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RESEARCH PAPER

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Migratory birds as vehicles for parasite dispersal? Infection by avian haemosporidians over the year and throughout the range of a long-distance migrant

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Abstract

Aim: The role of migratory birds in the spread of parasites is poorly known, in part because migratory strategies and behaviours potentially affecting transmission are not easy to study. We investigated the dynamics of infection by blood parasites through the annual cycle of a long-distance Nearctic–Neotropical migratory songbird to examine the role of this species in dispersing parasites between continents.

Location: The Americas.

Taxon: Grey-cheeked Thrush (*Catharus minimus*, Aves, Passeriformes, Turdidae), Birds.

Methods: We used molecular and microscopy screening of haemosporidian parasites (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) to examine the prevalence, distribution, and diversity of lineages through the annual cycle (breeding, migration, and wintering) of the grey-cheeked thrush in North and Central America, Santa Marta mountains, the Andes, and the Amazon. We aimed to identify transmission areas, to examine the degree of sharing of haemosporidian lineages with resident birds in various areas and to assess the potential role of immunologically naïve juvenile individuals in parasite transmission.

Results: Prevalence and lineage diversity of haemosporidians varied significantly over time, being higher during breeding and fall and spring migration, and declining during wintering. Grey-cheeked thrush shared few parasite lineages with tropical resident birds and slightly more lineages with other migratory and resident boreal species. We detected gametocytes in blood during spring migration stopover, but these were of lineages not found in resident tropical birds, indicating relapses of parasites transmitted elsewhere. Transmission likely occurs mostly on the breeding grounds, where juveniles and adults carried lineages restricted to closely related species of thrushes and other species of boreal birds.

Main conclusions: Long-distance migratory songbirds are likely not important dispersers of blood parasites because there are ecological and evolutionary barriers to the interchange of parasites across vastly separated areas. Further work with

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thorough spatial and temporal sampling across other species, and considering the role of vectors, is necessary to understand the ecological and evolutionary factors explaining the distribution of parasites over broad scales.

KEYWORDS
Grey-cheeked thrush, *Haemoproteus*, *Leucocytozoon*, migration, *Plasmodium*, stopover

1 | INTRODUCTION

Ecological and evolutionary factors explaining the distribution of parasites are poorly known despite their critical importance for understanding emerging infectious diseases (Fuller et al., 2012; Morand & Krasnov, 2010; Quammen, 2012). Migratory animals may be key players in the spread of parasites and in the structuring of assemblages (Altizer, Bartel, & Han, 2011; Garamszegi & Møller, 2007; Viana, Santamaría, & Figuerola, 2016). In particular, migratory birds may be vehicles for the dispersal of parasites among widely separated geographic areas (Altizer et al., 2011; Fourment, Darling, & Holmes, 2017; Rappole, Derrickson, & Hubálek, 2000; Waldenström, Bensch, Kiboi, Hasselquist, & Ottosson, 2002) and could play an important role in the transmission of parasites among species at breeding and wintering grounds (Cohen, Auckland, Marra, & Hamer, 2015; Fuller et al., 2014; Hellgren et al., 2013; Klaassen, Hoye, Nolet, & Buttemer, 2012; Levin et al., 2013; von Rönn, Harrod, Bensch, & Wolf, 2015; Valkiūnas, 2005; Waldenström et al., 2002). For example, in the Nearctic–Neotropical migratory system, millions of birds travel between temperate breeding and tropical nonbreeding areas, potentially allowing parasites to extend their geographical and host ranges (Durrant et al., 2008; Ricklefs, Fallon, Latta, Swanson, & Bermingham, 2005; Ricklefs et al., 2016); this may account for the broad distributions of some parasite lineages (Fallon, Fleischer, & Graves, 2006). The role of migratory birds in the spread of parasites is yet to be determined, however, and it has been speculated that variation in migratory strategies and behaviours among migrant species might affect host–parasite interactions and thus parasite transmission (Altizer et al., 2011; Clark, Clegg, & Klaassen, 2016; Møller & Szép, 2010).

Migratory birds are thought to become infected by haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) predominantly during the breeding period in the temperate zone, where vector abundance and parasite transmission are synchronized with reproductive activities of hosts (Beaudoin, Applegate, Davis, & McLean, 1971; Cosgrove, Wood, Day, & Sheldon, 2008; Valkiūnas, 2005). However, parasites may evolve strategies allowing year-round transmission in migrants (García-Longoria, Hellgren, Bensch, De Lope, & Marzal, 2015; Hasselquist, Östman, Waldenström, & Bensch, 2007; Hellgren et al., 2007; Pérez-Tris & Bensch, 2005a). For example, parasite gametocytes potentially transmissible from birds to dipteran vectors can remain in the blood stream of birds long after the initial acute phase of infection (Hammers et al., 2016; Valkiūnas, 2005; Wood et al., 2013). Relapses triggered by physiological changes associated with strenuous physical activities like migration (Applegate, Beaudoin, & Seeley, 1971; Atkinson & Van Riper, 1991; Dunn, Goodman, Benton, & Hamer, 2014) may potentially enhance the infectiousness and transmission of parasites during the migratory or stationary nonbreeding periods (Cosgrove et al., 2008; Valkiūnas, 2005). Whether migratory birds are in fact able to transmit diseases while travelling or on the wintering grounds is poorly known because few studies have sampled birds throughout their annual cycle (Hellgren et al., 2007, 2013; Munster et al., 2007; Rintamaki, Ojanen, Pakkala, & Tynjala, 1998; Valkiūnas, 2005; Waldenström et al., 2002). In addition, little is known about the extent to which migrant species share parasites with resident species with which they coexist at different times of the year (Cohen et al., 2015; Hall, Altizer, & Bartel, 2014; Ramey et al., 2016; von Rönn et al., 2015; Smith & Ramey, 2015; Viana et al., 2016).

To determine the extent to which migratory birds may disperse parasites, one must quantify the prevalence and diversity of lineages at different stages of the annual cycle (Wood & Cosgrove, 2006). For most resident and migratory birds from the temperate zone, prevalence and diversity of blood parasites are greater during the breeding period (Cornelius, Zylberberg, Breuner, Gleiss, & Hahn, 2014; Cosgrove et al., 2008; Valkiūnas, 2005) although this is not always the case (Barnard, Mettke-Hofmann, & Matsuoka, 2010; Deviche, Fokidis, Lerbour, & Greiner, 2010; Dunn et al., 2014). Temporal patterns in prevalence are likely lineage-specific due to particular host–parasite dynamics and variation in migratory strategy (Hellgren et al., 2013). For example, there is limited evidence that some migratory species may avoid tropical wintering areas where cross-species transmission rates might be higher or where acquiring new parasites might be more likely (Clark et al., 2016; D'Amico, Bertellotti, Baker, Tellino-Junior, & Gonzalez, 2008; Waldenström et al., 2002). Few studies have addressed questions related to transmission dynamics in wintering areas (Dodge, Guers, Sekercioglu, & Sehgal, 2013; Svensson, Ruegg, Sekercioglu, & Sehgal, 2007) or assessed whether or not migratory birds carry transmissible haemosporidian infections (i.e., gametocytes in blood) during migration (Boone, Rodewald, & DeGroote, 2010; DeGroote & Rodewald, 2010; Garvin, Szell, & Moore, 2006). Indeed, only a handful of migratory birds travelling

between North and South America have been screened thoroughly for blood parasites (Garvin et al., 2006; Greiner, Bennett, White, & Coombs, 1975).

