae	N. America
PIRLUD02 (*Haemoproteus*)	7	Cardinalidae[a], Parulidae[a], Turdidae[a]	Fringillidae	N. America
PIRUB01(*Haemoproteus*)	1	Cardinalidae[a]	Fringillidae	N. America
SETAUD05 (*Haemoproteus*)	3	Parulidae[a]	Parulidae	N. America
TOXRUF01 (*Haemoproteus*)	1	Mimidae	Mimidae	C. America[b]
VIGIL07 (*Haemoproteus*)	1	Vireonidae	Vireonidae	N. America
VIGIL09 (*Haemoproteus*)	1	Vireonidae	Certhiidae, Columbidae, Fringillidae, Thamnophilidae, Turdidae, Tyrannidae, Vireonidae	N. America, S. America
VIGRI02 (*Haemoproteus*)	3	Vireonidae	Vireonidae	C. America[b]
VIOLI06 (*Haemoproteus vireonis*)	4	Vireonidae	Dendrocolaptidae, Fringillidae, Vireonidae	N. America, S. America
VIOLI11 (*Haemoproteus*)	3	Parulidae[a], Vireonidae[a]	N/A	N/A

[a] Novel association between this lineage and this avian family.
[b] Novel association between this lineage and geographic region; not previously reported in North America.

Table 5
Among infected birds, bivariate analysis of variables for infection with *Plasmodium* versus *Haemoproteus* using chi-squared and Fisher's exact tests.

Variable	Categories	Sample size (N)	*Plasmodium* (%)	*Haemoproteus* (%)	p value
Wintering Grounds	North Am.	37	13 (35.1)	24 (64.9)	**0.000**
	Central Am.	190	157 (82.6)	33 (17.4)	
	South Am.	70	41 (58.6)	29 (41.4)	
Foraging guild	Ground	128	111 (86.7)	17 (13.3)	**0.000**
	Understory	72	55 (76.4)	17 (23.6)	
	Canopy	97	45 (46.4)	52 (53.6)	
Family	Turdidae	44	37 (84.1)	7 (15.9)	**0.000**
	Mimidae	42	22 (52.4)	20 (47.6)	
	Cardinalidae	85	59 (69.4)	26 (30.6)	
	Outgroup	25	8 (32)	17 (68)	
	Parulidae	61	56 (91.8)	5 (8.2)	
	Icteridae	16	7 (43.7)	9 (56.3)	
	Emberizidae	24	22 (91.7)	2 (8.3)	
Fat Score	0	95	67 (70.5)	28 (29.5)	0.149
	1	103	76 (73.8)	27 (26.2)	
	2	65	49 (75.4)	16 (24.6)	
	3 & 4	33	18 (54.5)	15 (45.5)	
Muscle Score	0 & 1	84	60 (71.4)	24 (28.6)	0.927
	2 & 3	213	151 (70.9)	62 (29.1)	

Fig. 3. Differences by wintering ground among infected birds 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.

