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DISEASE ECOLOGY

Disentangling the effects of host relatedness and elevation on haemosporidian parasite turnover in a clade of songbirds

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Citation: Starkloff, N. C., W. C. Turner, A. M. FitzGerald, M. C. Oftedal, E. S. Martinsen, and J. J. Kirchman. 2021. Disentangling the effects of host relatedness and elevation on haemosporidian parasite turnover in a clade of songbirds. Ecosphere. 12(5):e03497. 10.1002/ecs2.3497

Abstract. The persistence of a parasite species in an ecological community is determined both by its environmental tolerance and host breadth. The relative contribution of these niche characteristics to parasite community structure is challenging to parse because host persistence is also a consequence of extrinsic environmental factors. We investigated haemosporidian parasites (genera *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*) in a clade of avian hosts in eastern North America. Species in this clade of *Catharus* thrushes occupy specific elevational bands in a non-phylogenetically determined manner. This allowed us to tease apart the effects of host relatedness and elevation on parasite community structure, diversity, and infection prevalence. We screened blood and tissue samples from 414 adult birds from four mountain ranges in the Appalachian Highlands for blood parasites using a cytochrome-*b*-nested PCR protocol and identified parasite lineages by sequencing. We found an overall infection prevalence of 88.4% and identified a total of 38 parasite lineages including six novel lineages. Parasite community patterns varied by genus. Host relatedness rather than elevational zone predicted the beta turnover and phylobeta turnover of *Leucocytozoon* parasites, indicating that closely related rather than geographically proximate host species had more similar parasites. This pattern was not seen in *Plasmodium* parasites because the diversity of this parasite genus varied considerably in the high elevational zones among mountain ranges, that is, a sky-island effect. *Haemoproteus* parasites were rare in this study system. Our study suggests that the mechanisms that underlie community structuring vary between haemosporidian genera due to differences in the degree of host sharing among lineages.

Key words: avian malaria; beta diversity; *Catharus*; community turnover; elevational turnover; Haemospororida; host relatedness; *Leucocytozoon*; *Plasmodium*.

Received 6 April 2020; revised 17 October 2020; accepted 22 December 2020; final version received 24 February 2021. Corresponding Editor: Sarah M. Zohdy.

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INTRODUCTION

Characterizing parasite community structure is fundamental to predicting the potential impact of global change on host–parasite interactions. The presence of a parasite species in a particular ecological community is largely determined by its environmental tolerances, but also the specificity with which it infects host species in that community (Krasnov and Poulin 2010, Poulin and Krasnov 2010). For example, vector-transmitted parasites must survive outside their thermoregulated vertebrate hosts (Gage et al. 2008) and ectoparasites spend their entire life cycle outside of their hosts' body (Poulin and Krasnov 2010). The niche of these parasite groups is, thus, strongly determined by environmental tolerances. There is considerable variation in environmental tolerances of different parasite species (Lindsay and Birley 1996, Santiago-Alarcon et al. 2012), affecting both the geographic range and the infection prevalence of parasites across that range (Sehgal et al. 2011, Zamora-Vilchis et al. 2012). Parasites also display a broad spectrum of host specialization strategies, and specificity itself can be affected by the variability in environmental conditions, such as rainfall seasonality and wetness of dry seasons, encountered by parasites throughout their hosts' ranges (Fecchio et al. 2019, Wells and Clark 2019). Thus, evolutionary and ecological factors also interact to determine the success of host switching or host range expansion by parasites, making predictions of parasite community structure difficult.

With the absence of ecological and/or phylogenetic barriers, parasites are more likely to expand their host range resulting in zoonotic diseases, such as those spilling over into humans from rodent or bat reservoirs (Han et al. 2015, Morens et al. 2020) or from contact with domesticated animals (Woolhouse et al. 2001). Due to the potential fitness consequences of host switches to novel hosts (Liu et al. 2010, Kamath et al. 2019), it is important to understand the specificity with which parasite species infect their hosts and the contribution of host phylogenic relatedness and environmental factors to the membership of parasite species in ecological communities. The ecological and evolutionary constraints of specialization result in a trade-off in which host generalists may achieve broader geographic distributions through the parasitism of many different host species (Medeiros et al. 2014), whereas host specialist parasites may achieve higher infection rates by maximizing resource acquisition and immune evasion within co-evolved host species (Longdon et al. 2014, Poulin and Morand 2014). Specialization, however, is not typically the result of co-speciation of parasites with their hosts. Indeed, we rarely see parasite phylogenies mirroring host phylogenies (Nieberding et al. 2010), suggesting that host switching—rather than co-speciation—is the dominant form of host–parasite evolution (De Vienne et al. 2013). Although sharing of host species is more likely to occur whether the novel host species is phylogenetically proximate to an existing host due to similarities in immune systems and resources available (Tinsley and Majerus 2007, De Vienne et al. 2009, Longdon et al. 2011, Clark and Clegg 2017), parasites are also able to jump into distantly related host species with high encounter rates due to shared geographic space. If so, ecological factors affecting host proximity may be more important in predicting parasite community structure, especially for host generalist parasites.

Still, it is challenging to tease apart the relative contribution of host breadth and environmental tolerances to parasite community structure because the presence of a host species in an ecological community is also largely a result of environmental tolerances (Sexton et al. 2009). A promising approach to separate the effects of host evolutionary history and ecological characteristics on parasite community structure is to focus on factors (e.g., relatedness and habitat characteristics of host species) that do not covary within the study system. For example, Locke et al. (2013) identified host phylogeny, rather than ecological characteristics, as the key predictor of metazoan parasite community similarity in six freshwater fish species by quantifying diet and habitat characteristics that did not mirror their phylogeny. In contrast, a study on 15 species of desert rodents found that host phylogeny was not a good predictor of ectoparasite, intracellular parasite, or helminth beta turnover in comparison with host environment characteristics such as host range size and trophic status (Dallas and Presley 2014). By focusing on ecological factors that do not covary with host phylogeny, these studies were able to separate the effects of host evolutionary history and ecological characteristics on parasite community structure. The contradictory conclusions from these studies demonstrate the lack of universality among taxonomic groups in the importance of these niche characteristics. As parasite groups often do not share an evolutionary history, it is important to characterize the great diversity of host–parasite relationships across the tree of life.

The elevational distribution and phylogenetic relationships of *Catharus* thrushes (family Turdidae) in eastern North American present a unique opportunity to tease apart the relative contributions of host relatedness (a proxy for host immunological similarity) and elevation (a proxy for extrinsic environmental factors) on the degree of parasite sharing. The two most closely related host species in this clade, the Bicknell's Thrush (*Catharus bicknelli*), and Veery (*Catharus fuscescens*), breed at opposite ends of the sampled elevational gradients, while the Swainson's Thrush (*Catharus ustulatus*) and Hermit Thrush (*Catharus guttatus*) have broad elevational ranges (Fig. 1; Able and Noon 1976). Haemosporidian parasites (order Haemospororida) have been studied in *Catharus* thrushes across large geographic scales and through their circannual cycle (Dodge et al. 2013, Pulgarín-R et al. 2019; Starkloff et al., 2020); however, little is known about the variability in infection among host species. The diversity of this parasite group allows for a thorough exploration of the variation in host sharing and specialization. Parasites in the genus *Plasmodium* (transmitted by Culicidae mosquitoes and traditionally referred to as "malaria parasites") tend to be host generalists (Valkiunas 2004). Contrastingly, *Leucocytozoon* parasites (transmitted by Simulidae blackflies) and *Haemoproteus* parasites (subgenus *Parahaemoproteus* transmitted by Ceratopogonidae midges) tend to infect a single host species or genus (Valkiunas 2004, Moens and Pérez-Tris 2016, Galen et al. 2018). However, haemosporidian host specificity can change over evolutionary time (Galen et al. 2019) and across environmental gradients (Fecchio et al. 2019), and this may affect variation at the community level (Garcia-Longoria et al. 2019).