Missing from most studies examining the potential of migratory birds to transmit diseases is consideration of the role of birds of different ages. Because of their immunological naiveté, juvenile birds are expected to be especially susceptible to infection on the breeding grounds and may thus be important vehicles for long-distance parasite dispersal to wintering areas during their first migration. Juveniles may also be likely to become infected with parasites while migrating or wintering and to subsequently disperse parasites to breeding areas upon their return (García-Longoria et al., 2015; Hammers et al., 2016; Marzal et al., 2015; Valkiūnas, 2005). However, little is known about the role of juvenile birds versus adults as vehicles for parasites over long distances (Gutiérrez-López et al., 2015) because information on the prevalence, transmission, and diversity of lineages carried by birds of different ages during migration and stationary nonbreeding periods is limited (Bensch & Akesson, 2003; Dodge et al., 2013; Hasselquist et al., 2007; Valkiūnas, 2005; Waldenström et al., 2002). Specifically, we are unaware of studies examining parasites carried by juveniles during their first fall migration compared to those carried during their first spring migration, which could inform us about their potential to disperse parasites acquired at breeding grounds into the tropics and to disperse parasites acquired at stopover and wintering quarters into the temperate zone (Valkiūnas, 2005).

We investigated the dynamics of infection by haemosporidian parasite lineages through the annual cycle of a long-distance migratory songbird, the Grey-cheeked Thrush (*Catharus minimus*), to examine its role in dispersing parasites among geographically distant areas. Although a breeding population of this species has been screened for haemosporidian infections (Oakgrove et al., 2014), no study has sampled individuals through the annual cycle. Grey-cheeked Thrushes travel more than 10,000 km annually between breeding grounds in the boreal region of Canada and Alaska, and wintering grounds in northern South America including areas of the Amazon basin (Gómez et al., 2017; Ungvari-Martin, Heckscher, & Hobson, 2016). Grey-cheeked Thrushes enter South America in the fall primarily through the Darién between Panama and Colombia, whereas during spring migration, potentially hundreds of thousands of individuals stopover in northern Colombia before crossing the Caribbean en route to their breeding grounds (Bayly, Gómez, & Hobson, 2013). Our goals were to (a) examine the prevalence, diversity, and distribution of parasite lineages through the annual cycle (breeding, migration, and wintering) of the Grey-cheeked Thrush; (b) assess the degree of sharing of haemosporidian lineages infecting the Grey-cheeked Thrush with local resident birds in breeding, migration, and wintering areas; and (c) describe infection patterns and lineage diversity of parasites in juvenile individuals during migration to make inferences about the role of young birds in parasite dispersal.

We examined evidence in favour of the hypothesis that Grey-cheeked Thrushes exchange haemosporidian parasites with other birds during their seasonal migratory movements. If Grey-cheeked Thrushes spread blood parasites over long distances, then one should detect individuals (a) sharing haemosporidian lineages with bird species with which they coexist during different periods in their annual cycle and (b) carrying transmissible gametocytes in peripheral blood during migration. One would further expect stopover sites to be especially important for parasite transmission because the stress of migration can cause weakening of the immune system and potentially trigger relapses in infections from breeding or wintering grounds (Altizer et al., 2011; Atkinson, Dusek, Woods, & Iko, 2000; Ricklefs et al., 2005; Waldenström et al., 2002); also, high densities of birds at stopover sites may increase transmission rates (Hall et al., 2014). Finally, if the putatively naive immune system of young birds makes them prone to disperse parasites between regions, then one would expect them to carry several parasite lineages acquired in the temperate zone at high prevalence during fall migration and to carry additional lineages acquired in the tropics during spring migration.

2 | MATERIALS AND METHODS

2.1 | Sampling

The Grey-cheeked Thrush breeds in northern taiga forest and low-arctic shrubs across North America and Siberia (FitzGerald, 2017; Whitaker, Taylor, & Warkentin, 2015). Individuals start their migration from the breeding grounds in mid-August to September, pass through northern South America in September–October, and reach their wintering grounds in South America in October–November (Lowther, Rimmer, Kessel, Johnson, & Ellison, 2001). Spring migration starts in April–May and individuals arrive at their breeding grounds by late May to early June (Lowther et al., 2001). We obtained blood (n = 469) or museum tissue samples (n = 72) from 541 Grey-cheeked Thrushes in different periods of the annual cycle (breeding n = 106; fall migration n = 105; spring migration n = 273; and wintering n = 57; Figure 1 and Appendix S1). We conducted intensive field sampling at two key stopover sites. During fall migration, we worked in the Darién region of northwestern Colombia near the Panama border, which is the main entry route of the Grey-cheeked Thrush to South America in the fall (Gómez, Bayly, & Rosenberg, 2014). During spring migration, we worked in the Sierra Nevada de Santa Marta, northern Colombia, the main gateway out of northern South America in the spring (Bayly et al., 2013; Gómez et al., 2017). We also obtained samples from Peru, Central America, the central United States, and, especially, Alaska and Eastern Canada. Because of their remoteness, we were unable to obtain samples from areas in the central part of the species range in Canada (e.g., Northwest Territories, Nunavut).

