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Diversity, abundance, and host relationships in the avian malaria community of New Mexico pine forests

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Avian malaria parasites (genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) affect bird demography, distribution limits, and community structure, yet most bird communities and populations remain unsurveyed. We conducted a community-level survey of these vector-transmitted parasites in New Mexico, USA, to describe the diversity, abundance, and host associations. We focused on the breeding-bird community in the transition zone between piñon-juniper woodland and ponderosa pine forests (elevational range: 2150–2460 meters). We screened 186 birds representing 49 species using both standard PCR and microscopy techniques to detect infections of all three avian malaria genera. The combined infection rate was 36.6%, with the highest infection rate for *Haemoproteus* (20.9%), followed by *Leucocytozoon* (13.4%), then *Plasmodium* (8.0%). We sequenced mtDNA for 77 infections representing 43 haplotypes (25 *Haemoproteus*, 12 *Leucocytozoon*, 6 *Plasmodium*). When compared to all previously known lineages in the MalAvi and GenBank databases, 65% (28) of the haplotypes that we recovered were novel. We found evidence for host specificity at the avian clade and species level, but this specificity was variable among parasite genera. *Haemoproteus* and *Leucocytozoon* were each restricted to three avian host-clades or groups (out of six), while *Plasmodium* occurred in all groups except non-passerines. We found striking variation in infection rate among host species, with nearly universal infection among vireos and no infection among nuthatches. Using rarefaction and extrapolation, we estimated the total avian malaria diversity to be 70 haplotypes (95% CI: 43–98); thus, we may have already sampled ~60% of the diversity of avian malaria in New Mexico pine forests. It is possible that future studies will find higher diversity in microhabitats or host species that are under-sampled or unsampled in the present study. Fortunately, this study is fully extendable via voucher specimens, frozen tissues, blood smears, parasite images, and documentation provided in open-access databases (MalAvi, Genbank, and ARCTOS).

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Diversity, abundance, and host relationships in the avian malaria community of New Mexico pine forests

Short title: Avian malaria diversity in New Mexico

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Abstract

Avian malaria parasites (genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) affect bird demography, distribution limits, and community structure, yet most bird communities and populations remain unsurveyed. We conducted a community-level survey of these vector-transmitted parasites in New Mexico, USA, to describe the diversity, abundance, and host associations. We focused on the breeding-bird community in the transition zone between piñon-juniper woodland and ponderosa pine forests (elevational range: 2150–2460 meters). We screened 186 birds representing 49 species using both standard PCR and microscopy techniques to detect infections of all three avian malaria genera. The combined infection rate was 36.6%, with the highest infection rate for *Haemoproteus* (20.9%), followed by *Leucocytozoon* (13.4%), then *Plasmodium* (8.0%). We sequenced mtDNA for 77 infections representing 43 haplotypes (25 *Haemoproteus*, 12 *Leucocytozoon*, 6 *Plasmodium*). When compared to all previously known lineages in the MalAvi and GenBank databases, 65% (28) of the haplotypes that we recovered were novel. We found evidence for host specificity at the avian clade and species level, but this specificity was variable among parasite genera. *Haemoproteus* and *Leucocytozoon* were each restricted to three avian host-clades or groups (out of six), while *Plasmodium* occurred in all groups except non-passerines. We found striking variation in infection rate among host species, with nearly universal infection among vireos and no infection among nuthatches. Using rarefaction and extrapolation, we estimated the total avian malaria diversity to be 70 haplotypes (95% CI: 43–98); thus, we may have already sampled ~60% of the diversity of avian malaria in New Mexico pine forests. It is possible that future studies will find higher diversity in microhabitats or host species that are under-sampled or unsampled in the present study. Fortunately, this study is fully extendable via voucher specimens, frozen tissues, blood smears,

parasite images, and documentation provided in open-access databases (MalAvi, Genbank, and ARCTOS).

Introduction

Parasites are influential components of biotic communities, yet the vast majority of parasite diversity remains undescribed (Dobson et al., 2008; Poulin, 2014). A striking example is provided by the haemosporidian parasites (Protozoa: Apicomplexa: Haemosporida) that infect primates, rodents, bats, lizards, and birds. Avian haemosporidians of the genera *Haemoproteus, Plasmodium,* and *Leucocytozoon* (hereafter, 'avian malaria') are known to affect bird community structure (Atkinson et al., 2013; Kulma et al., 2013; Clark, Clegg & Lima, 2014), immune function (Atkinson et al., 2001; Beadell et al., 2007), telomere length and senescence (Asghar et al., 2015), survivorship (Atkinson et al., 2000), and fecundity (Knowles, Palinauskas & Sheldon, 2010). Over 250 avian malaria species have been described based on morphology (Valkiūnas, 2005), but mitochondrial (mtDNA) sequences have revealed that at least one order of magnitude higher diversity exists (Bensch, Hellgren & Pérez-Tris, 2009; Clark, Clegg & Lima, 2014). Nearly two decades since the introduction of mtDNA 'barcode' survey methods (Bensch et al., 2000), many geographic regions and the vast majority of avian populations remain unsurveyed for haemosporidians. New community-level surveys of avian malaria will be critical to understanding their diversity, biogeography, and coevolutionary dynamics.

The need for new descriptive data on avian malaria communities is vital, particularly in under-sampled regions and habitats, for several reasons. Interacting bird, dipteran, and avian malaria species underlie the avian malaria transmission cycle (Valkiūnas, 2005; LaPointe, Goff & Atkinson, 2010), and these are likely to be susceptible to range shifts driven by climate warming. This situation creates the potential for novel host-parasite interactions. When naïve hosts encounter novel malarial strains, the consequences can be severe, as illustrated by the decimation of native Hawaiian honeycreepers after the introduction of *Plasmodium relictum*

(Warner, 1968; van Riper et al., 1986; Atkinson et al., 2000). Furthermore, increased temperatures can result in multiple reproductive cycles for the dipteran definitive hosts (Robinet & Roques, 2010), potentially causing increased infection risk or parasitemia, with negative consequences for bird population growth (Scott et al., 1983; Brown et al., 2001; Garamszegi, 2011). Increased contact between hosts and parasites may also facilitate host-switching, which appears to be a common mode of lineage diversification in this group (Ricklefs & Fallon, 2002; Galen & Witt, 2014; Ricklefs et al., 2014). Descriptions of avian malaria communities will elucidate the ecological niches, host relationships, and host-switching potential of parasite lineages, providing information that will be critical for wildlife management and will provide a basis for predicting climate change impacts.