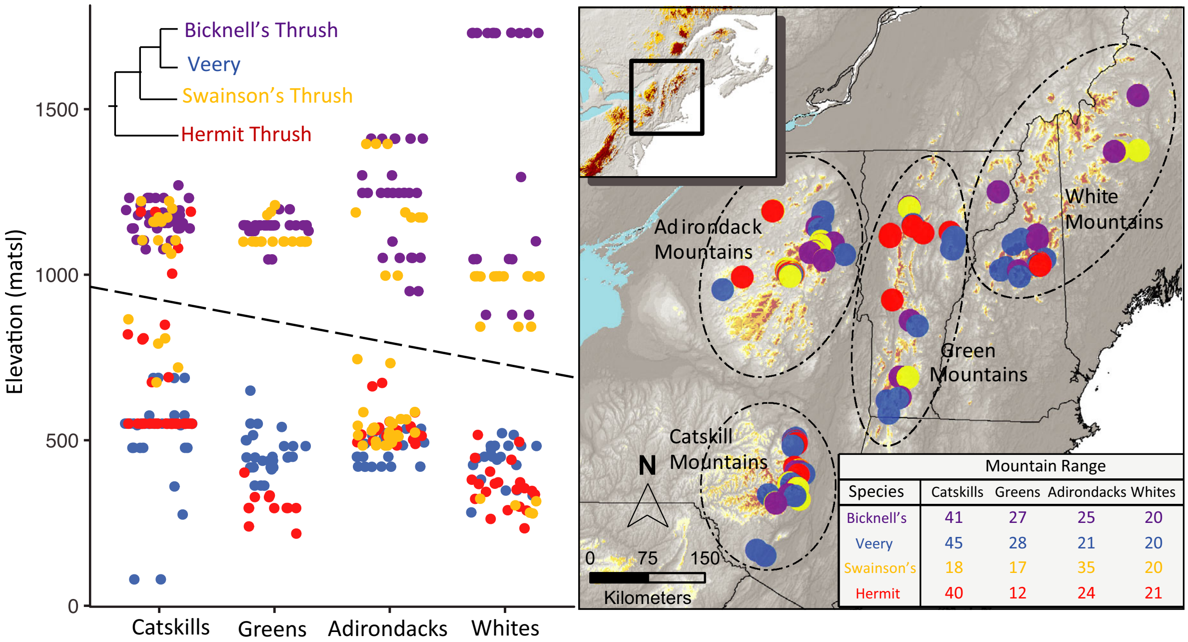
First, we tested alternative hypotheses regarding parasite variation among species and across elevational zones using community similarity (or beta diversity), which can refer to the variation in the identity of community members or can also account for the phylogenetic relationship among community members (Webb 2000). As closely related host species tend to have more similar immune systems and resources for exploitation by parasites, we expected more closely related host species to harbor more similar parasite communities than less closely related host species. This effect should be stronger when looking at parasite groups that tend to have a smaller host breadth (e.g., *Leucocytozoon* and *Haemoproteus*) than groups that tend to have more generalized host relationships (e.g., *Plasmodium*). Alternatively, if host specialization is less important to infection than environmental tolerance, parasite communities are likely to be more variable across elevational zones than within the same elevational zone. If both independent variables are equally (un)important to parasite community structuring in thrushes, we are unlikely to see either of the above patterns.

Second, we document parasite lineage alpha diversity and infection prevalence to evaluate parasite patterns within species and elevational zones. Prevalence is commonly used as a measure of parasite infection rates across host populations or communities and is essential to disease ecology as it provides an indication of the relative success of a parasite species in infecting a population or community of hosts (Krasnov and Poulin 2010), but does not provide any indication of the fitness consequences the parasite may have on its hosts (Palinauskas et al. 2018). Although ecogeographic patterns of free-living organisms are well understood, large-scale patterns of parasite biodiversity are still being established (Preisser 2019). Based on previous findings of latitudinal and elevation gradients of haemosporidian parasites (Merino et al. 2008, Zamora-Vilchis et al. 2012, Cuevas et al. 2020, Fecchio et al. 2020), we expect the prevalence and diversity of *Leucocytozoon* to increase, and of *Plasmodium* and *Haemoproteus* to decrease, with increasing elevation.

MATERIALS AND METHODS

Host sampling

Adult birds were captured in mist nets along established hiking trails or near roads in four



Species	Mountain Range Catskills	Greens	Adirondacks	Whites
Bicknell's	41	27	25	20
Veery	45	28	21	20
Swainson's	18	17	35	20
Hermit	40	12	24	21

Fig. 1. Host sampling by elevation (meters above sea level) and mountain range. Left panel: High and low elevational zones are separated by the dashed line. An approximation of the phylogenetic relationship of host species is shown in the inset based on Everson et al. (2019). Right panel: geographic localities and sample numbers across the four mountain ranges. Colors signify host species sampled at each site: Bicknell's Thrush (purple), Veery (blue), Swainson's Thrush (yellow), and Hermit Thrush (red). Inset shows the locality of the sampling in eastern North America.

mountain ranges in the Appalachian Highlands: the Catskill, Green, Adirondack, and White Mountains (Fig. 1). All samples were collected during the breeding seasons of 1993–2018 (late-May to early-July), in the form of blood or frozen tissue samples (Appendix S2). Blood samples were obtained using targeted mist netting and brachial venipuncture from banded and released birds. The tissue samples are associated with voucher specimens collected by researchers at the Cleveland Museum of Natural History, American Museum of Natural History, and New York State Museum. Previous studies conclude that blood samples and tissue samples have similar detectability of haemosporidian parasites (Svensson et al. 2007, Ramey et al. 2013, Pulgarín-R et al. 2019). Birds were sampled opportunistically, and samples were categorized into either high or low elevational zones based on the natural gap in our sampling that corresponds to the locality of the ecotonal change from deciduous to boreal forest in each mountain range (Fig. 1). Ecotones occur at lower elevations with increasing latitude along the Appalachian highlands (Cogbill and White 1991), precluding use of a single elevational threshold throughout our study region.