We captured birds using mist nets, collected blood (~35 μL) from the brachial vein using small gauge needles and nonheparinized capillary tubes, and then released all birds after banding them. Samples were stored in 90–95% ethanol or 3% SDS lysis buffer. We complemented our blood sampling with tissue samples from voucher specimens deposited in museum collections (Appendix S1). For each individual captured (and for specimens, when available), we recorded

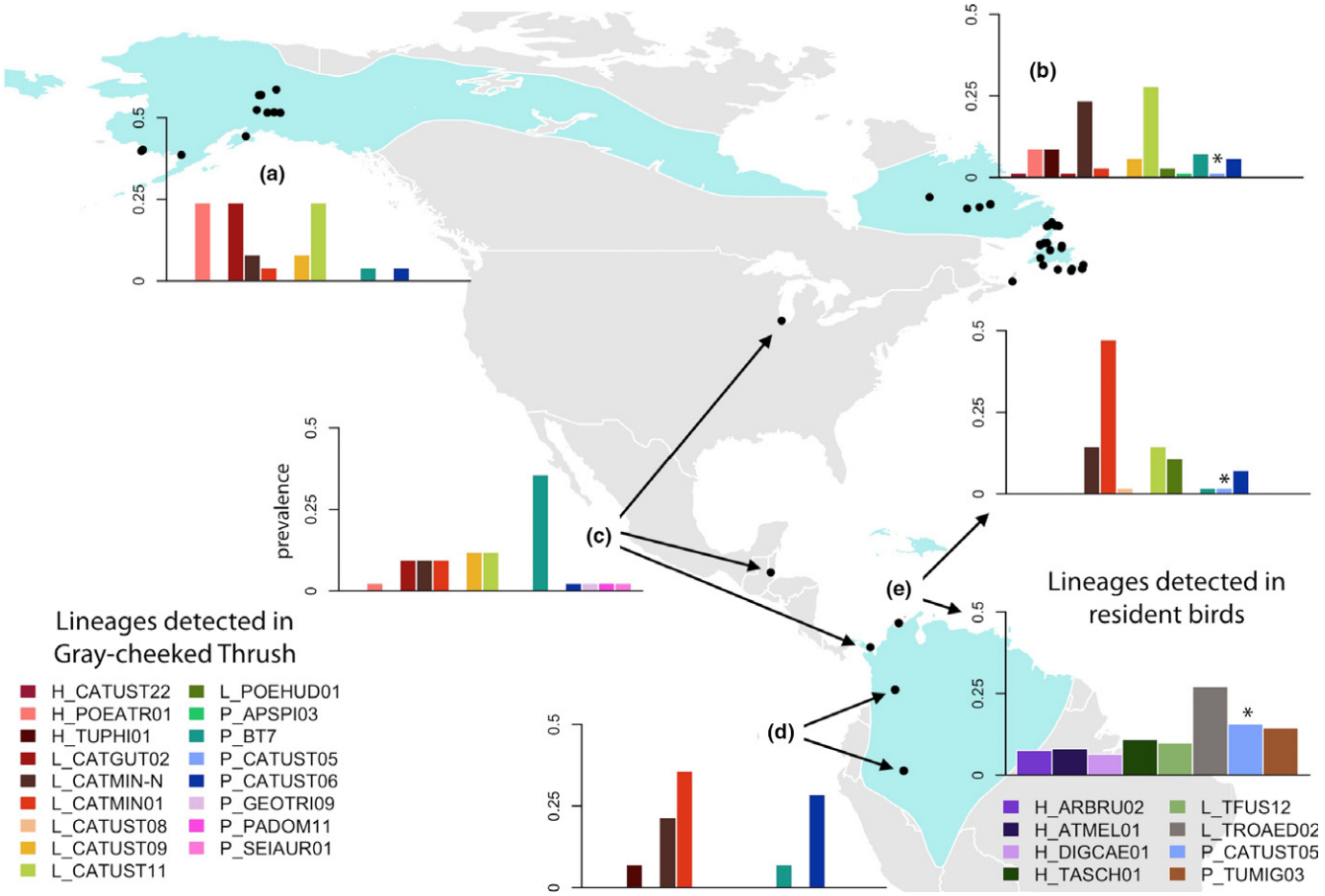

FIGURE 1 Distribution of haemosporidian lineages (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) at main sampling areas during the breeding season: (a) Alaska and (b) eastern Canada; fall migration: (c) Chicago, Belize, and Darien in northern Colombia; wintering: (d) Amazonia and Cordillera Oriental of Colombia; and spring migration: (e) Sierra Nevada de Santa Marta in northern Colombia. Plots show the distribution and prevalence of parasite lineages infecting Grey-cheeked Thrush except for the one in the lower right, which shows lineages detected in resident birds in Santa Marta. The only lineage shared by Grey-cheeked Thrush and resident birds is indicated by asterisks

data on age date, latitude, longitude, elevation, body mass, and wing chord (Pyle, 1997). No individual was sampled more than once. Although our sampling spanned from 1988 to 2016 (older samples are tissues from collections), 77% (n = 417) of the samples were obtained by us between 2013 and 2016. Because we sought to characterize the diversity and distribution of parasite lineages across geographic areas and not to evaluate the turnover of lineages or variation in prevalence across years, the extent of the sampling window was appropriate for the questions asked. Nonetheless, we checked whether the patterns in parasite prevalence and identity varied with age of samples.

To assess whether birds had gametocytes in their blood stream during migration and hence were potentially able to transmit parasites to local birds at fall stopover sites, we collected thin blood smears for 90 individuals in Santa Marta during 2015. Smears were fixed and stained and examined in the laboratory following standard methods for parasite screening (Santiago-Alarcón & Carbó-Ramírez, 2015; Valkiūnas, 2005). To determine the extent to which haemosporidian lineages infecting Grey-cheeked Thrushes were shared with resident species, we sampled local birds (n = 852 individuals, 111 resident species) in locations and habitats occupied by Grey-cheeked Thrushes during stopover (Appendix S2). Although our sampling of local birds spanned from 2007 to 2016, the majority of the samples (n = 366 individuals) were obtained on the same dates (March to May 2015) and locations where migrating Grey-cheeked Thrushes were sampled.

2.2 | Molecular methods and lineage designation

We extracted genomic DNA using a modified version of the phenol–chloroform method (Gutiérrez-Pinto et al., 2012; Sambrook & Russell, 2001) or a DNeasy Blood and Tissue Kit according to manufacturer's instructions using the spin column protocol for either nucleated blood or animal tissue (Qiagen, Valencia, California). DNA was eluted with a single addition of 200 μL of AE Buffer. We examined DNA quality using gel electrophoresis; only high-quality DNA was used in the following steps to reduce the chance of false negatives. We used a nested PCR protocol to detect haemosporidian infections (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) using the HAEMFI/HAEMR, HAEMF/HAEMR2, and HAEMFL/HAEMR2L

primers (Hellgren, Waldenström, & Bensch, 2004). PCR products were run on 1.5–2% agarose gels using 0.5× TBE and visualized by GelRed under ultraviolet light to check for positive infections. In all PCR reactions, we included at least two positive controls and one negative control to confirm amplification and to check for contamination. We did not assess whether the probability of infection varied among tissues (pectoral muscle, liver, or heart), but previous work indicates that this is not the case; (Ramey, Fleskes, Schmutz, & Yabsley, 2013; Svensson-Coelho et al., 2016); however, we did examine whether our results were consistent when including all samples and when restricting analyses only to blood samples.