The southwestern United States, in particular, is mostly unsurveyed and is likely to harbor a distinct avian malaria assemblage, in part because its arid environment imposes challenges for the dipteran definitive hosts that serve as vectors (Yohannes et al., 2005; Lachish et al., 2011). The few previous community-level surveys of avian malaria parasites in western North America have been conducted in California (Martinsen et al., 2008; Walther et al., 2016) and Alaska (Loiseau et al., 2012; Oakgrove et al., 2014). Here we report on the first community-level avian malaria survey in New Mexico, USA. New Mexico's arid climate and broad elevation gradients provide a compelling and untapped system in which to investigate avian malaria diversity and ecology. We focus specifically on the breeding-season community in the elevational zone between 2150–2460 meters, which is characterized by the transition from forests dominated by piñon pine to those dominated by ponderosa pine. Our objectives were: (1) To compare infection rates for each of the three avian malaria genera (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) among a suite of breeding bird species using microscopy and mtDNA; (2) To describe associations between avian malaria lineages and their host species in a phylogenetic context; (3) To evaluate lineage-richness (α-diversity) of the avian malaria community in a previously unsurveyed region and habitat, including the proportion of lineages that are novel (never found in previous surveys). The survey results that we report are fully extendable via voucher specimens, frozen tissues, blood smears, parasite images, and documentation in open-access databases (MalAvi, Genbank, and ARCTOS).

Methods

Field Sampling

We conducted fieldwork during June and July 2016 at three sites in northern New Mexico within the jurisdiction of the Rio Puerco Field Office of the Bureau of Land Management (BLM), an agency within the United States Department of Interior. The three sites included: (1) Mesa Chivato (McKinley and Sandoval Counties), which is on the northern flank of Mt. Taylor; (2) El Malpais National Conservation Area (Cibola County), which is on the southern side of the Zuni Mountains; and (3) Elk Springs (Sandoval County), which is on the western slope of the Jemez Mountains (Fig. 1). Sampling was conducted within a narrow elevational band (2150–2460 m) at the upper elevational extent of piñon-juniper woodland, where it transitions to ponderosa pine forest. These pine-dominated habitats were interspersed with patches of grassland and occasional Gambel oak, Douglas fir, or aspen. Permanent water was scarce in the sampled habitats, consisting of a tiny, spring-fed creek in the Elk Springs site, a natural spring (Ojo de los Indios) that has been developed in Mesa Chivato, and a few widely-dispersed watering troughs and earthen tanks for cattle or wildlife in Mesa Chivato and El Malpais National Conservation Area.

We focused on the breeding-season community in order to characterize locally-transmitted parasites. Sampling during the breeding season may also maximize detection because breeding birds experience an increase in glucocorticoid stress hormones (Romero, 2002), which can cause relapse of latent avian malaria infections (Applegate, 1970; Valkiūnas et al., 2004; Garvin & Schoech, 2006). Blood smears were prepared at the time of collection and were later fixed and stained in the lab (details below). Whole avian specimens were preserved on dry ice and transported to the Museum of Southwestern Biology (MSB) at the University of New Mexico for specimen preparation and preservation of tissues for genetic analysis. All samples were collected under Institutional Animal Care and Use Protocol 16-200406-MC and appropriate state and federal scientific collecting permits (New Mexico Department of Game and Fish Authorization Number 3217; U.S. Fish and Wildlife Permit Number MB094297-0). Complete details on each specimen, including precise locality, collection method, and necropsy data are available in Table S1 and its embedded links to the ARCTOS database. Additionally, all novel haplotypes, host species infected, and occurrence sites were documented in the MalAvi database (Bensch, Hellgren & Pérez-Tris, 2009).

Genetic data collection

We extracted genomic DNA from frozen pectoral muscle tissue of 186 avian specimens using a QIAGEN DNeasy Blood and Tissue Kit, following the manufacturer’s protocol. We used three nested polymerase chain reaction (PCR) protocols to amplify a 478 base pair (bp) fragment of cytochrome b (*cytb*) in the haemosporidian mitochondrial genome. We followed the protocols of Hellgren, Waldenström & Bensch (2004) and Waldenström et al. (2004) to maximize detection of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* parasites. We used the outer primer pairs

HaemNFI/HaemNR3 and HaemNF/HaemNR2 with the nested primer pair HaemF/HaemR2 to screen for *Haemoproteu*s and *Plasmodium*. We used the outer primer pair HaemNFI/HaemNR3 with the nested primer pair HaemFL/HaemR2L to screen for *Leucocytozoon*. Each outer PCR contained 1.25 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 1X PCR Buffer II, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM each primer, and 20 ng template DNA in a total reaction volume of 25 μl. The thermal profile of this reaction was modified following Galen & Witt (2014) and consisted of an initial 8-min denaturation step at 95°C, followed by 20 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 45 sec, with a final 10-min extension at 72°C. The nested PCR used the outer PCR product as the template (1 μl for *Haemoproteus* and *Plasmodium*; 2 μl for *Leucocytozoon*). Reaction conditions were the same for nested PCR except the number of cycles was increased to 35. Negative controls were included in each PCR reaction to check for contamination. Once identified, positive controls were included in each subsequent PCR to verify successful DNA amplification. All PCR reactions were visualized on 2% agarose gels using SYBR Safe Gel Stain (Invitrogen) and a Kodak Gel Logic 200 Digital Imaging System to identify positive samples and verify the presence of PCR product of the expected length. All successful amplifications were purified using ExoSap-IT (Affymetrix, Inc.) and sequenced in both directions using dye terminator cycle sequencing on an ABI 3130 sequencer at the UNM Molecular Biology Core Facility.