Samples were also collected from juvenile birds of three host species in the Green Mountains, Vermont, July: Bicknell's Thrush (*n* = 76, collected in 2000–2002), Hermit Thrush (*n* = 7, collected in 2003–2004), and Swainson's Thrush (*n* = 3, collected in 2004). These samples were only used to identify haemosporidian lineages that were locally transmitted to birds before their initial migration and were not included in any analyses. The majority of the juvenile Bicknell's Thrush samples (*n* = 74) were collected from nestlings as described in Goetz et al. (2003). The remaining juvenile birds were fledged (but still adorned juvenile plumage) and sampled using the same methods as for adults. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Parasite sampling

We extracted genomic DNA from all blood and tissue samples using a DNeasy Blood and Tissue extraction kit (QIAGEN, Valencia, CA) and targeted a 478 base pair fragment of mitochondrial gene cytochrome-*b* (cyt-*b*) using nested PCR (Hellgren et al. 2004). All negative PCRs (indicated by the absence of bands on 1.5% agarose gels) were repeated twice (for a total of three times) to establish that they were not false negatives. We cleaned positive amplicons using Exosap (ExoSAP-IT; Amersham Biosciences, Arlington Heights, Illinois, USA) and Sanger-sequenced amplicons on an ABI3700. We aligned sequences and edited them using Geneious Prime 2019.1.3. (https://www.geneious.com). We identified lineages by BLASTing to known sequences from MalAvi (Bensch et al.) and GenBank (Clark et al. 2016). A single, unambiguous nucleotide difference between sequences denoted different, potentially reproductively isolated lineages (Bensch et al. 2004, Pérez-Tris and Bensch 2005), and we treated each unique lineage as a separate unit of diversity for our analyses. We submitted novel lineages that did not match previously identified lineages to MalAvi (CATGUT04-06, CATUST40-42).

Analyses

We examined the effects of host relatedness and elevation on parasite community turnover (compositional and phylogenetic) within each haemosporidian genus and in all three genera combined. Linear regression models (lm function in the stats R package (R Core Team [2018]) were used to evaluate the variation in both turnover metrics among host species pairs and between elevational zones. In host models, host species pairs were assigned into three different categories of host divergence based on the Everson et al. (2019) phylogeny (Fig. 1): (1) Bicknell's Thrush and Veery are the most closely related pair, (2) followed by their comparisons to the Swainson's Thrush, and (3) lastly, all comparisons to the most distantly related Hermit Thrush. In elevation models, comparisons were made between high and low elevational zones (high–low) within each mountain range, and among high (high–high) and among low (low–low) elevational zones across mountain ranges. Individuals of any species were pooled into high or low elevational zones in each mountain range. We quantified beta diversity (compositional) and phylobetadiversity (phylogenetic) to evaluate dissimilarity among parasite communities using community matrices of parasite lineages and their abundances in each host species and elevational zones in each mountain range. We calculated beta diversity (chao dissimilarity index) among pairs of host species and between elevational zones in each mountain range using the vegdist function with chao index in vegan R package (Oksanen et al. 2013). Similarly, we calculated phylobetadiversity (mean pairwise distance) using the comdist function in picante R package (Kembel et al. 2010). This function incorporated the branch lengths from a maximum likelihood (ML) General Time Reversible (GTR), Gamma distributed tree with a proportion of invariant sites (GTR+G+I, 1000 replicate bootstrap) based on parasite mitochondrial cyt-*b* sequences constructed in MEGA7 (Kumar et al. 2016). It was rooted at the *Leucocytozoon* clade based on Borner et al. (2016).

We tested the effects of host species and elevational zone on alpha diversity using a linear regression model. We estimated alpha diversity (Shannon diversity index) in each host species and for each elevational zone from each mountain range. This diversity metric was calculated using rarefaction in the R package iNext (Hsieh et al. 2016), accounting for community abundance and evenness of lineages per genus (Spellerberg and Fedor 2003).

Lastly, to evaluate the effects of elevational zone and host species on the parasite prevalence (host infected or uninfected), we used a binomial generalized linear mixed-effects model (GLMM, glmer function in lme4 R package (Bates et al. 2007), logit link function). Elevational zone and host species were included as fixed effects in the model, with mountain range and a time variable as random effects. For this temporal variable, we partitioned sampling years into half decadal categories (e.g., 2001–2005 and 2006–2010), to account for non-continuous sampling. For each parasite genus, we ran models of varying combinations of predictive variables (see Table 1) and used ΔAIC_c to find the best model for predicting the infection status of birds (Burnham and Anderson 2004). For all analyses in R, we used version 3.5.2.

Table 1. Comparison of binomial GLMM models predicting the prevalence of *Leucocytozoon* and *Plasmodium* parasites in four closely related species of *Catharus* thrushes (n = 414).

Parasite genus	Model	AIC$_c$	ΔAIC$_c$
Leucocytozoon	**Host species + elevation**	**327.4651**	**0**
	Host species × elevation	329.4216	1.9565
	Host species	330.5176	3.0525
	Elevation	331.4047	3.9396
	Random effect only	335.6027	8.1376
Plasmodium	**Host species**	**464.5979**	**0**
	Host species + elevation	466.4389	1.841
	Host species × elevation	467.0612	2.4633
	Random effects only	476.5932	11.9953
	Elevation	477.8024	13.2045

Notes: Models are ranked in ascending order of ΔAIC$_c$, relative to the model with the lowest AIC$_c$ for that parasite genus (bolded). All models included the temporal variable (half decadal category) and site as random effects in addition to fixed variables listed. We were unable to model *Haemoproteus* infection status due to the rarity of infection (2.4% infected).

RESULTS

We identified a total of 38 haemosporidian lineages (Fig. 2), infecting 366 of the 414 birds sampled (88.4%; 25.1% coinfected by parasites in two genera). These infections were caused primarily by parasites in the genus *Leucocytozoon* (85.3% infection rate, 23 lineages). *Plasmodium* parasites comprised 10 lineages (26.8% of infections). *Haemoproteus* parasites (5 lineages) were rare (2.4%) and so were excluded from individual genus statistical comparisons. We identified six new *Leucocytozoon* lineages, all occurring as singleton infections (Fig. 2). While rare lineages (<2% prevalence) were typically found as singletons in species within the three closely related host species, most of the more common lineages (n = 8) infected all four host species at similar prevalence. We identified four lineages in the juvenile birds, confirming local transmission on the breeding grounds: P_CATUST05 (n = 1), P_BT7 (n = 1), L_CATUST11 (n = 3), and L_CATMIN07 (n = 3).

Beta diversity analyses indicated a trend of higher chao dissimilarity and higher mean pairwise distance of *Leucocytozoon* communities with increasing host genetic divergence, whereas *Plasmodium* communities did not significantly vary in either beta diversity measure with host divergence (Fig. 3, left panel). *Leucocytozoon* and *Plasmodium* communities did not have higher dissimilarity between low and high elevations within a mountain range than in high or low elevations across mountain ranges (Fig. 3, right panel). However, *Plasmodium* communities were most dissimilar at high elevations across mountain ranges, though this effect was less pronounced when looking at parasite phylogenetic dissimilarity (Fig. 3, right panel). When including all three genera, both beta diversity measures largely mirrored patterns of *Leucocytozoon* (Appendix S1: Fig. S1). Alpha diversity of *Plasmodium* and *Leucocytozoon* parasites did not differ significantly among host species or between elevational zones (Appendix S1: Fig. S2). However, thrushes in this study system (across all four mountains) were infected by a more diverse assemblage of *Leucocytozoon* parasites than *Plasmodium* parasites (Fig. 2).