For all successful amplifications, we sequenced a c. 479 bp fragment of the cytochrome-*b* gene (cyt *b*) to determine the parasite identity of each infection (Hellgren et al., 2004). We edited and aligned DNA sequences in GENEIOUS PRO 6.1.6 with default settings. We detected several double infections of *Plasmodium-Haemoproteus*, evidenced by multiple peaks in the chromatograms (Pérez-Tris & Bensch, 2005b); we were able to identify one or two of the involved lineages in six coinfections using the package *sangerseqR* in R. To identify lineages, we compared cyt *b* DNA sequences to the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi/). Sequences not matching lineages in MalAvi with 100% identity were regarded as new lineages when they differed by ≥0.2% (1 base pair) from published cyt *b* sequences (Bensch, Hellgren, & Pérez-Tris, 2009; Bensch, Pérez-Tris, Waldenström, & Hellgren, 2004).

2.3 | Statistical analyses

We examined variation in prevalence throughout the annual cycle for all parasites, for *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* separately, and for the five most prevalent lineages (i.e., those with >10 infections, Figure 1). We used generalized additive models (GAM) with binomial errors and a logit link to analyse infection status (positive/negative) using Julian date as a smoothed function (Hellgren et al., 2013; Wood, 2004, 2006) in the R package *mgcv*. This approach has been used to examine nonlinear relationships between infection status and predictor variables (Cosgrove et al., 2008; Hellgren et al., 2013; Wood et al., 2007).

2.4 | Distribution, diversity, and lineage sharing

Using the MalAvi database (Bensch et al., 2009), we collected information on host identity and geographic distribution of parasites found in the Grey-cheeked Thrush to assess parasite sharing between this species and tropical- and boreal-breeding birds. To account for differences in sampling effort among periods of the annual cycle and to determine whether our sampling had been sufficient to characterize the diversity of parasite lineages infecting the Grey-cheeked Thrush, we performed rarefaction analyses to estimate undiscovered lineage diversity for all the samples and for samples collected in each period (Colwell et al., 2012). The analysis was conducted with 1000 randomizations using the R package iNEXT (Hsieh, Ma, & Chao, 2016). Finally, we described variation in prevalence and lineage diversity in juvenile birds during fall and spring migration to assess the potential of young birds to (a) transmit lineages acquired in their natal areas to tropical species and (b) transmit lineages acquired in the tropics to species in the breeding grounds after completing their first full migration cycle.

3 | RESULTS

3.1 | Prevalence, diversity, and distribution of parasites in space and time

Of the 541 Grey-cheeked Thrush sampled, 188 individuals were infected with haemosporidian parasites (34.8% prevalence). We detected *Leucocytozoon* in 154 individuals, *Plasmodium* in 44 individuals, and *Haemoproteus* in 21 individuals. Pooled haemosporidian prevalence differed among periods of the annual cycle (χ^2 = 58.6, *df* = 3, $p < 0.001$), being higher during the breeding period (65.0%) and lower during the wintering period (21.0%, Figure 2). Because prevalence patterns did not differ when we restricted analyses to blood samples or to samples of different ages (Appendix S3), in the following, we focus on results obtained using all samples. A smoothed function of Julian sampling date significantly predicted variation in the pooled prevalence of all parasites (χ^2 = 54.15, $p < 0.001$, Figure 3a) and for each genus independently (*Leucocytozoon* χ^2 = 63.5, $p < 0.001$; *Plasmodium* χ^2 = 23.0, $p < 0.001$; *Haemoproteus* χ^2 = 20.3, $p < 0.001$, Figure 3b). In general, prevalence increased during spring on arrival to breeding grounds, peaked in the breeding months of June and July, and decreased as fall migration progressed. The overall circannual pattern in prevalence was largely driven by the modal pattern of *Leucocytozoon*, whereas patterns in prevalence of *Haemoproteus* and *Plasmodium* were bimodal, with peaks during and after the breeding period (Figure 3b).

We identified 17 cyt-b parasite lineages of which seven were *Leucocytozoon*, seven *Plasmodium*, and three *Haemoproteus*. We detected 40 coinfections: 19 by *Plasmodium*/*Leucocytozoon*, 15 by *Haemoproteus*/*Leucocytozoon*, and 6 by *Plasmodium*/*Haemoproteus*. Only five of 17 lineages (three *Leucocytozoon*, one *Plasmodium*, and one *Haemoproteus*) had been previously found infecting Grey-cheeked Thrush (Figure 1, Appendix S4). Our rarefaction analyses suggested that five lineages were not detected for the entire annual cycle, and less than four lineages are yet to be detected for each of the different periods. Therefore, only a few, rare lineages are yet to be found across the range of the Grey-cheeked Thrush (Appendix S5 and Appendix S6, Figure 1). When doubling the reference sample size for each period using the iNEXT function, the extrapolated lineage diversity was higher during the breeding period (*n* = 15) and fall migration (*n* = 15), followed by spring migration (*n* = 10) and the wintering period (*n* = 6; Appendix S5 and S6, Figure 1).

Presence/absence patterns and prevalence during breeding, migration, and wintering periods varied among the 17 parasite lineages. The most prevalent lineages were also the most widespread: six lineages accounted for 78% of the infections and four of them were detected in individuals in all periods of the annual cycle

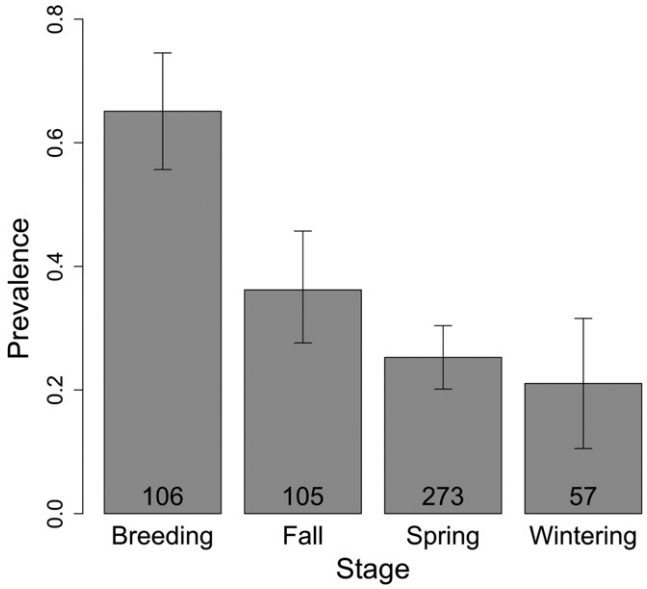

FIGURE 2 Combined prevalence of *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* in the Grey-cheeked Thrush. The highest prevalence of haemosporidian parasites was detected during the breeding period in Alaska and Canada. Numbers in bars represent sample sizes for each period. Error bars represent 95% bootstrap confidence intervals

(Figure 1, Appendix S6). In contrast, six lineages were detected only once at single sites (Figure 1, Appendix S6). None of the more prevalent lineages were restricted to a single period (breeding, fall migration, spring migration, or wintering), but their prevalences varied among periods (Figure 4, Appendix S6). For instance, *Leucocytozoon* lineage CATMIN01 was more prevalent during spring migration in Santa Marta and during wintering, and was rarely detected in other periods; in contrast, *Plasmodium* BT7 was detected mainly during fall migration in the USA and the Darién (Figure 1 and Figure 4, Appendix S6). Most of the less prevalent lineages (i.e., those infecting 1–8 individuals) were detected on the breeding grounds and during fall migration, but rarely during wintering or spring migration (Figure 1).