Microscopic examination

Blood smears were air dried in the field and, within six months, were fixed using absolute methanol and stained for 50 minutes with phosphate-buffered Giemsa solution (7.0 pH). We examined each blood smear for evidence of haemosporidan blood parasites using either a Leica

DM5000 B or a Nikon Labophot-2 light microscope, following identification protocol described by Valkiūnas (2005). We scanned at least 10,000 erythrocytes in all viable smears at 1000X magnification using an oil immersion lens. We did not attempt to identify gametocytes to morphospecies; rather, we took digital photographs to archive in the ARCTOS database. We re-screened 76 (45%) of the blood smears to confirm negative or positive identifications after an initial comparison with PCR results.

Genetic data analysis

Parasite sequences were edited and aligned using the default alignment algorithm in Geneious version 8.0 (Biomatters Ltd; Kearse et al., 2012). We compared our sequences to previously sequenced infections in the public databases GenBank (National Center for Biotechnology Information, U.S. National Library of Medicine) and MalAvi (Bensch, Hellgren & Pérez-Tris, 2009) using the Basic Local Alignment Search Tool (BLAST). We used the closest match to determine the parasite genus for each haplotype. Studies have indicated that avian malaria sequences differing by a single bp can differ in host association and in transmission (Bensch, Hellgren & Pérez-Tris, 2009). We therefore characterized parasite haplotypes differing by one or more bp from existing sequences in the GenBank and MalAvi databases as novel and named them following MalAvi naming conventions (first three letters of the genus and species of the first bird host species from which the haplotype was sequenced, followed by a haplotype number for that bird species). Some authors have suggested combining haplotypes into lineages based on a 1% divergence rule (Outlaw & Ricklefs, 2014) and considering geographic distributions and hosts infected (Svensson-Coelho et al., 2013). In this study we tentatively treat each haplotype as a unique lineage; additional sampling will be required to determine whether some of these closely related haplotype groups may represent segregating variants within single populations.

We estimated the phylogenetic relationships among New Mexico haemosporidian parasites based on *cytb* using maximum likelihood in RAxML version 8.2 (Stamatakis, 2014). Given the modest size of the dataset, we analyzed all codon positions as a single partition. We used the GTR+G model of nucleotide substitution and conducted a rapid bootstrap analysis with 1000 bootstrap replicates, after which we searched for the best-scoring maximum-likelihood tree. We did not specify an outgroup for the ML analysis and instead rooted the tree at the *Leucocytozoon* clade based on the relationships for Haemosporida determined using several loci and taxa (Borner et al. 2016). We generated a phylogenetic tree for the avian species sampled using BirdTree.org, which uses calibrated backbone trees of well-supported avian clades and generates trees for all bird species by partially constraining them to their respective clade (expanded methods in Jetz et al., 2012, 2014). We used the phylogeny subsets tool to download a tree including only the species we sampled from the 'Ericson All Species' source of trees (Ericson et al., 2006).

Estimates of lineage diversity

We used EstimateS version 9.1.0 (Colwell, 2013) to generate an estimate of undiscovered lineage diversity present in northern New Mexico avian malaria communities. This approach estimates species richness in a community based on rarefaction and extrapolation of reference samples (Colwell et al., 2012). We used counts for each parasite haplotype as individual-based abundance data regardless of host species identity, which should result in a conservative estimate of species richness. Rarefaction was conducted with 100 randomizations and the rarefaction curve was extrapolated with unconditional 95% confidence intervals to a total of 400 individuals, at which point the species richness curve reached an asymptote.

Results

Parasite abundance

We collected 186 individuals from 49 species and representing 19 families of New Mexico birds (Table S1). Twenty-six species tested positive for one or more of the three genera of avian malaria parasites. In total, 65 out of the 186 birds were infected based on PCR (34.9%). These include 39 birds infected with one or more lineages of *Haemoproteus* (20.9%), 15 birds infected with *Plasmodium* (8.0%), and 25 birds infected with *Leucocytozoon* (13.4%). Two additional individuals tested positive for either *Haemoproteus* or *Plasmodium* in the PCR screening, but we were unable to identify these lineages to genus because of poor sequence quality. Infection rates were variable among parasite genera, as well as among host clades and host species (Fig. 2). We defined co-infection as testing PCR positive for more than one genus of malaria parasite (i.e. possessing both *Leucocytozoon* and *Haemoproteus/Plasmodium*), or testing positive for more than one haplotype within a parasite genus (i.e. two distinct *Haemoproteus* or *Plasmodium* lineages), either in separate nested PCR reactions or by presence of double peaks in sequence chromatograms. A total of 18 (9.7%) of individuals were co-infected, including two mixed infections comprised of *Leucocytozoon* with *Plasmodium*, 12 of *Leucocytozoon* with *Haemoproteus*, two of *Haemoproteus* with *Haemoproteus*, and one of *Plasmodium* with *Plasmodium*.

We completed microscopic examination for 168 individuals (90%) that had blood smears of adequate quality. We detected evidence of positive haemosporidian infection in 43 (25.6%) of

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the individuals we screened. The rate of detection was higher using PCR than microscopy (Table 1). In 21 cases, PCR was positive with a negative microscopy result, and in three cases, microscopy was positive with a negative PCR result. Parasitemia (defined as the proportion of red blood cells infected out of 10,000) was < 1% for the majority of slides examined. The highest level of infection was in an individual of *Empidonax oberholseri* (Tyrannidae) with ~2% of red blood cells infected. Combining both PCR and microscopy results, 68 birds in total (36.6%) were infected.

Parasite diversity

We identified 43 distinct haplotypes of avian malaria parasites, including 25 *Haemoproteus*, six *Plasmodium*, and 12 *Leucocytozoon* haplotypes (Fig. 2; Table S2). Based on published sequences in the MalAvi and GenBank databases, 28 haplotypes (65%) identified were novel, which consisted of 18 novel haplotypes for *Haemoproteus* (meaning 72% of the haplotypes we found for the genus were novel), two for *Plasmodium (*33%), and eight for *Leucocytozoon* (67%). We identified a total of 83 positive PCR infections, and obtained unambiguous sequences from 77 of them. We excluded four sequences positively identified as *Leucocytozoon* and two sequences for which we were unable to distinguish between *Haemoproteus* and *Plasmodium* from the parasite phylogeny. These infections were included for the calculation of overall and *Leucocytozoon* infection rates. Additionally, we found evidence of infection in three juvenile birds; two with *Leucocytozoon* and one with a novel *Haemoproteus* lineage, providing evidence for local transmission.