The best prevalence model for *Leucocytozoon* contained host species and elevational zone without an interaction term (Table 1). The high level of variability suggests that *Catharus* tends to have high *Leucocytozoon* infection prevalence regardless of species, though prevalence was typically lower at high elevation (mean = 0.754; 95% CI lower = 0.512, upper = 0.899) than low elevation (mean = 0.948; 95% CI lower = 0.858, upper = 0.982; Fig. 4). The best model for *Plasmodium* prevalence was the host species only model (Table 1), with the lowest prevalence in the closely related Bicknell's Thrush (mean = 0.125; 95% CI lower = 0.074, upper = 0.203) and Veery (mean = 0.240; 95% CI lower = 0.167, upper = 0.333), higher in the Swainson's Thrush (mean = 0.297; 95% CI lower = 0.208, upper = 0.0405), and highest in the most distant host species, the Hermit Thrush (mean = 0.449; 95% CI lower = 0.346, upper = 0.557; Fig. 4).

DISCUSSION

A haemosporidian parasite community is impacted by each lineage's ability to infect different host species and a wide variety of abiotic and biotic factors (Poulin and Krasnov 2010, Sehgal et al. 2011, Fecchio et al. 2019, Wells and Clark

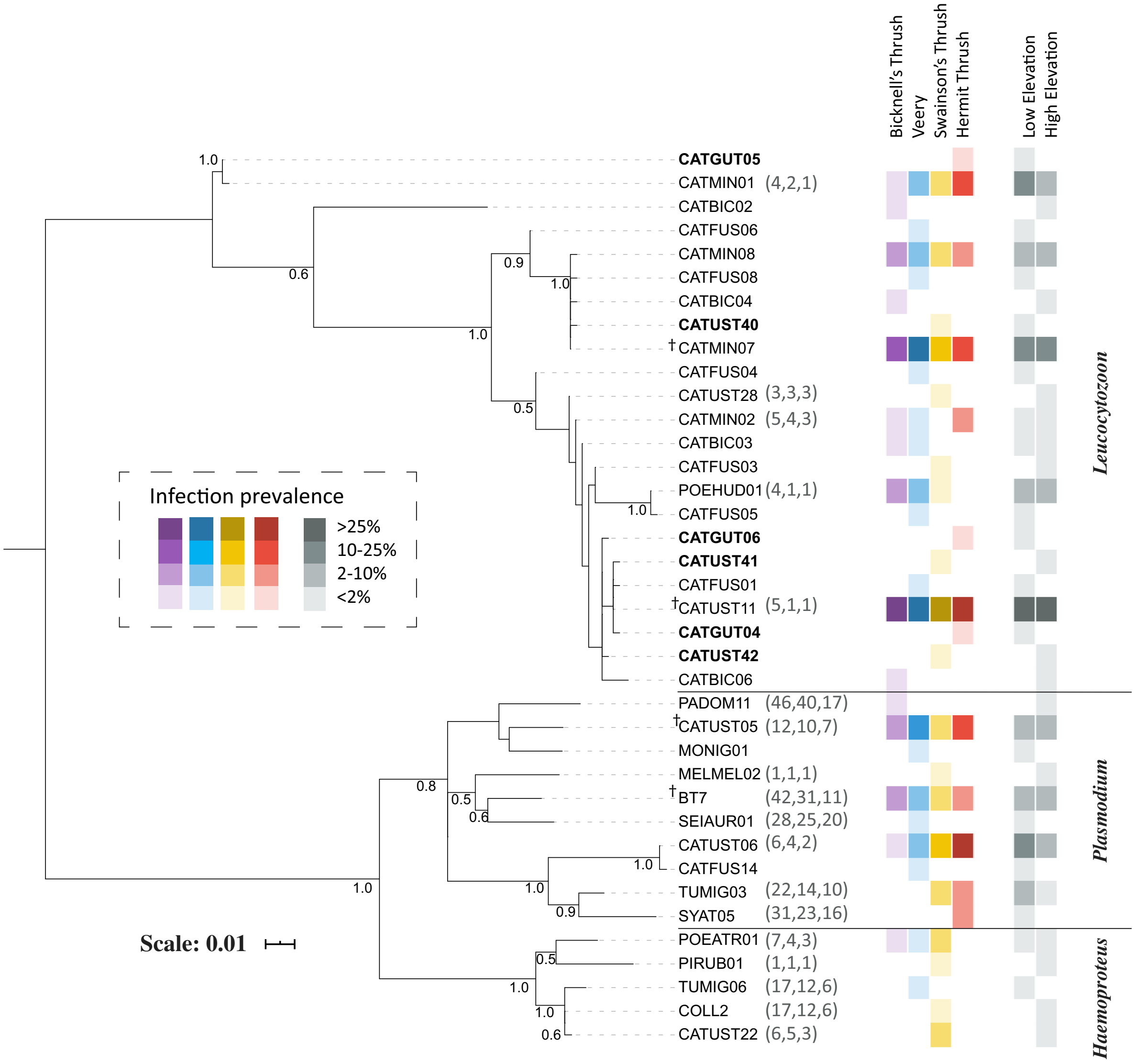


Fig. 2. RaxML phylogenetic tree showing relationship among haemosporidian parasites based on mitochondrial cyt-*b* and the infection rate of lineages in different host species (same colors as Fig. 1) and elevational zones (grays). The four shades in the legend indicate the variation in parasite infection rates (from light to dark: <2%, 2–10%, 10–25%, >25%). Lineages that were not previously reported in MalAvi are bolded. For lineages that MalAvi has reported host data, we include the number of host species, genera, and families in brackets after the lineage name. The four lineages identified in juvenile birds prior to their initial migration are marked with †. Bootstrap support values are provided on branches, and branch lengths are to scale based on substitution rates.

2019). We used a model avian host system of temperate forest-breeding thrushes in the Appalachian Highlands to tease apart the effects of host relatedness and elevation on haemosporidian community structure. *Leucocytozoon* parasites seem to show host delineation, leading to more similar parasite communities in more closely related host species despite the separation of hosts along elevational gradients. Contrastingly, *Plasmodium* communities were equally dissimilar among all host species comparisons, suggesting that the sharing of lineages is not determined by host relatedness.