3.2 | Parasites infecting Grey-cheeked Thrush and other birds at breeding, migration, and wintering areas

By comparing our data with information in the Malavi database, we found that Grey-cheeked Thrush shared some parasite lineages with other bird species year round. In particular, we found that Grey-cheeked Thrush shared few parasite lineages with tropical resident birds and more lineages with other species breeding in the temperate zone. Next, we describe these overall patterns in more detail.

Of the 13 lineages we found infecting Grey-cheeked Thrush during the breeding period (Figure 1, Appendix S6), 11 had been previously detected in 22 bird species in North America, and one (*Haemoproteus* TUPHI01) in one species (Song Thrush, *Turdus philomelos*) from Europe and Central Asia (Appendix S4). One

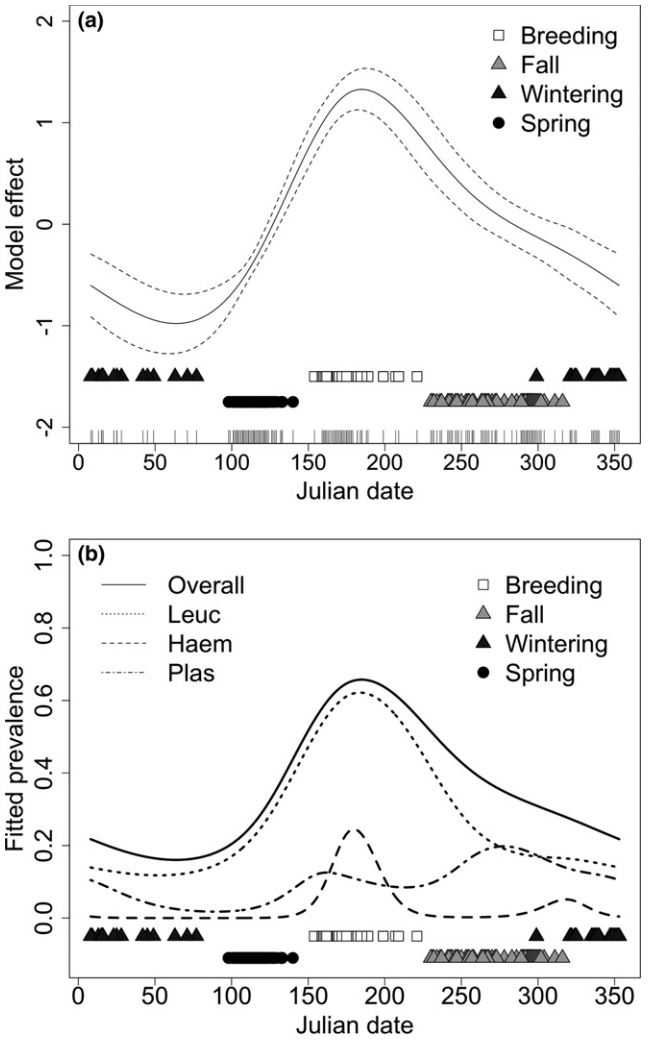

FIGURE 3 Predictive GAM models of seasonal variation in prevalence across the year both for pooled (a) and for genus-specific prevalence (b), indicate that the peak of prevalence and therefore transmission in Grey-cheeked Thrush occur during the breeding period. The curves represent the estimated model effect (±1 SE) as predicted by Julian date (using penalized squared regression), and the fitted prevalence for overall infections. *Leucocytozoon* (Leuc), *Haemoproteus* (Haem), and *Plasmodium* (Plas). Prevalence is expressed as the proportion (between 0 and 1) of birds infected. Julian date starts with 0 on the 1 January and ends at 365, 31 December every year. Overlapping dates for fall and wintering reflect that some individuals arrive earlier to wintering areas, while others are still migrating

Leucocytozoon lineage had not been detected before in Grey-cheeked Thrush or any other bird species. Of the 22 host species sharing parasites with Grey-cheeked Thrush during the breeding period, 21 share at least part of their breeding ranges with this species (Appendix S4).

Of the 11 lineages detected during fall migration, eight were also detected during the breeding period. Three *Plasmodium* lineages (GEOTRI09, PADOM11, and SEIAUR01) were only found in the fall and are known to collectively infect 46 bird species: 27 species from North America, and 19 from South America. All five lineages