Parasite phylogeny and host associations

The parasite phylogeny indicated strong support for the sister group relationship between avian *Haemoproteus* and *Plasmodium* (bootstrap value = 100). We recovered monophyletic relationships for each genus with strong to moderate support (bootstrap values: 100 for *Leucocytozoon*, 85 for *Haemoproteus*, 67 for *Plasmodium*). We found evidence for associations between host clades and parasite genera. All *Haemoproteus* haplotypes were restricted to three avian clades: Passerides clade 1b, Corvides, and Suboscines (Fig. 2). Similarly, all *Leucocytozoon* haplotypes were restricted to Passerides clade 1b, Passerides clade 2, and Corvides. *Plasmodium* infections occurred in all avian clades or groups sampled except non-passerines. We found no infections in non-passerine species, which may be due to low sample size (n = 8). Although node-support values were modest, some monophyletic *Haemoproteus* groups appear to be restricted to single avian clades (Fig. 2). Notably, the clade containing VIRPLU04 to VIGIL07 included 14 infections, 12 of which were recovered only from avian hosts in the genus *Vireo*.

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Estimates of lineage diversity

A rarefaction curve generated in EstimateS using the 77 infections and 43 haplotypes identified suggested that the total haplotype richness is ~70 (95% CI: 43–98; Fig. 3). According to this method, we have identified ~60% of the lineage diversity present at these sites, and sampling a total of ~240 infections should be sufficient to capture > 95% of the lineage diversity in this avian malaria community. Based on our PCR-derived infection rate of 34.9%, this projection suggests we will need to screen ~690 birds, or ~500 additional samples to adequately characterize the avian malaria community of New Mexico pine forests. This estimate should be regarded as a conservative minimum estimate of the sampling needed, as explained below.

Discussion

Haemosporidian abundance in New Mexico pine forest breeding bird communities

We detected high levels of infection in the first community-wide survey of blood parasites in New Mexico breeding birds, with over one third (36.6%) of individuals infected with at least one of the three parasite genera. This level of infection is comparable to community surveys in other parts of the U.S. including California (39.8% of 399 birds; Walther et al., 2016), Alaska (53% of 903 birds; Oakgrove et al., 2014), and Missouri (38.6% of 757 birds; Ricklefs et al., 2005). Community-level surveys in other parts of the world vary widely in avian malaria infection rates, from 17.4% of 2661 birds in Brazil (excludes *Leucocytozoon*; Fecchio et al., 2017), to 79.1% of 532 birds in east Africa (Lutz et al., 2015). In our New Mexico study, *Haemoproteus* was the most prevalent parasite genus (20.9% of birds infected), followed by *Leucocytozoon* (13.4%), then *Plasmodium* (8.0%). This generic composition was strikingly different from that found by some previous studies in western North America. For example, Walther et al. (2016) found much higher *Plasmodium* infection rates compared to the other two genera in a California songbird community, and Oakgrove et al. (2014) found *Leucocytozoon* to be the most abundant genus in an Alaska survey. The relative absence of standing water in ponderosa pine forest and piñon-juniper woodland habitats and differences in vector ecology may contribute to these patterns. Interestingly, Elk Springs, the site with a spring-fed creek, had a higher *Haemoproteus* infection rate and nearly three times the *Leucocytozoon* infection rate compared to the Mesa Chivato and El Malpais National Conservation Area, but this pattern remains to be confirmed with additional sampling.

The variation in prevalence that we detected among host species suggests intriguing avenues for further investigation. We uncovered extremely high parasite prevalence and high levels of co-infection in two *Vireo* species, *Vireo gilvus* and *V. plumbeus*. Of 13 individuals collected, 12 (92%) were positive for either *Haemoproteus* or *Leucocytozoon*, and 8 (61.5%) were co-infected. Walther et al. (2016) also identified high infection rates in *Vireo gilvus* (n = 11) and identified *V. gilvus* as the only study species to be co-infected with more than three parasite lineages. The high prevalence and rate of co-infection indicates that *Vireo* species will be important to investigate as potential reservoirs for *Haemoproteus* and *Leucocytozoon* parasites (e.g., Möens et al., 2016). In contrast, the three species of nuthatches (*Sitta pygmaea*, *S. canadensis*, *S. carolinensis*) in our survey were completely uninfected (n = 12). It is possible that immune function or ecological characteristics such as cavity-nesting (Fecchio et al., 2011; Svensson-Coelho et al., 2013; Lutz et al., 2015) minimize infection in these species.

The number of positive infections we detected with PCR differed somewhat from microscopy results, consistent with previous studies that have compared the two methods (Valkiūnas et al., 2008; Möens et al., 2016). Differences between PCR and microscopy detection are expected for at least three reasons. First, PCR can identify a positive infection with fewer than a single parasite per one million host cells (Hellgren, Waldenström & Bensch, 2004), thus these low-level infections are unlikely to be detected using standard microscopic examinations of 10,000–100,000 cells. Second, it is conceivable that infections detected by PCR may be abortive infections, which would not develop into gametocytes in the blood stream (Valkiūnas et al., 2013). Third, parasitemia is easiest to identify by microscopy at peak levels of infection (Atkinson et al., 2000); however, we are unlikely to sample birds experiencing this level of parasitemia because of reduced activity in infected birds (Knowles, Palinauskas & Sheldon, 2010). Furthermore, detection by PCR appears to be sensitive to tissue type, with higher detection probability for heart, liver, or pectoral muscle tissue compared to blood (Svensson-Coelho et al., 2016). We sampled pectoral muscle tissue, for which Svensson-Coelho et al. (2016) found fewer false negatives compared to other tissue types, although no tissue type had perfect detection.

Novel parasite diversity and apparent host clade associations

Our survey revealed high diversity of avian malaria parasites in northern New Mexico including several novel lineages. Of the 43 haplotypes we sampled, 14 have previously been identified and published in the MalAvi database. Eight of these have only been documented in the U.S., and five have only been identified within the western U.S., suggesting restricted geographical ranges within continental North America for at least some lineages. *Haemoproteus* was the most diverse lineage in our study with 25 haplotypes identified, 18 of which were novel. Similar to the variation in patterns of prevalence, other surveys have found either *Plasmodium* (California: Walther et al., 2016; South America: Svensson-Coelho et al., 2013; Fecchio et al., 2017) or

Leucocytozoon (Alaska: Oakgrove et al., 2014; eastern Africa: Lutz et al., 2015) to have higher diversity compared to other genera. We found more novel lineages (28 total, or 65%) than similar community-level surveys in California (40% novel; Walther et al., 2016) and Alaska (49% novel; Oakgrove et al., 2014) despite having a much smaller sample size thus far (186 birds compared to 399 and 913; 46–49 species sampled in all three surveys).