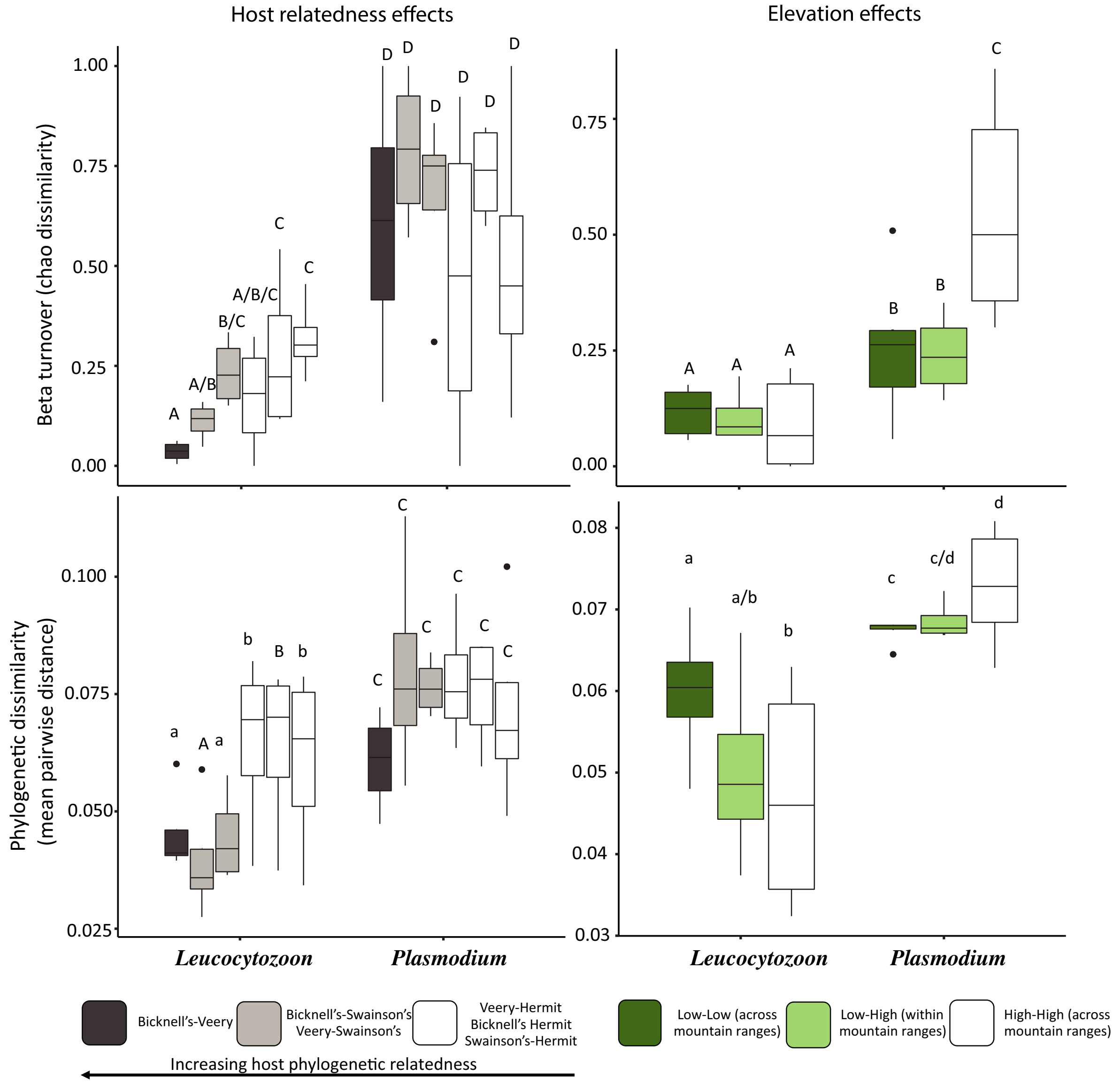


Fig. 3. Variation in community (top panels) and phylogenetic (bottom panels) dissimilarity of two genera of haemosporidian parasites with host relatedness (left panels) and elevational variation (right panels). For host relatedness models, we compared parasite communities between each pair of hosts (genetic relatedness between host pairs increases from right to left) within each mountain range. For elevation models, we compared the two diversity measures between high and low zones within the same mountain site (low–high), and across the four high zones (high–high) and the four low zones (low–low). Uppercase/lowercase letters indicate relationships of $P < 0.05/0.10$.

Leucocytozoon parasites are typically more prevalent and abundant in colder, temperate climates, as are their black fly vectors (Haas et al. 2012, McCreadie and Adler 2014, Oakgrove et al. 2014, McCreadie et al. 2018, Cuevas et al. 2020, Fecchio et al. 2020). The temperate, forested breeding habitats of *Catharus* thrushes provide ideal conditions for high *Leucocytozoon* infection rates across their geographic ranges (Galen et al. 2018; Starkloff et al., 2020). Consistent with these

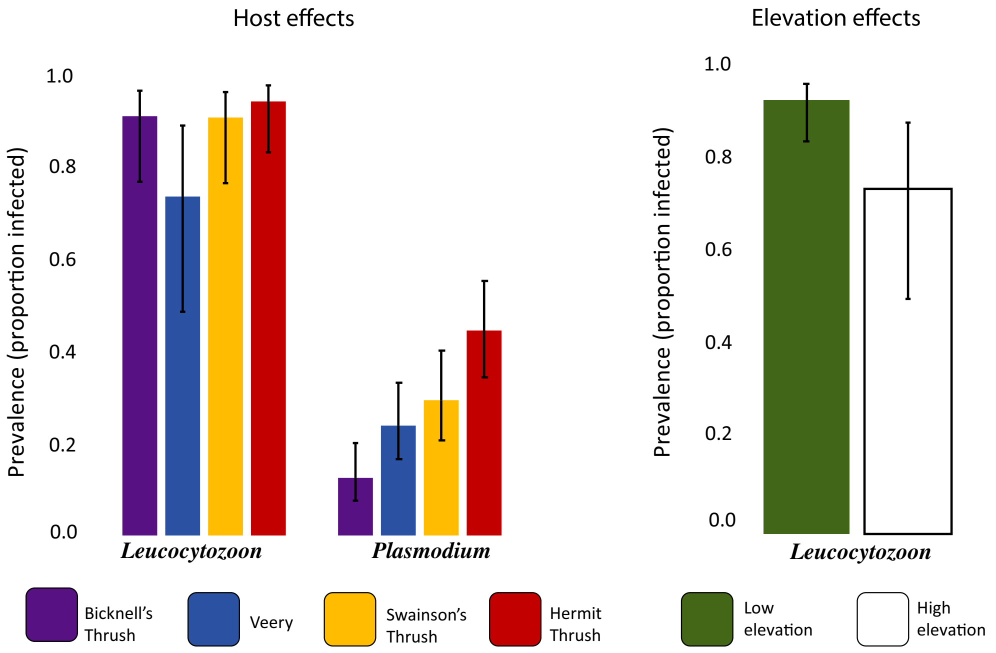


Fig. 4. Bar plots of model effects of best binomial GLMM model for each haemosporidian parasite genus based on adjusted mean and 95% confidence intervals (calculated using the function all.effects in R package effects). Prevalence of *Plasmodium* was best explained by just host species, while *Leucocytozoon* prevalence was best explained by both host species and elevational zone (Table 1).

patterns, we found the *Catharus* thrushes in this study infected at a very high prevalence by *Leucocytozoon* parasites (88.4% on average). We also found a high diversity of *Leucocytozoon* lineages across the host species and sites sampled including a total of 23 lineages, six of which have not been discovered before. Optimal climate conditions in northern latitudes likely provide increased resource stability for *Leucocytozoon* parasites and their vectors, which may lead to higher rates of competition among individual lineages (Futuyma and Moreno 1988). While we observed increased dissimilarity of *Leucocytozoon* parasite communities with decreased host relatedness, overall beta turnover of *Leucocytozoon* was low (mean chao dissimilarity = 0.19) as these communities were overrepresented by the highly prevalent L_CATUST11 (>25%) and L_CATMIN07 (10–25%) in all host species (Fig. 2). The classic trade-off hypothesis states that a specialist parasite achieves higher prevalence within its co-evolved host and generalist parasites infect a greater diversity of host species at lower prevalence (Medeiros et al. 2014). However, our findings provide evidence for the resource-breadth hypothesis which states that the breadth of hosts reflects the parasite species' host use proficiency resulting in higher prevalence by parasites with larger host breadths (Medeiros et al. 2014). Indeed, L_CATUST11 and L_CATMIN07 infect all four host species with high prevalence. These two lineages may be in competition with one another as we see a fluctuation in their dominance at larger spatial scales, where L_CATMIN07 is more prevalent than L_CATUST11 further south of our study system (Starkloff et al., 2020).