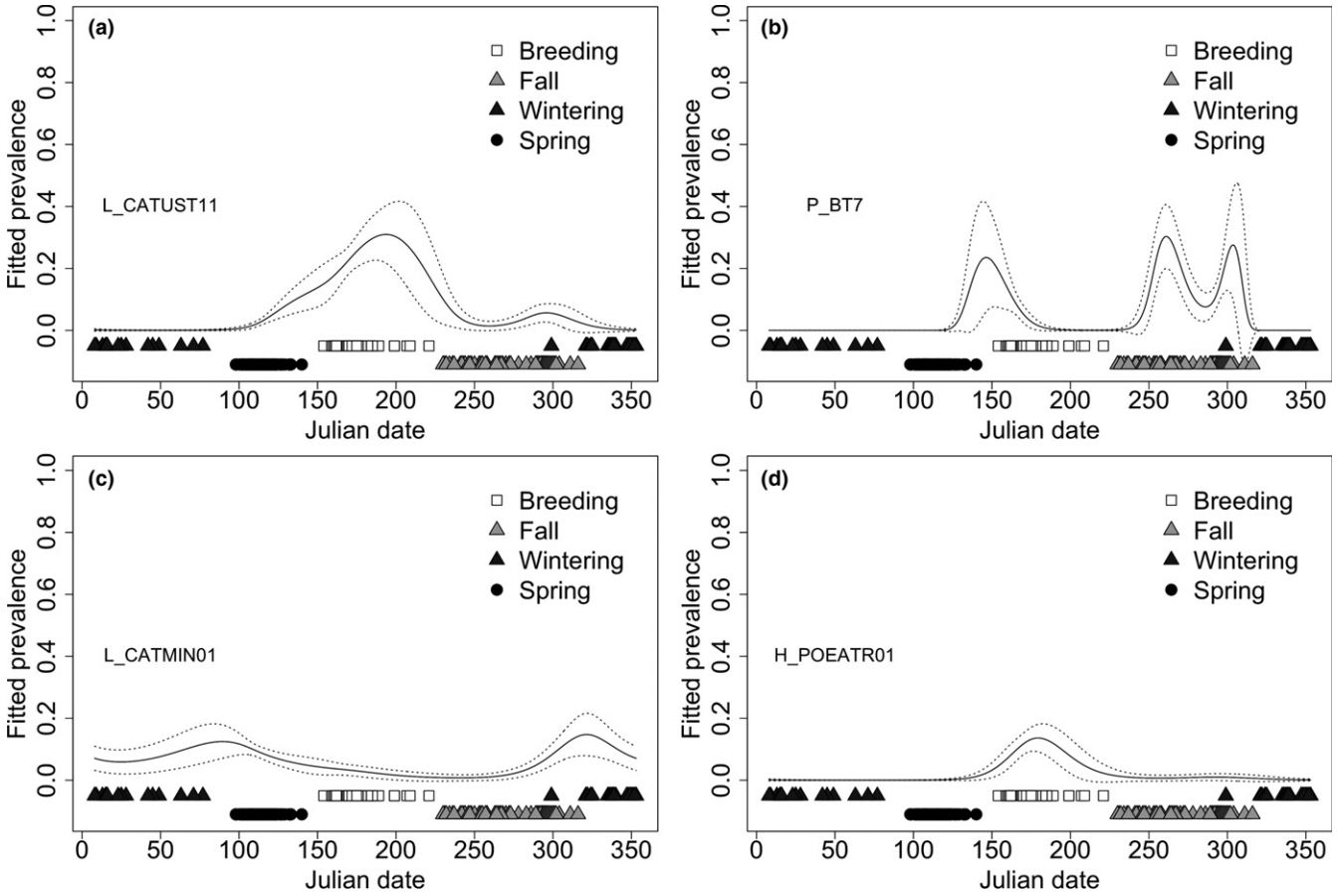

FIGURE 4 The most prevalent lineages show different temporal patterns of infection across the annual cycle of Grey-cheeked Thrush. Fitted prevalence for (a) *Leucocytozoon* lineage CATUST11; (b) *Plasmodium* lineage BT7; (c) *Leucocytozoon* lineage CATMIN01; (d) *Haemoproteus* lineage POEATR01. Smooth functions are solid lines and dotted lines are ±1SE

detected during the wintering period were also detected in the breeding and fall migration periods (Figure 1), but none of them were previously detected in any tropical resident bird occurring in the wintering range of the Grey-cheeked Thrush in South America. Finally, during spring migration, we detected eight lineages, seven of which were also found during the breeding, fall, and wintering periods and one *Leucocytozoon* lineage was only detected during spring (Figure 1, Appendix S6).

At the Santa Marta stopover site, we found transmissible macro- and microgametocytes in peripheral blood of 10 Grey-cheeked Thrush individuals (out of 90 sampled), of which nine were of *Leucocytozoon* and one of *Plasmodium*. Morphologically, gametocytes resembled those of *L. dubreuili*, which has previously been found with high prevalence in Grey-cheeked Thrush and other boreal birds in Newfoundland, Canada (Bennett, Campbell, & Cameron, 1974; Valkiūnas, 2005), and also in the Great Thrush (*Turdus fuscater*) at high elevations of the Eastern Andes of Colombia (Lotta, Matta, Torres, Moreno-de, & Moncada, 2013).

Four of the 17 haemosporidian lineages detected in Grey-cheeked Thrush (23%, three *Plasmodium* and one *Haemoproteus*) were shared with four species of resident birds (out of 114 species screened) at the Santa Marta stopover site: *Haemoproteus* H_POEATR01 was detected in one of 16 (6.2%) Mountain Elaenia (*Elaenia frantzii*) and in one of 11 (9%) Slate-throated Redstart (*Myioborus miniatus*); *Plasmodium* P_CATUST06 in one of 134 (0.75%) Grey-breasted Wood-Wren (*Henicorhina leucophrys*); *Plasmodium* P_CATUST05 in 27 of 202 (13.3%) Grey-breasted Wood-Wren, and one of 12 (8.3%) Black-hooded Thrush (*Turdus olivater*); and *Plasmodium* P_PADOM11 in one Blue-black Grassquit (*Volatinia jacarina*). The above four shared lineages corresponded to 7.5% of the 53 lineages (16 *Plasmodium*, 16 *Haemoproteus*, 21 *Leucocytozoon*) we detected in the local bird assemblage in Santa Marta. None of the five *Leucocytozoon* lineages detected in Grey-cheeked Thrushes at this stopover site were shared with resident birds despite the presence of eight lineages of *Leucocytozoon* in the local bird assemblage at elevations (900–1500 m) where Grey-cheeked Thrush stopover during spring migration. Moreover, of the most common lineages found in the local bird assemblage in Santa Marta (i.e., those detected ≥10 times, Figure 1, Appendix S7), only one (*Plasmodium* P_CATUST05) was shared with Grey-cheeked Thrush (Figure 1, Appendix S7). Six lineages were also present at elevations where Grey-cheeked Thrush stopover during spring migration. According to the Malavi database, *Plasmodium* P_CATUST05 has also been detected in the Great Thrush (*Turdus fuscater*) and the House Wren

(*Troglodytes aedon*) in Colombia and Peru (Galen & Witt, 2014; González, Lotta, García, Moncada, & Matta, 2015).

The relatively low sharing of lineages between Grey-cheeked Thrush and other species particularly in stopover and wintering regions may reflect that several of the birds with which this species shares geographic areas are distantly related to it and that there are regional differences in the phylogenetic composition of host assemblages. For example, because tropical and boreal avifaunas differ in the large amount of suboscine passerines in the former region, low sharing of lineages with species in the tropics may indicate that comparisons involve species distantly related to our focal species across which parasite transmission would be unlikely due to host specificity. Therefore, we assessed parasite sharing with birds more closely related to the Grey-cheeked Thrush, namely (a) oscines passerines (suborder Passeres) and (b) thrushes (family Turdidae). Sharing of parasites with all oscines was low both in North America (6% of lineages) and South America (0.6% of lineages), but was considerably higher with thrushes both in North America (21.1% of lineages) and in South America (6.2% of lineages).