The apparent host breadth and geographic range of the lineages we sampled provides evidence for some generalist parasites, mostly within *Plasmodium*. For example, LAIRI01 was found in four different avian clades in our study, and has previously been reported in the Philippines, Ecuador, and Mexico (Silva-Iturriza, Ketmaier & Tiedemann, 2012; Levin et al., 2013). We found one occurrence of WW3, which is distributed across Africa, Europe, and other parts of the U.S. (Waldenström et al., 2002; Bensch & Åkesson, 2003; Hellgren et al., 2007). One *Haemoproteus* lineage, SIAMEX01, also appears to be wide-ranging across the U.S. and has been identified in several avian hosts (Ricklefs & Fallon, 2002; Levin et al., 2013). The majority of avian malaria lineages found in our study, however, seem to be host specific at the species or clade level. One example includes the six *Haemoproteus* and three *Leucocytozoon* lineages that were specific to *Vireo* species, three of which were also identified in *Vireo* species in California (Walther et al., 2016). Among the major host clades, lineage diversity was highest for Passerides clade 1b and Corvides. Most *Haemoproteus* and *Leucocytozoon* lineages were sampled from a single avian host clade, but additional sampling is needed to confirm the patterns of host specificity in New Mexico pine forest breeding bird communities.

Our estimates of total avian malaria lineage diversity indicate that a substantial number of haplotypes, ~25, remain to be sampled in these communities; this is likely to be an underestimate. Although there was clearly some host specificity, we were not able to account for host-species identity in our rarefaction procedure in EstimateS. Our sampling of host species was uneven and biased toward common species. Considering the preponderance of unsampled and under-sampled bird species in the community, the avian malaria diversity in New Mexico pine forests is likely much higher than the estimate presented here. For the same reasons, uneven sampling among the three sites may have exacerbated our underestimation of diversity, particularly if there is species-turnover among mountain ranges.

Conclusions

We uncovered a diverse community of avian malaria parasites in New Mexico pine forests, with the majority of infections representing novel mtDNA haplotypes. We found evidence for host-specificity at the level of avian species and clades. There was also striking variation in infection rates among avian species and clades, typified by near universal infection of vireos and absence of infection in nuthatches. This study underscores the need for further sampling in southwestern North America in order to discover the diversity of ecologically important symbionts that are interacting with birds. Follow-up studies should extend on the open-data provided here to test our extrapolation of the total avian malaria haplotype diversity, and to determine the extent to which the avian malaria community varies among neighboring ‘sky island’ mountain ranges or at elevations above or below the zone sampled here.

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References

Applegate JE. 1970. Population Changes in Latent Avian Malaria Infections Associated with Season and Corticosterone Treatment. *Journal of Parasitology* 56:439–443.

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

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

Atkinson CT., Lease JK., Drake BM., Shema NP. 2001. Pathogenicity, serological responses, and diagnosis of experimental and natural malarial infections in native hawaiian thrushes. *The Condor* 103:209–218. DOI: 10.1650/0010-5422(2001)103[0209:psrado]2.0.co;2.

Atkinson CT., Saili KS., Utzurrum RB., Jarvi SI. 2013. Experimental evidence for evolved tolerance to avian malaria in a wild population of low elevation Hawai'i 'Amakihi (Hemignathus virens). *EcoHealth* 10:366–375. DOI: 10.1007/s10393-013-0899-2.

Beadell JS., Atkins C., Cashion E., Jonker M., Fleischer RC. 2007. Immunological change in a parasite-impoverished environment: Divergent signals from four island taxa. *PLoS ONE* 2. DOI: 10.1371/journal.pone.0000896.

Bensch S., Åkesson S. 2003. Temporal and Spatial Variation of Hematozoans in Scandinavian Willow Warblers. *Journal of Parasitology* 89:388–391. DOI: 10.1645/0022-3395(2003)089[0388:TASVOH]2.0.CO;2.

Bensch S., Hellgren O., Pérez-Tris J. 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. DOI: 10.1111/j.1755-0998.2009.02692.x.

Bensch S., Stjernman M., Hasselquist D., Orjan O., Hannson B., Westerdahl H., Pinheiro RT. 2000. Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings of the Royal Society B: Biological Sciences* 267:1583–1589. DOI: 10.1098/rspb.2000.1181.

Borner J., Pick C., Thiede J., Kolawole OM., Kingsley MT., Schulze J., Cottontail VM., Wellinghausen N., Schmidt-Chanasit J., Bruchhaus I., Burmester T. 2016. Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. *Molecular Phylogenetics and Evolution* 94:221–231. DOI: 10.1016/j.ympev.2015.09.003.

Brown CR., Komar N., Quick SB., Sethi RA., Panella NA., Brown MB., Pfe M., Pfeffer M. 2001. Arbovirus infection increases with group size. *Proc R Soc Lond B Biol Sci* 268:1833–1840. DOI: 10.1098/rspb.2001.1749.

Clark NJ., Clegg SM., Lima MR. 2014. A review of global diversity in avian haemosporidians (Plasmodium and Haemoproteus: Haemosporida): New insights from molecular data. *International Journal for Parasitology* 44:329–338. DOI: 10.1016/j.ijpara.2014.01.004.

Colwell RK. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. *User's Guide and application published at: http://purl.oclc.org/estimates.*

Colwell RK., Chao A., Gotelli NJ., Lin SY., Mao CX., Chazdon RL., Longino JT. 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *Journal of Plant Ecology* 5:3–21. DOI: 10.1093/jpe/rtr044.

Dobson A., Lafferty KD., Kuris AM., Hechinger RF., Jetz W. 2008. Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the United States of America* 105:11482–11489. DOI: 10.1073/pnas.0803232105.