The differences that we see among host pairs in *Leucocytozoon* community turnover are a result of variability in parasite sharing of rarer lineages that are typically absent in the Hermit Thrush. For example, L_POEHUD01 (only reported in *Catharus minimus*, the sister species to the Bicknell's Thrush, in MalAvi; Bensch et al.) is present in the Swainson's Thrush (<2% prevalence) and is often detected (2–10%) in the closely related Bicknell's Thrush and Veery (Fig. 2), but was absent in the Hermit Thrush (Fig. 2, Bensch et al. 2009). The two closest relatives of L_POEHUD01 are also absent from the Hermit Thrush in our study (Fig. 2), suggesting that this clade of parasites may not be able to infect the more distantly related species despite being transmitted in the same low elevation habitats. The absence of this parasite clade in the Hermit Thrush, which is more closely related to non-migratory, neotropical *Catharus* thrushes than to the other migratory *Catharus* thrushes (Everson et al. 2019), may be a result of considerable differentiation in molecular pathways limiting successful infections by more specialized parasites (Longdon et al. 2014). Future work should examine molecular pathways crucial to host cell invasion and parasite evasion to determine the genetic underpinnings of parasite sharing among hosts of differing relatedness.

When combining all three haemosporidian genera, community turnover patterns largely mirror those of *Leucocytozoon*, demonstrating the importance of looking at a smaller taxonomic scale to elucidate genus-level patterns. *Plasmodium* parasite communities are not determined by host relatedness, or (like *Leucocytozoon*) by elevation. *Plasmodium* community composition was, however, most heterogeneous among high elevation zones of the four mountain ranges, suggesting a sky-island effect (Williamson et al. 2019). *Plasmodium* parasites and their mosquito vectors are less abundant and diverse in colder climates (Foley et al. 2007, Merino et al. 2008), which could limit their transmission between the tops of mountain ranges. *Plasmodium* parasite communities were substantially less diverse and more genetically divergent in our study system than *Leucocytozoon* parasite communities which are comprised of many closely related parasites. Thus, the addition or removal of a single *Plasmodium* lineage can have a larger effect on community turnover measures than a *Leucocytozoon* lineage. Patterns of substantially higher turnover of *Plasmodium* communities than *Leucocytozoon* communities are also seen over a larger spatial scale in *Catharus* thrushes (Starkloff et al., 2020).

Plasmodium communities have high dissimilarity across all species comparisons (mean chao dissimilarity = 0.63). As all four host species winter allopatrically in Central and South America (Clement 2000), we must consider the possibility that *Plasmodium* infections could have been acquired on the wintering grounds or during migratory stopovers (Waldenström et al. 2002, Altizer et al. 2011). However, Soares et al. (2019) found that neotropical migrants sampled on their wintering grounds have parasite assemblages that are distinct from those of resident species, suggesting little to no transmission between these two groups of seasonally co-occurring birds. Within the *Catharus* study system, Pulgarín-R et al. (2019) showed that the sister species of the Bicknell's Thrush, the migratory Gray-cheeked Thrush (*C. minimus*), showed limited potential as a long-distance disperser of haemosporidian parasites. Additionally, the four lineages (P_CATUST05, P_BT7, L_CATMIN07, L_CATUST11) that we found in juvenile birds sampled prior to their first migration confirms their local transmission on the breeding grounds. The latter three locally transmitted lineages have previously been documented in juvenile birds in western North America, suggesting widespread transmission across the continent (Dodge et al. 2013, Cozzarolo et al. 2018, Pulgarín-R et al. 2019). The comparable similarity of *Leucocytozoon* communities both within and between closely related *Catharus* species (Starkloff et al., 2020) suggests that *Leucocytozoon* transmission outside of their temperate breeding grounds is improbable and unlikely to impact parasite community structure.

The prevalence of *Plasmodium* varies considerably among the four host species, with the highest prevalence in the Hermit Thrush. High variability in *Plasmodium* community structure among all species pairs may, thus, be driven more so by large disparities among host species in infection prevalence of lineages than the presence/absence of lineages (Fig. 2). High *Plasmodium* prevalence in the Hermit Thrush may be a result of higher host susceptibility or increased host tolerance of the infection (Miller et al. 2006, Råberg et al. 2009), or higher transmission rates achieved in a host species typically found at high density (Arneberg et al. 1998). The Hermit Thrush occupies the largest elevational breadth (Able and Noon 1976) and the broadest range of habitat types (Dellinger et al. 2012) of our host species, likely exposing it to a wider variety of parasitic organisms (Johnson et al. 2016). As a result, a specialized immune response may be less beneficial than tolerance mechanisms that may lower the fitness costs of infection for species harboring diverse assemblages of parasites. Contrastingly, *Leucocytozoon* prevalence was similar among the four host species. We did, however, find a slight decrease with elevation, despite the tendency of *Leucocytozoon* prevalence to be higher in colder conditions (Haas et al. 2012, Oakgrove et al. 2014, Cuevas et al. 2020, Fecchio et al. 2020). Haemosporidian communities in *Catharus* thrushes are clearly not structured by thermal tolerances alone, and the elevational decrease in *Leucocytozoon* prevalence is likely the result of some other factor associated with high elevation habitat, such as smaller streams that are typical of high elevations harboring a lower abundance of black fly larvae than larger streams and rivers (Malmqvist 2002). Alternatively, the host breadth of haemosporidian lineages may have decreased as climatic conditions change with elevation (Fecchio et al. 2019), thus, decreasing the probability of lineages infecting birds at high elevations. While this is unlikely at our spatial scale, our four host species study system is not suitable to evaluate whether host breadth of parasites varied with elevational climatic differences. Screening all avian host species across these elevational gradients would provide a better understanding of how the host breadth of a parasite lineage varies across its range.

As host breadth is typically variable across much larger spatial scales (Fecchio et al. 2019), the regional scale of our study was informative in revealing the disparate importance of host relatedness in structuring communities of two haemosporidian genera sampled across an environmental gradient. The collaborative archiving of avian haemosporidian parasites and their known avian host associations (Bensch et al.) makes these parasites an ideal taxonomic group to test whether these patterns are consistent in different climatic conditions and host communities in the future.

ACKNOWLEDGMENTS

We would like to thank Christina Carlson and Eric Diaz for their assistance in the field and laboratory, and Yen-Hua Huang for his statistical guidance. We are grateful to Kent McFarland, Christopher Rimmer, Andy Jones, and Ben Winger for contributing blood samples to this study. This research was funded by the University at Albany, the New York State Museum, an Edmund Niles Huyck Preserve Research Grant, and an American Museum of Natural History Frank M. Chapman Research Award. NCS was supported by a New York State Museum Graduate Research Fellowship. We would also like to thank the administrators at the Edmund Niles Huyck Preserve, Catskill Mountains Forest Preserve, the Huntington Wildlife Forest research station, and Bartlett Research Forest for allowing collection of blood samples on their premises. All new sampling was conducted under the IACUC permit #16-001 from the University at Albany, SUNY.

LITERATURE CITED

Able, K. P., and B. R. Noon. 1976. Avian community structure along elevational gradients in the Northeastern United States. Oecologia 26:275–294.