3.3 | Infection patterns and lineage diversity of parasites in juvenile birds during fall and spring migration

We were able to age 431 Grey-cheeked Thrushes: 264 adults and 167 juveniles. The overall prevalence of haemosporidians in juvenile birds (46 infected individuals, 27.5%) was similar to that in adults (76 infected individuals, 28.7%). However, we found infected juvenile individuals during breeding, migration, and wintering, suggesting they are potentially able to disperse parasites year round. Prevalence in juveniles was higher during fall migration (35.2%) and spring migration (26.3%), and was substantially lower during breeding (3.3%), rising again during wintering (22.5%). The number of lineages detected in juvenile birds (n = 13) was higher than in adults (n = 11), and juveniles carried five *Plasmodium*, one *Haemoproteus*, and all seven *Leucocytozoon* lineages detected in Grey-cheeked Thrush in this study (Table 1). Four of the 13 lineages detected in juvenile birds (two *Leucocytozoon* and two *Plasmodium*) accounted for 65% of the infections (Table 1). Because most lineages found in juvenile birds (10 of 13) were detected during fall migration, most parasites are likely being transmitted in temperate areas during the breeding period and infected juvenile birds may potentially act as sources of parasite transmission to the tropics (Table 1). However, none of the lineages infecting juvenile birds during breeding and fall migration have been detected in tropical resident birds according to Malavi (Table 1, Appendix S5). Finally, during spring migration, we detected eight lineages in juveniles, seven of which shared with the breeding, fall, and wintering periods. The remaining lineage (*Leucocytozoon* lineage CATUST08) has only been detected previously in another species of thrush (*Catharus ustulatus*) in Alaska (Appendix S3), which suggests it was likely not acquired from resident birds in South America.

4 | DISCUSSION

Migratory birds may play an important role in spreading parasites across geographically distant areas due to their cyclical exposure to different parasite and avian assemblages during their annual journeys (Møller & Erritzøe, 1998; Valkiūnas, 2005; Viana et al., 2016). However, explicit evidence of long-distance dispersal of parasites by migratory birds is scant (Fuller et al., 2012; Kilpatrick et al., 2006;

TABLE 1 The number of parasite lineages detected in juvenile birds was higher during fall than spring migration. Four lineages were shared with adult birds during fall (a), and four lineages where detected during both fall and spring migrations (*). Breeding birds were sampled in Alaska and eastern Canada; fall migration: Chicago, Belize, and Darien in northern Colombia; wintering: Amazonia and Cordillera Oriental of Colombia; and spring migration: Sierra Nevada de Santa Marta in northern Colombia. Details of localities are provided in Appendix S1

Lineage	Breeding	Fall	Spring	Wintering	Total Infections	Prevalence (%)
H_POEATR01	0	1	0	0	1	2.17
L_CATMIN01*	0	4	5	2	11	23.91
L_CATMIN-N*	2	1	2	2	7	15.22
L_CATGUT02[a]	0	1	0	0	1	2.17
L_CATUST09	0	3	0	0	3	6.52
L_CATUST11[a]*	0	1	2	0	3	6.52
L_POEHUD01	0	0	3	0	3	6.52
L_CATUST08	0	0	1	0	1	2.17
P_CATUST06	0	0	3	4	7	15.22
P_BT7[a]*	0	5	1	0	6	13.04
P_GEOTRI09	0	1	0	0	1	2.17
P_PADOM11	0	1	0	0	1	2.17
P_SEIAUR01	0	1	0	0	1	2.17
Total	2	19	17	8	46	100

Smith et al., 1996). We asked whether the Grey-cheeked Thrush, a long-distance migrant which breeds in boreal latitudes and winters in the tropics, had the potential to exchange parasites with other avian species based on an extensive sample of individuals assayed through much of its range in stationary periods of the annual cycle and during both fall and spring migration.

As a first step to determine the extent to which a long-distance migratory bird may disperse parasites among regions, we quantified the prevalence and diversity of lineages at different stages of the annual cycle of the Grey-cheeked Thrush. We found that the prevalence and diversity of haemosporidian parasites in this species vary over time, being higher in the breeding period and during fall migration, then declining strongly during wintering, and increasing again during spring migration. These temporal patterns of variation in prevalence and diversity of haemosporidians are consistent with findings of previous studies on other species of migrants in North America and Europe (Beaudoin et al., 1971; Hellgren et al., 2013). Therefore, the potential for haemosporidian dispersal by Grey-cheeked Thrushes to bird assemblages outside its breeding areas is greatest during fall and spring migration and is more limited during wintering. Similar seasonal patterns of parasite prevalence have been documented in other migratory birds (García-Longoria et al., 2015; Pérez-Tris & Bensch, 2005a; Sorensen et al., 2016).

A necessary condition for migratory birds to be vehicles for long-distance dispersal of parasites is that they should share parasitic lineages with bird species with which they coexist during different periods in their annual cycle. Because we found that Grey-cheeked Thrushes are infected by only ca. 0.6% of parasite lineages known to infect tropical resident birds (four of 709 of the lineages reported for South America in Malavi) and by only ca. 4% of the lineages infecting resident and migratory birds from the temperate zone (16 of 397 lineages reported for North America in Malavi), our work suggests that if this species indeed disperses parasites over long distances, then such dispersal is taxonomically restricted to few lineages. Furthermore, the potential for dispersal of lineages across areas is likely lower than suggested by the above figures because some of the infections we detected might be abortive; in such cases, parasite lineages are detectable by PCR but because they do not continue their life cycle in the host they cannot be transmitted to other birds via vectors.

While sharing of a few parasite lineages with other bird species reveals that there is some limited potential for Grey-cheeked Thrush to disperse parasites to other species and across areas, the extent to which such potential can be realized will depend on temporal patterns of prevalence of different parasites. Because Grey-cheeked Thrushes carry some parasite lineages at relatively high prevalence during migration (and less so during wintering), there is indeed potential for this species to disperse them between regions. For instance, because the common *Plasmodium* lineage P_BT7 is highly prevalent during fall migration, chances are that Grey-cheeked Thrushes will arrive to the tropics with active infections of this lineage which they may transmit. In contrast, *Haemoproteus* H_POEATR01 is more prevalent during the summer, so the odds of individuals reaching the tropics with transmissible infections are lower.

The potential for parasite dispersal across species and regions mediated by Grey-cheeked Thrush appears greater when one restricts analyses to avian hosts in its same family, with which our focal species shared a much greater percentage of parasite lineages relative to comparisons involving all bird species. Indeed, most of the lineages we detected in the Grey-cheeked Thrush have been found in closely related species of thrushes such as *Catharus ustulatus* and *C. guttatus*, which suggests relatively high host specificity might play an important role in parasite dispersal (Ellis et al., 2015). However, although we found that Grey-cheeked Thrush shared more than 21% of parasite lineages with other thrushes from the temperate zone, only one of the shared lineages was observed in tropical birds, suggesting that these parasites are not extensively transmitted between breeding and wintering areas.