Ericson PGP., Zuccon D., Ohlson JI., Johansson US., Alvarenga H., Prum RO. 2006. Higher-level phylogeny and morphological evolution of tyrant flycatchers, cotingas, manakins, and their allies (Aves: Tyrannida). *Molecular Phylogenetics and Evolution* 40:471–483. DOI: 10.1016/j.ympev.2006.03.031.

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

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

Galen SC., Witt CC. 2014. Diverse avian malaria and other haemosporidian parasites in Andean house wrens: Evidence for regional co-diversification by host-switching. *Journal of Avian Biology* 45:374–386. DOI: 10.1111/jav.00375.

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

Garvin MC., Schoech SJ. 2006. Hormone levels and infection of Haemoproteus danilewskyi in free-ranging blue jays (Cyanocitta cristata). *The Journal of parasitology* 92:659–662. DOI: 10.1645/GE-759R.1.

Hellgren O., Waldenström J., Bensch S. 2004. a New Pcr Assay for Simultaneous Studies of Leucocytozoon, Plasmodium, and Haemoproteus From Avian Blood. *Journal of Parasitology* 90:797–802. DOI: 10.1645/GE-184R1.

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

Jarvis A., Reuter HI., Nelson A., Guevara E. 2008. Hole-filled seamless SRTM data V4. *International Centre for Tropical Agriculture (CIAT)*:available from http://srtm.csi.cgiar.org.

Jetz W., Thomas GH., Joy JB., Hartmann K., Mooers AO. 2012. The global diversity of birds in space and time. *Nature* 491:444–448. DOI: 10.1038/nature11631.

Jetz W., Thomas GH., Joy JB., Redding DW., Hartmann K., Mooers AO. 2014. Global Distribution and Conservation of Evolutionary Distinctness in Birds. *Current Biology* 24:919–930. DOI: 10.1016/j.cub.2014.03.011.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P., Drummond A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. DOI: 10.1093/bioinformatics/bts199.

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

Kulma K., Low M., Bensch S., Qvarnström A. 2013. Malaria infections reinforce competitive asymmetry between two Ficedula flycatchers in a recent contact zone. *Molecular Ecology*

22:4591–4601. DOI: 10.1111/mec.12409.

Lachish S., Knowles SCL., Alves R., Wood MJ., Sheldon BC. 2011. Infection dynamics of endemic malaria in a wild bird population: Parasite species-dependent drivers of spatial and temporal variation in transmission rates. *Journal of Animal Ecology* 80:1207–1216. DOI: 10.1111/j.1365-2656.2011.01893.x.

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

Levin II., Zwiers P., Deem SL., Geest EA., Higashiguchi JM., Iezhova TA., Jiménez-Uzcátegui G., Kim DH., Morton JP., Perlut NG., Renfrew RB., Sari EHR., Valkiunas G., Parker PG. 2013. Multiple Lineages of Avian Malaria Parasites (Plasmodium) in the Galapagos Islands and Evidence for Arrival via Migratory Birds. *Conservation Biology* 27:1366–1377. DOI: 10.1111/cobi.12127.

Loiseau C., Harrigan RJ., Cornel AJ., Guers SL., Dodge M., Marzec T., Carlson JS., Seppi B., Sehgal RNM. 2012. First Evidence and Predictions of Plasmodium Transmission in Alaskan Bird Populations. *PLoS ONE* 7:7–11. DOI: 10.1371/journal.pone.0044729.

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

Martinsen ES., Blumberg BJ., Eisen RJ., Schall JJ. 2008. Avian hemosporidian parasites from northern California oak woodland and chaparral habitats. *Journal of wildlife diseases* 44:260–268. DOI: 10.7589/0090-3558-44.2.260.

Moens MAJ., Valkiūnas G., Paca A., Bonaccorso E., Aguirre N., Pérez-Tris J., Cotter S. 2016. Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood parasites of Andean birds. *Journal of Animal Ecology* 85:1234–1245. DOI: 10.1111/1365-2656.12550.

Moyle RG., Oliveros CH., Andersen MJ., Hosner PA., Benz BW., Manthey JD., Travers SL., Brown RM., Faircloth BC. 2016. Tectonic collision and uplift of Wallacea triggered the global songbird radiation. *Nature Communications* 7:1–7. DOI: 10.1038/ncomms12709.

Oakgrove KS., Harrigan RJ., Loiseau C., Guers S., Seppi B., Sehgal RNM. 2014. Distribution, diversity and drivers of blood-borne parasite co-infections in Alaskan bird populations. *International Journal for Parasitology* 44:717–727. DOI: 10.1016/j.ijpara.2014.04.011.

Outlaw DC., Ricklefs RE. 2014. Species limits in avian malaria parasites (Haemosporida): how to move forward in the molecular era. *Parasitology* 141:1223–32. DOI: 10.1017/S0031182014000560.

Poulin R. 2014. Parasite biodiversity revisited: Frontiers and constraints. *International Journal for Parasitology* 44:581–589. DOI: 10.1016/j.ijpara.2014.02.003.

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

Ricklefs RE., Outlaw DC., Svensson-Coelho M., Medeiros MCI., Ellis VA., Latta S. 2014. Species formation by host shifting in avian malaria parasites. *Proceedings of the National Academy of Sciences of the United States of America* 111:14816–21. DOI: 10.1073/pnas.1416356111.

Ricklefs RE., Swanson BL., Fallon SM., Martinez-Abrain A., Scheuerlein A., Gray J., Latta S.

2005. Community Relationships of Avian Malaria Parasites. *Ecological Monographs* 75:543–559. DOI: 10.1890/04-1820.

van Riper C., van Riper SG., Goff ML., Laird M. 1986. The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. *Ecological Monographs* 56:327–344. DOI: 10.2307/1942550.

Robinet C., Roques A. 2010. Direct impacts of recent climate warming on insect populations. *Integrative Zoology* 5:132–142. DOI: 10.1111/j.1749-4877.2010.00196.x.

Romero LM. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology* 128:1–24. DOI: 10.1016/S0016-6480(02)00064-3.

Scott TW., McLean RG., Bruce Francy D., Card CS. 1983. A Simulation Model for the Vector-Host Transmission System of a Mosquito-Borne Avian Virus, Turlock (Bunyaviridae) 1. *Journal of Medical Entomology* 20:625–640.