Altizer, S., R. Bartel, and B. A. Han. 2011. Animal migration and infectious disease risk. Science 331:296–302.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society of London. Series B: Biological Sciences 265:1283–1289.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2:74. http://btr0x2.rz.uni-bayreuth.de/math/statlib/R/CRAN/doc/packages/lme4.pdf

Bensch, S., O. Hellgren, and J. Pérez-Tris. 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Molecular Ecology Resources 9:1353–1358.

Bensch, S., J. Péarez-Tris, J. Waldenströum, and O. Hellgren. 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? Evolution 58:1617–1621.

Borner, J., et al. 2016. Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. Molecular Phylogenetics and Evolution 94:221–231.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research 33:261–304.

Clark, K., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2016. GenBank. Nucleic Acids Research 44:D67–D72.

Clark, N. J., and S. M. Clegg. 2017. Integrating phylogenetic and ecological distances reveals new insights into parasite host specificity. Molecular Ecology 26:3074–3086.

Clement, P. 2000. Thrushes. Princeton University Press, Princeton, New Jersey, USA.

Cogbill, C. V., and P. S. White. 1991. The latitude-elevation relationship for spruce-fir forest and treeline along the Appalachian mountain chain. Vegetatio 94:153–175.

Cozzarolo, C.-S., T. Jenkins, D. P. Toews, A. Brelsford, and P. Christe. 2018. Prevalence and diversity of haemosporidian parasites in the yellow-rumped warbler hybrid zone. Ecology and Evolution 8:9834–9847.

Cuevas, E., J. A. Vianna, E. Botero-Delgadillo, D. Doussang, D. González-Acuña, O. Barroso, R. Rozzi, R. A. Vásquez, and V. Quirici. 2020. Latitudinal gradients of haemosporidian parasites: prevalence, diversity and drivers of infection in the Thorn-tailed Rayadito (*Aphrastura spinicauda*). International Journal for Parasitology: Parasites and Wildlife 11:1–11.

Dallas, T., and S. J. Presley. 2014. Relative importance of host environment, transmission potential and host phylogeny to the structure of parasite metacommunities. Oikos 123:866–874.

De Vienne, D. M., M. E. Hood, and T. Giraud. 2009. Phylogenetic determinants of potential host shifts in fungal pathogens. Journal of Evolutionary Biology 22:2532–2541.

De Vienne, D. M., G. Refrégier, M. López-Villavicencio, A. Tellier, M. E. Hood, and T. Giraud. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytologist 198:347–385.

Dellinger, R., P. B. Wood, P. W. Jones, and T. M. Donovan 2012. Hermit Thrush (*Catharus guttatus*), version 2.0. The Birds of North America, Cornell Lab of Ornithology, Ithaca, New York, USA.

Dodge, M., S. L. Guers, Ç. H. Sekercioğlu, and R. N. Sehgal. 2013. North American transmission of Hemosporidian parasites in the Swainson's thrush (*Catharus ustulatus*), a migratory songbird. Journal of Parasitology 99:548–553.

Everson, K. M., J. F. McLaughlin, I. A. Cato, M. M. Evans, A. R. Gastaldi, K. K. Mills, K. G. Shink, S. M. Wilbur, and K. Winker. 2019. Speciation, gene flow, and seasonal migration in *Catharus thrushes* (Aves: Turdidae). Molecular Phylogenetics and Evolution 139:106564.

Fecchio, A., J. A. Bell, M. Bosholn, J. A. Vaughan, V. V. Tkach, H. L. Lutz, V. R. Cueto, C. A. Gorosito, D. González-Acuña, and C. Stromlund. 2020. An inverse latitudinal gradient in infection probability and phylogenetic diversity for Leucocytozoon blood parasites in New World birds. Journal of Animal Ecology 89:423–435.

Fecchio, A., K. Wells, J. A. Bell, V. V. Tkach, H. L. Lutz, J. D. Weckstein, S. M. Clegg, and N. J. Clark. 2019. Climate variation influences host specificity in avian malaria parasites. Ecology Letters 22:547–557.

Foley, D. H., L. M. Rueda, and R. C. Wilkerson. 2007. Insight into global mosquito biogeography from country species records. Journal of Medical Entomology 44:554–567.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207–233.

Gage, K. L., T. R. Burkot, R. J. Eisen, and E. B. Hayes. 2008. Climate and vectorborne diseases. American Journal of Preventive Medicine 35:436–450.

Galen, S. C., R. Nunes, P. R. Sweet, and S. L. Perkins. 2018. Integrating coalescent species delimitation with analysis of host specificity reveals extensive cryptic diversity despite minimal mitochondrial divergence in the malaria parasite genus Leucocytozoon. BMC Evolutionary Biology 18:128.

Galen, S. C., K. A. Speer, and S. L. Perkins. 2019. Evolutionary lability of host associations promotes phylogenetic overdispersion of co-infecting blood parasites. Journal of Animal Ecology 88:1936–1949.

Garcia-Longoria, L., A. Marzal, F. De Lope, and L. Garamszegi. 2019. Host-parasite interaction explains variation in the prevalence of avian haemosporidians at the community level. PLOS ONE 14:e0205624.

Goetz, J. E., K. P. McFarland, and C. C. Rimmer. 2003. Multiple paternity and multiple male feeders in Bicknell's Thrush (*Catharus bicknelli*). The Auk 120:1044–1053.

Haas, M., M. Lukáň, J. Kisková, and Z. Hrehová. 2012. Occurrence of blood parasites and intensity of infection in *Prunella modularis* in the montane and subalpine zone in the Slovak Carpathians. Acta Parasitologica 57:221–227.

Han, B. A., J. P. Schmidt, S. E. Bowden, and J. M. Drake. 2015. Rodent reservoirs of future zoonotic diseases. Proceedings of the National Academy of Sciences of the United States of America 112:7039–7044.

Hellgren, O., J. Waldenström, and S. Bensch. 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. Journal of Parasitology 90:797–802.

Hsieh, T. C., K. H. Ma, and A. Chao. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). Methods in Ecology and Evolution 7:1451–1456.

Johnson, P. T., C. L. Wood, M. B. Joseph, D. L. Preston, S. E. Haas, and Y. P. Springer. 2016. Habitat heterogeneity drives the host-diversity-begets-parasite-diversity relationship: evidence from experimental and field studies. Ecology Letters 19:752–761.

Kamath, P. L., K. Manlove, E. F. Cassirer, P. C. Cross, and T. E. Besser. 2019. Genetic structure of Mycoplasma ovipneumoniae informs pathogen spillover dynamics between domestic and wild Caprinae in the western United States. Scientific Reports 9:1–14.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464.

Krasnov, B. R., and R. Poulin 2010. Ecological properties of a parasite: species-specific stability and geographical variation. Pages 99–113 *in* S. Morand, and B. R. Krasnov, editors. The biogeography of host–parasite interactions. Oxford University Press, Oxford, UK.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Lindsay, S. W., and M. H. Birley. 1996. Climate change and malaria transmission. Annals of Tropical Medicine & Parasitology 90:573–588.

Liu, W., Y. Li, G. H. Learn, R. S. Rudicell, J. D. Robertson, B. F. Keele, J.-B.-N. Ndjango, C. M. Sanz, D. B. Morgan, and S. Locatelli. 2010. Origin of the human malaria parasite Plasmodium falciparum in gorillas. Nature 467:420–425.

Locke, S. A., J. D. McLaughlin, and D. J. Marcogliese. 2013. Predicting the similarity of parasite communities in freshwater fishes using the phylogeny, ecology and proximity of hosts. Oikos 122:73–83.

