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Diversity and host assemblage of avian haemosporidians in different terrestrial ecoregions of Peru

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Abstract

Characterizing the diversity and structure of host–parasite communities is crucial to understanding their eco-evolutionary dynamics. Malaria and related haemosporidian parasites are responsible for fitness loss and mortality in bird species worldwide. However, despite exhibiting the greatest ornithological biodiversity, avian haemosporidians from Neotropical regions are quite unexplored. Here, we analyze the genetic diversity of bird haemosporidian parasites (*Plasmodium* and *Haemoproteus*) in 1,336 individuals belonging to 206 bird species to explore for differences in diversity of parasite lineages and bird species across 5 well-differentiated Peruvian ecoregions. We detected 70 different haemosporidian lineages infecting 74 bird species. We showed that 25 out of the 70 haplotypes had not been previously recorded. Moreover, we also identified 81 new host–parasite interactions representing new host records for these haemosporidian parasites. Our outcomes revealed that the effective diversity (as well as the richness, abundance, and Shannon–Weaver index) for both birds and parasite lineages was higher in Amazon basin ecoregions. Furthermore, we also showed that ecoregions with greater diversity of bird species also had high parasite richness, hence suggesting that host community is crucial in explaining parasite richness. Generalist parasites were found in ecoregions with lower bird diversity, implying that the abundance and richness of hosts may shape the exploitation strategy followed by haemosporidian parasites. These outcomes reveal that Neotropical region is a major reservoir of unidentified haemosporidian lineages. Further studies analyzing host distribution and specificity of these parasites in the tropics will provide important knowledge about phylogenetic relationships, phylogeography, and patterns of evolution and distribution of haemosporidian parasites.

Key words: Amazonia, avian malaria, generalist parasite, habitat specificity, *Haemoproteus*, *Plasmodium*, specialist parasite

The study of biodiversity (defined as species, genetic, and ecosystem diversity in an area) has recently gained relevance in community ecology and conservation research because of its economic importance and its essential role in the functioning of ecosystems (Wall et al. 2015; Butchart et al. 2016; Perrigo et al. 2020; Rana et al. 2020). In this sense, one of the major goals for the scientific community is to identify mechanisms that produce and maintain biological diversity in ecosystems through ecological and evolutionary studies (e.g., Kondratyeva et al. 2019). Interactions between organisms have been proposed as one of the major drivers shaping the distribution and abundance of species (Thomas et al. 2005). However, most of the studies on this topic have focused on competition and predation as the main determinants driving species diversity, whereas parasites have comparatively received less attention (Thomas et al. 2005). This is particularly striking given the abundance, ubiquity, and extraordinary diversity of parasites (Antao 2011; Poulin and Randhawa 2015).

Avian malaria and related haemosporidians (genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) are diverse and abundant protozoan parasites, with more than 4,100 parasite lineages infecting more than 1,900 species representing most bird clades (MALAVI database version 2.4.7, October 2020, Bensch et al. 2009). They are transmitted by hematophagous dipteran vectors, the definitive hosts (Valkiūnas 2005; Santiago-Alarcon et al. 2012). These blood parasites exert pathogenic effects on their avian host by provoking tissue damage (Ilgunas et al. 2019), reducing their survival (Marzal et al. 2005; Martínez-De La Puente et al. 2010; Asghar et al. 2015) and decreasing their reproductive success (Merino et al. 2000; Marzal et al. 2005), thus decimating host populations and eventually being responsible for extinctions after their introduction beyond their natural range (Lapointe et al. 2012; Marzal and Garcia-Longoria 2020). One of the most interesting features of haemosporidian parasites is their high variability in host specificity and parasite exploitation strategies. In this sense, it has been shown that haemosporidians may range from infecting a small number of related host species (specialist parasite) to a wide range of unrelated bird species (generalist parasite) (Clark et al. 2015; Moens and Pérez-Tris 2016). Moreover, it has also been reported that the same parasite lineage may change its parasite exploitation strategy depending on the community biodiversity (Garcia-Longoria et al. 2019).