Silva-Iturriza A., Ketmaier V., Tiedemann R. 2012. Prevalence of avian haemosporidian parasites and their host fidelity in the central Philippine islands. *Parasitology International* 61:650–657. DOI: 10.1016/j.parint.2012.07.003.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. DOI: 10.1093/bioinformatics/btu033.

Svensson-Coelho M., Blake JGJG., Loiselle B a., Penrose a. SAS., Parker PG., Ricklefs RE. 2013. *Diversity, Prevalence, and Host Specificity of Avian Plasmodium and Haemoproteus in a Western Amazon Assemblage*. DOI: 10.1525/om.2013.76.1.1.1.

Svensson-Coelho M., Silva GT., Santos SS., Miranda LS., Araujo-Silva LE., Ricklefs RE., Miyaki CY., Maldonado-Coelho M. 2016. Lower Detection Probability of Avian Plasmodium in Blood Compared to Other Tissues. *Journal of Parasitology* 102:559–561. DOI: 10.1645/16-8.

Valkiunas G. 2005. *Avian malaria parasites and other haemosporidia*. Boca Raton, Florida, USA: CRC Press.

Valkiūnas G., Bairlein F., Iezhova TA., Dolnik O V. 2004. Factors affecting the relapse of Haemoproteus belopolskyi infections and the parasitaemia of Trypanosoma spp. in a naturally infected European songbird, the blackcap, Sylvia atricapilla. *Parasitology Research* 93:218–222. DOI: 10.1007/s00436-004-1071-2.

Valkiūnas G., Iezhova T a., Križanauskienė A., Palinauskas V., Sehgal RNM., Bensch S. 2008. A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *The Journal of Parasitology* 94:1395–401. DOI: 10.1645/GE-1570.1.

Valkiunas G., Kazlauskiene R., Bernotiene R., Palinauskas V., Iezhova TA. 2013. Abortive long-lasting sporogony of two Haemoproteus species (Haemosporida, Haemoproteidae) in the mosquito Ochlerotatus cantans, with perspectives on haemosporidian vector research. *Parasitology Research* 112:2159–2169. DOI: 10.1007/s00436-013-3375-6.

Waldenström J., Bensch S., Hasselquist D., Östman Ö. 2004. A New Nested Polymerase Chain Reaction Method Very Efficient in Detecting Plasmodium and Haemoproteus Infections from Avian Blood A New Nested Polymerase Chain Reaction Method Very Efficient in Detecting Plasmodium and Haemoproteus Infections From Avian B. *Journal of Parasitology* 90:191–194. DOI: 10.1645/GE-3221RN.

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

Walther EL., Carlson JS., Cornel A., Morris BK., Sehgal RNM. 2016. First molecular study of prevalence and diversity of avian haemosporidia in a central California songbird community. *Journal of Ornithology* 157:549–564. DOI: 10.1007/s10336-015-1301-7.

Warner RE. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *The Condor* 70:101–120. DOI: 10.2307/1365954.

Yohannes M., Haile M., Ghebreyesus TA., Witten KH., Getachew A., Byass P., Lindsay SW. 2005. Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Tropical Medicine and International Health* 10:1274–1285. DOI: 10.1111/j.1365-3156.2005.01512.x.

Table 1 Positive infections identified from microscopy and PCR. Total screened indicates the number of individual birds screened by each method. Of those screened, the number and proportion of individuals that were positive are reported.

		Haemoproteus/Plasmodium	*Leucocytozoon*	Overall
Method	Total screened	No. positive (%)	No. positive (%)	No. positive (%)
Microscopy	168	40 (23.8%)	7 (4.2%)	43 (25.6%)
PCR	186	55 (29.6%)	25 (13.4%)	65 (34.9%)

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Figure 1 Map of study areas and 2016 sampling localities. Fieldwork was conducted in three sites located in piñon-juniper and ponderosa pine woodland habitats (elevational range: 2150-2460 meters). The number of individuals found positive for *Haemoproteus* (H), *Plasmodium* (P), and *Leucocytozoon* (L) is shown. Elevation is based on the SRTM Digital Elevation Database (Jarvis et al., 2008).