Longdon, B., M. A. Brockhurst, C. A. Russell, J. J. Welch, and F. M. Jiggins. 2014. The evolution and genetics of virus host shifts. PLoS Path 10: e1004395.

Longdon, B., J. D. Hadfield, C. L. Webster, D. J. Obbard, and F. M. Jiggins. 2011. Host phylogeny determines viral persistence and replication in novel hosts. PLoS Path 7:e1002260.

Malmqvist, B. 2002. Aquatic invertebrates in riverine landscapes. Freshwater Biology 47:679–694.

McCreadie, J. W., and P. H. Adler. 2014. Abundance–occupancy relationships of larval black flies (Diptera: Simuliidae) in temperate Nearctic streams. Insect Conservation and Diversity 7:523–532.

McCreadie, J. W., R. H. Williams, S. Stutsman, D. S. Finn, and P. H. Adler. 2018. The influence of habitat heterogeneity and latitude on gamma diversity of the Nearctic Simuliidae, a ubiquitous group of stream-dwelling insects. Insect Science 25:712–720.

Medeiros, M. C., V. A. Ellis, and R. E. Ricklefs. 2014. Specialized avian Haemosporida trade reduced host breadth for increased prevalence. Journal of Evolutionary Biology 27:2520–2528.

Merino, S., J. Moreno, R. A. Vásquez, J. Martínez, I. Sánchez-Monsálvez, C. F. Estades, S. Ippi, P. Sabat, R. Rozzi, and S. Mcgehee. 2008. Haematozoa in forest birds from southern Chile: latitudinal gradients in prevalence and parasite lineage richness. Austral Ecology 33:329–340.

Miller, M. R., A. White, and M. Boots. 2006. The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. Evolution 60:945–956.

Moens, M. A., and J. Pérez-Tris. 2016. Discovering potential sources of emerging pathogens: South America is a reservoir of generalist avian blood parasites. International Journal for Parasitology 46:41–49.

Morens, D. M., J. G. Breman, C. H. Calisher, P. C. Doherty, B. H. Hahn, G. T. Keusch, L. D. Kramer, J. W. LeDuc, T. P. Monath, and J. K. Taubenberger. 2020. The origin of COVID-19 and why it matters. American Journal of Tropical Medicine and Hygiene 103:955–959.

Nieberding, C., E. Jousselin, and Y. Desdevises 2010. The use of co-phylogeographic patterns to predict the nature of host-parasite interactions, and vice versa. Pages 59–70 *in* S. Morand, and B. R. Krasnov, editors. The biogeography of host-parasite interactions. Oxford University Press, Oxford, UK.

Oakgrove, K. S., R. J. Harrigan, C. Loiseau, S. Guers, B. Seppi, and R. N. Sehgal. 2014. Distribution, diversity and drivers of blood-borne parasite co-infections in Alaskan bird populations. International Journal for Parasitology 44:717–727.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner 2013. Package 'vegan'. Community ecology package, version 2:1-295. https://cran.ism.ac.jp/web/packages/vegan/vegan.pdf

Palinauskas, V., R. Žiegytė, J. Šengaut, and R. Bernotienė. 2018. Different paths–the same virulence: experimental study on avian single and co-infections with *Plasmodium relictum* and *Plasmodium elongatum*. International Journal for Parasitology 48:1089–1096.

Pérez-Tris, J., and S. Bensch. 2005. Dispersal increases local transmission of avian malarial parasites. Ecology Letters 8:838–845.

Poulin, R., and B. R. Krasnov 2010. Similarity and variability of parasite assemblages across geographical space. Pages 115–127 *in* S. Morand, and B. R. Krasnov, editors. The biogeography of host–parasite interactions. Oxford University Press, Oxford, UK.

Poulin, R., and S. Morand 2014. Parasite biodiversity. Smithsonian Institution, Washington, DC, USA.

Preisser, W. 2019. Latitudinal gradients of parasite richness: a review and new insights from helminths of cricetid rodents. Ecography 42:1315–1330.

Pulgarín-R, P. C., C. Gómez, N. J. Bayly, S. Bensch, A. M. FitzGerald, N. Starkloff, J. J. Kirchman, A. M. González-Prieto, K. A. Hobson, and J. Ungvari-Martin. 2019. Migratory birds as vehicles for parasite dispersal? Infection by avian haemosporidians over the year and throughout the range of a long-distance migrant. Journal of Biogeography 46: 83–96.

R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Råberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society B: Biological Sciences 364:37–49.

Ramey, A. M., J. P. Fleskes, J. A. Schmutz, and M. J. Yabsley. 2013. Evaluation of blood and muscle tissues for molecular detection and characterization of hematozoa infections in northern pintails (*Anas acuta*) wintering in California. International Journal for Parasitology: Parasites and Wildlife 2:102–109.

Santiago-Alarcon, D., V. Palinauskas, and H. M. Schaefer. 2012. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. Biological Reviews 87:928–964.

Sehgal, R. N. M., W. Buermann, R. J. Harrigan, C. Bonneaud, C. Loiseau, A. Chasar, I. Sepil, G. Valkiūnas, T. Iezhova, and S. Saatchi. 2011. Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. Proceedings of the Royal Society B: Biological Sciences 278:1025–1033.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics 40:415–436.

Soares, L., S. C. Latta, and R. E. Ricklefs. 2019. Neotropical migratory and resident birds occurring in sympatry during winter have distinct haemosporidian parasite assemblages. Journal of Biogeography 47:748–759.

Spellerberg, I. F., and P. J. Fedor. 2003. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener' Index. Global Ecology and Biogeography 12:177–179.

Starkloff, N. C., J. J. Kirchman, A. W. Jones, B. M. Winger, Y. H. Huang, P. C. Pulgarín-R, and W. C. Turner. 2020. Drivers of community turnover differ between avian hemoparasite genera along a North American latitudinal gradient. Ecology and Evolution 10:5402–5415.

Svensson, L. M. E., K. C. Ruegg, C. H. Sekercioglu, and R. N. M. Sehghal. 2007. Widespread and structured distributions of blood parasite haplotypes across a migratory divide of the Swainson's thrush (*Catharus ustulatus*). Journal of Parasitology 93:1488–1495.

Tinsley, M. C., and M. E. Majerus. 2007. Small steps or giant leaps for male-killers? Phylogenetic constraints to male-killer host shifts. BMC Evolutionary Biology 7:238.

Valkiunas, G. 2004. Avian malaria parasites and other Haemosporidia. CRC Press, Boca Raton, Florida, USA.

Waldenström, J., S. Bensch, S. Kiboi, D. Hasselquist, and U. Ottosson. 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Molecular Ecology 11:1545–1554.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. American Naturalist 156:145–155.

Wells, K., and N. J. Clark. 2019. Host specificity in variable environments. Trends in Parasitology 35:452–465.

Williamson, J. L., C. J. Wolf, L. N. Barrow, M. J. Baumann, S. C. Galen, C. J. Schmitt, D. C. Schmitt, A. S. Winter, and C. C. Witt. 2019. Ecology, not distance, explains community composition in parasites of sky-island Audubon's Warblers. International Journal for Parasitology 49:437–448.

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Zamora-Vilchis, I., S. E. Williams, and C. N. Johnson. 2012. Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. PLOS ONE 7:e39208.

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