Although avian haemosporidians are present in almost all geographical regions, these parasites have been irregularly studied across different biogeographical regions (Valkiūnas 2005). This is especially apparent in the case of haemosporidians of Neotropical birds, which have received comparatively less attention than bird malaria parasites from temperate regions (González et al. 2015; Lotta et al. 2019; Santiago-Alarcon and Marzal 2020). Although the Neotropical region exhibits the greatest ornithological biodiversity with 3,578 bird species described (BirdLife 2020), only 969 avian haemosporidian lineages have been recorded in this region (MALAVI database version 2.4.4, 24 March 2020, Bensch et al. 2009). These records are remarkably lower than numbers from temperate regions such as Europe and North America, with 604 and 643 haemosporidian lineages infecting 550 and 643 bird species, respectively (BirdLife 2020; MALAVI database version 2.4.4, 24 March 2020, Bensch et al. 2009). Moreover, due to the greater diversity of parasites in the tropics (Dobson et al. 2008; Møller et al. 2009), these differences in the number of bird haemosporidian parasites between biogeographical regions should be even greater.

Ecoregions are defined as large areas with exceptional biodiversity and representative communities (Noss 1992). These areas contain distinct assemblages of natural communities and species (Olson et al. 2001; Dinerstein et al. 2017), thus providing a unique framework for the identification of species clusters (e.g., host–parasite interactions) and comparisons of biodiversity among units. Peru exhibits high species richness of vertebrates, invertebrates, and

plants, and remarkable levels of endemism at the species level and also higher taxa (e.g., genera and families). In fact, 19 out of the 867 terrestrial ecoregions recognized in the world are present in this Neotropical country (Olson et al. 2001). Moreover, Peru also has about 84 out of the 104 existing ecosystems and 28 out of the 32 climates on the planet, which has classified Peru as 1 of the 17 megadiverse countries in the world (Williams et al. 2001). About 1,870 bird species have been identified in Peru, representing 20% of the global avian diversity and more than 62% of the bird species richness in South America (Plenge 2020). Because of their high degree of endemism or their risk of extinction, many of these bird species are considered a priority in conservation policies (Schulenberg et al. 2010). However, notwithstanding the huge number of bird species present in this tropical country, very few studies have explored the genetic diversity of its bird malaria parasites (see Fecchio et al. [2018a] and Galen and Witt [2014] for some exceptions), identifying in its ornithofauna only 4.4% of the known avian haemosporidian parasites (MALAVI database version 2.4.4, 24 March 2020, Bensch et al. 2009). Moreover, these studies have revealed that, among the identified parasite lineages infecting Peruvian birds, there is a high number of previously unidentified haemosporidian lineages, representing about 30–100% of overall recorded blood parasites (Marzal et al. 2015; Fecchio et al. 2019). These outcomes suggest that a great part of Peruvian haemosporidian diversity remains unexplored.

Here, we present a molecular-based study to explore the infection by haemosporidian parasites in more than 200 bird species from Peru. Because the presence of haemosporidian parasites may vary according to abiotic and biotic factors determining vector abundance (Lalubin et al. 2013) and bird community composition (Fecchio et al. 2018b; Pulgarín-R et al. 2018), we analyzed more than 1,300 birds from 5 Peruvian ecoregions to explore host–parasite assemblages and to determine differences in diversity of parasite lineages and bird species across ecoregions.