A final, critical condition for migratory birds to be considered vehicles for parasite dispersal is that they should carry transmissible forms during migration. In agreement with this, we found Grey-cheeked Thrushes carrying gametocytes of *Leucocytozoon* and *Plasmodium* in peripheral blood at the Santa Marta site, suggesting there is some potential for transmission at stopover sites (Valkiūnas, 2005). However, whether such potential effectively results in transmission depends on gametocyte transmission rates, which are essentially unknown in birds (they are low in humans; (Mideo & Day, 2008). An indication that transmissions may seldom materialize is our finding that none of the lineages detected in blood in Grey-cheeked Thrush during stopover were found among resident birds. The only shared lineages were not detected in blood smears (i.e., there was no indication of gametocytes in peripheral blood), were found in low frequencies in resident birds, and were rarely detected during spring migration in the Grey-cheeked Thrush despite ample sampling during that period. A similar case occurs in the Galapagos Islands, where two *Plasmodium* lineages have been detected in a migratory species (Bobolink, *Dolichonyx oryzivorus*) and resident birds, but lineages are not actively transmitted in the archipelago (Levin et al., 2013).

We believe it is unlikely that lack of suitable vectors explains the seemingly limited transmission and sharing of lineages infecting the Grey-cheeked Thrush and resident birds during stopover in Santa Marta. The area is rich in fast-flowing streams favoured by simulid vectors of *Leucocytozoon* (Valkiūnas, 2005) and there is local transmission of different *Leucocytozoon* lineages in other species of birds, including thrushes (e.g., *Turdus fuscater*). Likewise, vectors of *Plasmodium* and *Haemoproteus* are available, which may enable transmission of lineages between resident and migratory birds. Therefore, unless there is high vector–parasite specificity for the lineages infecting Grey-cheeked Thrush, which is not the case in high-elevation areas in the Colombian Andes (Lotta et al., 2013, 2016), local transmission of lineages should be possible in both directions involving Grey-cheeked Thrushes and the local avifauna. Seasonal patterns of transmission of local parasites or immune resistance might explain the lack of cross-species infections at stopover sites (Pérez-Tris & Bensch, 2005a; Valkiūnas, 2005). For instance, asynchrony between

the window of peak transmission of haemosporidians infecting resident birds and the timing of stopover of Grey-cheeked Thrushes through Santa Marta might explain the paucity of shared lineages.

We found evidence that juvenile Grey-cheeked Thrushes carry haemosporidian parasites during their first fall migration, indicating a potential role of young birds as vehicles for parasite dispersal from temperate to tropical areas (García-Longoria et al., 2015; Waldenström et al., 2002). However, none of the lineages infecting juvenile Grey-cheeked Thrushes in the fall have been found in tropical resident birds and juveniles did not appear to become infected with tropical parasite lineages before spring migration, suggesting that the role of young birds in moving parasites between regions is limited and not different from that of adult birds. Our data on the distribution of lineages across periods in juvenile birds indicate that transmission is likely occurring mostly on the breeding grounds (García-Longoria et al., 2015; Sorensen et al., 2016), where the most prevalent lineages infecting Grey-cheeked Thrush are shared with other species of boreal birds (Dodge et al., 2013; Loiseau et al., 2012; Oakgrove et al., 2014).

Taken together, our data indicate that adult and juvenile Grey-cheeked Thrush are predominantly infected with haemosporidian parasites during the breeding period in the temperate zone. Individuals subsequently overwinter and stopover in tropical areas where the most common parasite lineages infecting them on their summer breeding grounds are absent or rare. Because we found that most parasites infecting the Grey-cheeked Thrush are specialist lineages or are restricted to bird assemblages with which this species shares breeding areas, transmission of parasites by this long-distance migrant to tropical birds is likely limited by ecological and evolutionary barriers disallowing the interchange of parasites between areas. The limited potential for parasite dispersal across latitudes observed in our study contrasts with observations suggesting the absence of barriers to parasite dispersal across the breeding range of the Grey-cheeked Thrush considering that the most common lineages were found all the way from the west coast of Alaska to Newfoundland. Because our study relied on the MalAvi database to infer parasite sharing among hosts and areas, and because many species and regions remain poorly sampled for parasites, it is likely that we underestimated the number of species of birds with which Grey-cheeked Thrush shares parasite lineages. However, ongoing studies in poorly explored areas such as the Andes and Amazonia have not detected most of the lineages found in this study (A. Fecchio pers. comms), suggesting that additional data on lineage sharing are unlikely to change our conclusion that potential for parasite transmission across species involving Grey-cheeked Thrush is limited.

A critical aspect to be addressed in future studies is the role of blood-sucking vectors in the transmission of parasites in areas where resident and migratory birds overlap in different periods of the year (Santiago-Alarcón, Palinauskas, & Schaefer, 2012). Information about vector diversity, distribution, and specificity is critical to understand whether the apparent barriers for parasite dispersal across regions and species evidenced in our study result from inability of parasites to infect novel hosts due to differences in factors such as host immunity or whether the potential for infection is not realized owing to restrictions imposed by vector availability and suitability. Work involving vector assemblages is thus an open field to make important advances in the understanding of parasite dispersal in the Nearctic–Neotropical migratory system, particularly at wintering and stopover sites (Ishtiaq, Bowden, & Jhala, 2017; Ishtiaq et al., 2008; Ricklefs et al., 2016). To our knowledge, ours is the first study addressing the dynamics of infection by haemosporidian parasites across the annual cycle of a long-distance migratory songbird in the Nearctic–Neotropical system and provides evidence consistent with previous findings in the Euro-African system that, aside from a few wide-ranging and generalist lineages, most parasites do not easily spread over continents and mostly remain confined to particular hosts or assemblages (Hellgren et al., 2007; Ricklefs et al., 2016; Valkiūnas, 2005). Additional work with thorough spatial and temporal sampling across other species is necessary to fully understand the ecological and evolutionary factors explaining the distribution of parasites over broad scales.

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DATA AVAILABILITY

Relevant data associated with this manuscript will be deposited in GenBank, Malavi database and Dryad.

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BIOSKETCHES

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Author contributions: P.C.P.R. and C.D.C. wrote the paper together with significant contributions from C.G., N.J.B., S.B., and J.K.K.; P.C.P.R., G.C., N.J.B., A.M.F., J.J.K., A.M.G., K.A.H., J.U.M., and H.S. collected samples; P.C.P.R., M.I.C., N.S., and H.S. conducted laboratory work and sequencing. P.C.P.R. conducted the parasite screening. P.C.P.R. and C.D.C. designed the study and performed statistical analysis. All authors contributed to revisions of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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