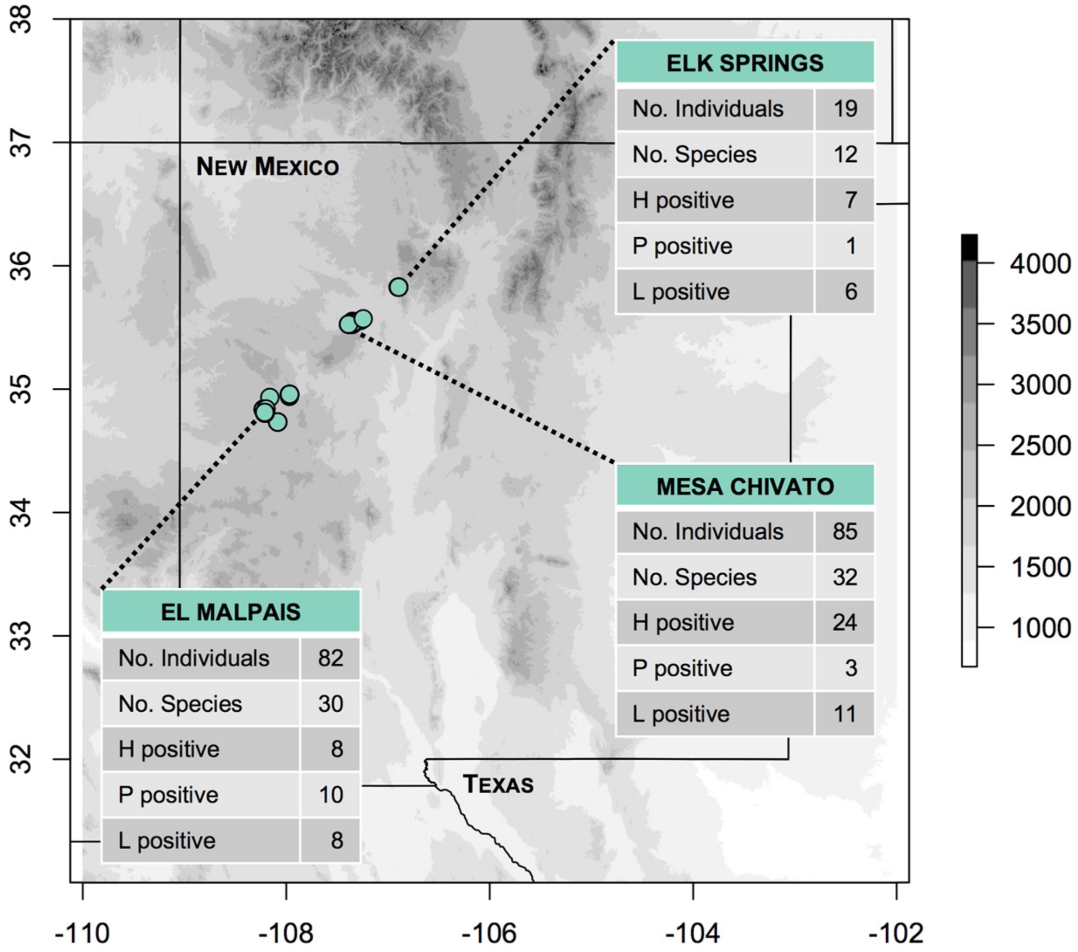

Figure 2 Phylogeny of haemosporidian haplotypes found in New Mexico birds. Columns represent host clades (left) and host species (right). Dotted line indicates non-monophyly of non-passerines, and clade names are based on the names and topology from Moyle et al. (2016). Host species phylogeny was generated from BirdTree.org and the colors of host clade branches correspond to host species in each clade. Bar plots depict the proportion of individuals infected for each parasite genus: *Haemoproteus* (turquoise), *Plasmodium* (dark brown), *Leucocytozoon* (light brown). Stars indicate novel malaria haplotypes. The parasite phylogeny was estimated in RAxML and branch labels indicate bootstrap values. The table is shaded to indicate which clade/species was infected with each lineage and the number of birds infected.

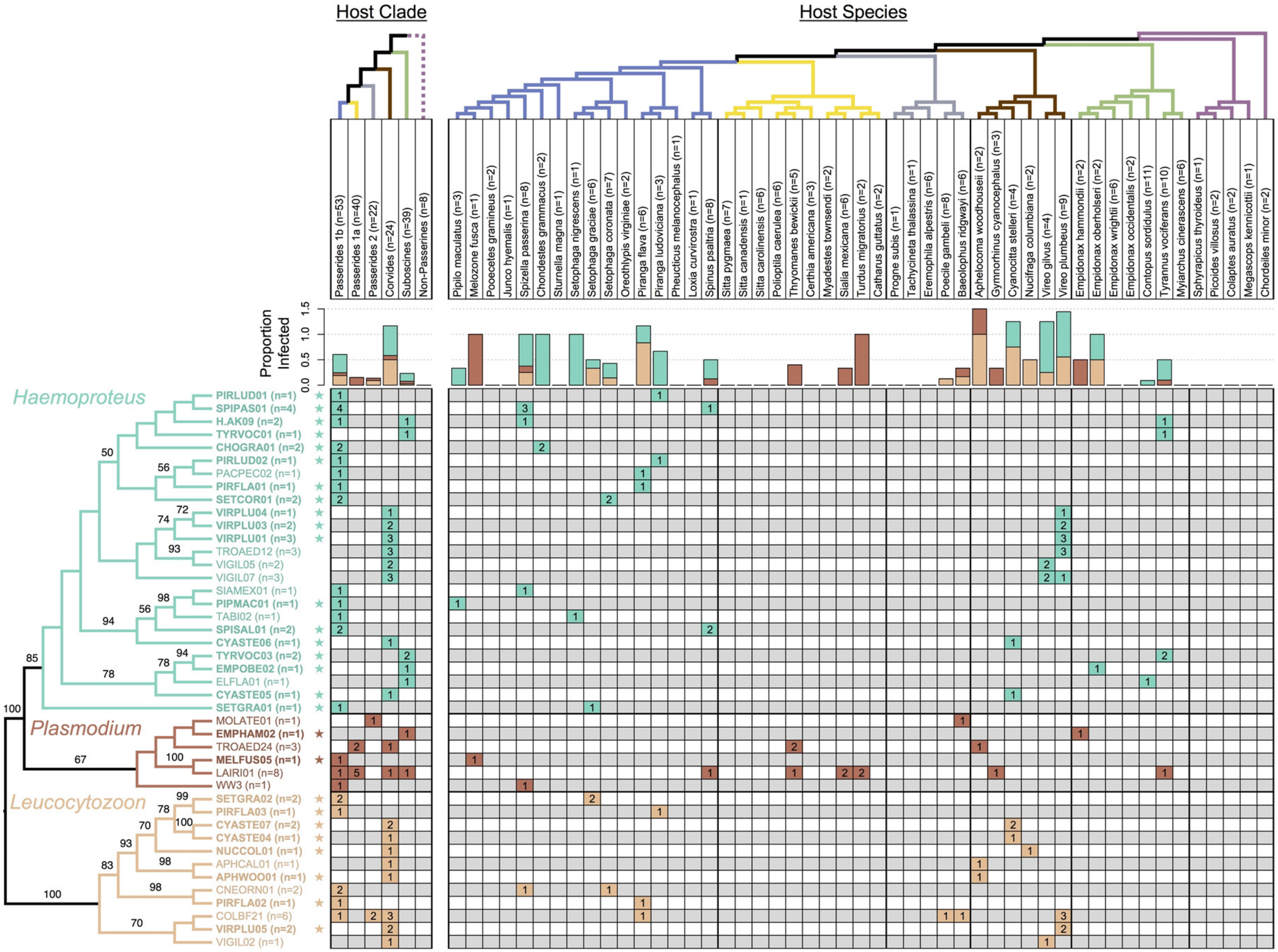

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Figure 3 Estimate of malaria lineage diversity in northern New Mexico based on EstimateS rarefaction and extrapolation using 77 avian malaria infections and 43 distinct haplotypes. The point indicates the reference sample, solid line the rarefaction, and dotted line the extrapolation. The analysis suggests that sampling approximately 240 total infections would capture > 95% of the haemosporidian lineage diversity in this community. The total haplotype richness is estimated to be 70 (95% CI: 43–98).