Plasmodium prevalence of 12.2% among migrating wood-warblers in Ohio. Variation in the infection prevalence among different avian communities and during different times of the year likely reflects variation in vector habitats in the areas where the birds occur, as well as seasonal changes, (e.g., elevated levels of breeding hormones) and other individual-level parameters (Bennett and Fallis, 1960; Desser et al., 1968; Rintamaki et al., 1997), in addition to different sensitivities of diagnostic approaches used for the detection of parasites.
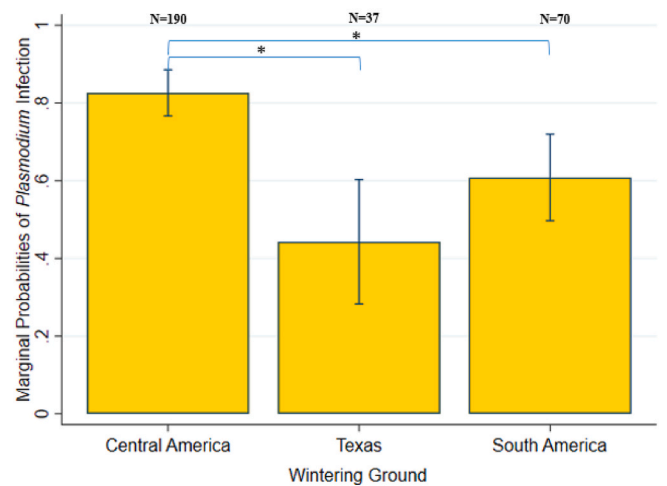
In our study, the assumed wintering origin of birds (US wintering vs. Central America vs. South America) was not a significant predictor of infection status. Prior studies of resident vs. migratory birds have reported mixed results in terms of the prevalence or diversity of parasites. For example, a resident population of Dark-eyed Junco (*Junco hyemalis*; a partially-migratory species) maintained higher parasite prevalence than a migratory population (Slowinski et al., 2018). In contrast, migrants in Brazil had both a higher prevalence and diversity of haemosporidian parasites as compared to resident birds, with limited evidence of lineage sharing among the migrants and residents (Anjos et al., 2021). Whereas we did not find associations among bird foraging guilds and haemosporidian infection status, among the infected birds, we did find
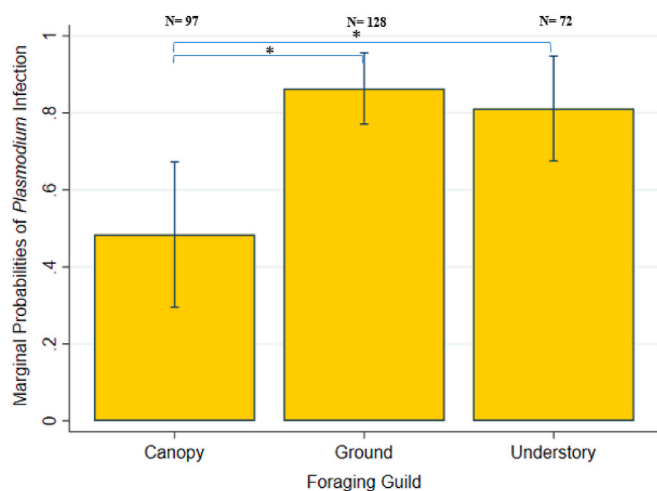
Fig. 4. Differences by foraging guild among infected birds in the probability of *Haemoproteus* versus *Plasmodium* infection adjusted for the significant predictors in the model. Single asterisks with brackets beneath denote significant differences between categories.

significant associations between certain life history traits and parasite genus. Infected ground and understory foragers were more likely to be infected with *Plasmodium* compared to infected canopy foragers, while the opposite was true for *Haemoproteus* infections. In support of these relationships between parasites and substrate heights, Fecchio et al. (2011) found a significant positive correlation between nest height and *Haemoproteus* prevalence. Svensson-Coelho et al. (2013), in Ecuador, and Gupta et al. (2020), in India, both found a negative relationship between foraging height and *Plasmodium* parasite prevalence. In contrast to our study, Astudillo et al. (2013) found higher *Haemoproteus* prevalence in birds foraging in lower forest strata while higher *Plasmodium* prevalence was found in birds foraging in the upper strata in Georgia, USA. These findings are likely related to variation in the frequency of encounters with infected vectors in different habitats. Because particular vector species will partition in vertical strata, some avian hosts may be more susceptible or more frequently exposed than others (Garvin and Greiner, 2003). For example, greater numbers of blood-fed *Culicoides* midges, the vector of *Haemoproteus,* have been found in canopy traps than in ground traps in Eastern Tennessee by McGregor et al. (2018).

The majority of infections in the birds of this study were *Plasmodium* (71% of infected birds). This is in contrast to most studies including one on the Gulf coast of Louisiana during spring migration, where birds were found to be infected in near equal proportions with *Haemoproteus* (33.1%) and *Plasmodium* infections (31.6%), with the remaining split between *Leucocytozoon* and *Trypanosoma* spp. (Garvin et al., 2006). In breeding birds in New Mexico, *Haemoproteus* infections were more than twice as common as *Plasmodium* infections (Marroquin-Flores et al., 2017). Among resident birds in Brazil, Fecchio et al. (2011) found, the most frequent infection was also by *Haemoproteus* (66.3%) followed by *Plasmodium* (33.7%). Furthermore, in a study of warblers stopping over in northwestern Ohio, 63.2% were infected with *Haemoproteus* spp. and 12.2% were infected with *Plasmodium* spp. (DeGroote and Rodewald, 2010). We further detected a mixed infection prevalence of 13.5% not much higher than other studies such as a Michigan study which found a mixed infection prevalence of 9.1% (Smith et al., 2018). Among infected birds, Central American migrants in this study had greater odds of infection with *Plasmodium* compared to birds that winter in other areas. While *Plasmodium* is considered the more generalist genus, it has further been cited as the more pathogenic and severe of the two genera (Atkinson and van Ripper, 1991). Perhaps our findings are the result of differences in vector abundance between *Culicoides* midges, the vectors of *Haemoproteus,* and *Culex* mosquitoes, the vectors of *Plasmodium*. The

birds of this study overwinter across the Neotropics, such as Panama, Trinidad, Yucatan, Honduras, and Venezuela (Moore and Kerlinger, 1987; Norris et al., 2006; Hobson et al., 2014), and a better understanding of the migratory connectivity of the populations that stop at our site combined with studies of avian malaria vectors in the areas where they winter would refine our understanding of infection risk and host-parasite relationships.

Some avian families were associated with a higher infection prevalence than others. For example, birds in the family Icteridae had higher probability of infection than birds in Turdidae, Mimidae, and Parulidae. Host-parasite relationships may be species specific for many reasons. Garvin et al. (2006) suggest this interspecific variability may be the result of differing abilities to cope with the stress of migration and the energetic cost of infection. Additionally, some avian families likely have ecological or behavioral traits that increase their exposure to vectors and therefore prevalence (Cote and Poulinb, 1995). For example, mixed species flocking has been suggested to reduce parasitism by diluting the numbers of conspecifics, thereby circumventing the increased parasitism typically associated with single species flocks (Moller and Allander, 1993; Pomara et al., 2007; Lutz et al., 2015; Hilario-Perez and Dowling, 2018). Individual-level factors may also contribute to the likelihood of infection, such as host immunity to infection, host behaviors that influence vector contact (i.e., anti-vector behavior) (Deviche et al., 2001), or even host size whereby larger hosts would have greater surface area for a vector to feed (Atkinson and van Ripper, 1991). Finally, these family level differences may be a result of high host specificity of some lineages of haemosporidian parasites. For example, Ellis et al. (2020) provided evidence that even generalist haemosporidian lineages infect closely related host species more often than would be expected by chance.

Of the 65 haemosporidian lineages detected in our study, 17 (26.1%) were novel lineages and 48 lineages were previously reported in MalAvi. Many studies report a much higher percentage of novel lineages, for example, 63% of the lineages recovered in a New Mexico study were novel and 59.1% of lineages in a Michigan study were novel (Marroquin-Flores et al., 2017; Smith et al., 2018). Of the 48 previously reported six (13%) have been reported from birds in South or Central America with no previous reports in North America. This is not uncommon as Svensson- Coelho et al. (2013) also detect novel geographic associations, with 14 lineages in birds in Orellana Province, Ecuador that had not been previously reported in Ecuador. Our data support common intercontinental transport of parasites between the Americas, a phenomenon which has been documented in Blue-winged Teals (*Anas discors*) migrating between the American continents (Ramey et al., 2016). Alternately, this result could reflect sampling bias due to low sampling effort of resident birds for blood parasites along the Texas Gulf coast. Future studies of parasite dispersal and transmission within North America will elucidate the degree to which these translocated parasite lineages may establish locally and infect resident birds, with the potential for clinical impacts.

The study has limitations in that birds captured in mist nets at coastal stopover sites may not be representative of the broader population of those species. For example, birds with severe clinical signs of malaria infection may not be capable of flying and our study may be biased toward uninfected and chronically infected individuals. Alternatively, uninfected migrating birds may be healthier with sufficient energy stores to overshoot coastal sites, stopping further inland after crossing the Gulf of Mexico (Cohen et al., 2021). Additionally, our study design does not allow for determination of if a bird was infected during the breeding, migratory, or wintering phases of the annual cycle. Not all PCR-positive samples were able to be sequenced and assigned to a genus; accordingly, the genus-specific infection prevalences should be interpreted as a minimum. Additionally, we did not test for the third genus of avian haemosporidians- *Leucocytozoon* spp. parasites. Nevertheless, in this study we sampled a snapshot of the abundant and diverse migrating birds coming from across the Neotropics *en route* to breeding areas

across the Nearctic.

5. Conclusions

This study presents the complex host-parasite relationships between trans-Gulf migrating birds and haemosporidian parasites, documenting the presence of several novel parasite lineages, and new geographic and host associations of established lineages, and their relationship to bird life history traits. We emphasize the need for further studies on avian blood parasite ecology throughout the full-annual cycle in order to answer further questions such as how host-parasite interactions and parasite dispersal may be impacted by global climate change and what impacts this phenomenon could have on migratory and resident avifauna.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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