Material and Methods

Data collection and database

The study was conducted in Neotropical localities from 5 different ecoregions in Peru (Olson et al. 2001; Dinerstein et al. 2017): 1) Sechura desert (Eco ID: 608), located on the coastal plain, it extends inland for over 100 km to the foothills of the Andes Mountains; it is characterized with xeric shrublands. 2) Peruvian Yungas (Eco ID: 493), characterized by tropical and sub-tropical montane deciduous and evergreen forests which flank the eastern slopes and central valleys of the central Andes from northernmost to southernmost Peru. 3) Ucayali moist forests (Eco ID: 512), a tropical forest ecoregion located at the foot of the Andes mountains which includes the basin west of the Ucayali river, a major tributary of the Amazon River. 4) Iquitos várzea (Eco ID: 469), seasonally flooded forests in the west of the Amazon biome, and 5) Southwest Amazon moist forests (Eco ID: 505), located in the Upper Amazon basin and characterized by a relatively flat landscape with alluvial plains dissected by undulating hills or high terraces (see Supplementary Table S1 for detailed locations and sampling years).

From 2012 to 2018, 1,336 adult birds belonging to 206 different species were captured and sampled. Most of the individuals ($N = 1{,}170$) were captured in their natural habitats using mist-nets. Additionally, we sampled 150 local psittacines that were recently seized from illegal wildlife trade by Autoridad Regional Ambiental (ARA) and Servicio Nacional Forestal y de Fauna Silvestre (SERFOR) in San Martín region. Moreover, we also analyzed blood parasites from 16 local wild birds kept in captivity in Quistococha zoo. All these additional samples (from the illegal wildlife trade recovery and the zoo) were not included in the comparison analyses among ecoregions, and were exclusively studied to report parasite diversity. From each individual, we took a blood sample (10–30 µL, according to its body size) in heparinized microcapillaries by puncturing the brachial vein and stored in SET-buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) until molecular analysis.

Detection and characterization of parasite lineages

Blood samples were examined using molecular methods to determine the presence and genetically characterize *Haemoproteus* and *Plasmodium* parasite lineages (Waldenström et al. 2004). DNA from blood samples was extracted using the GeneJETTM Genomic DNA Purification Kit (Thermo Scientific Inc., reference #K0722). Extracted DNA was diluted to 25 ng/µL and used as a template in a nested polymerase chain reaction (PCR) assay for detection of parasites following the protocols described by Waldenström et al. (2004). All PCR assays contained 1 negative control for every 8 samples, and 2 positive controls for every 82 samples. The amplification was evaluated by running 2.5 µL of the final PCR on a 2% agarose gel. Haemosporidians detected by a positive amplification were sequenced using the procedures described by Bensch et al. (2000). The obtained sequences of 478 bp of the cyt b were edited, aligned, and compared in a sequence identity matrix using the programs BioEdit (Hall 1999) and Geneious (Kearse et al. 2012). Finally, the aligned sequences were blasted against the MalAvi database (Version 2.4.4, March 2020; Bensch et al. 2009) in order to identify parasite lineages. Parasites with sequences differing by 1 nucleotide substitution were considered to represent evolutionarily independent lineages (Bensch et al. 2000). New lineages (sequences not previously published in GenBank) were also sequenced from the reverse end using the primer HaemR2 to confirm that they were unique. All new DNA sequences have been deposited in GenBank (see Supplementary Table S2). New host–parasite relationships were established by comparison of our results with public database (MalAvi database Version 2.4.5, 11 May 2020, Bensch et al. 2009) showing avian haemosporidians lineages (based on mitochondrial cytochrome b lineages) and host range. A new host record was established when the avian haemosporidian lineage has not been previously reported infecting this bird species in previous studies.

Some individuals showed haemosporidian mixed infection (identified as "double base calling" in the electropherogram). Because PCR-based assays underestimates the number of lineages found in mixed infections of the genera *Haemoproteus* and *Plasmodium* (Valkiūnas et al. 2006; Bernotienė et al. 2016; Ciloglu et al. 2019), only individuals showing a single infection were included in our analyses to accurately identify haemosporidian lineages.

Parasite lineage and avian diversity indices

Several estimates of species diversity are used in the wide range of literature on biological diversity. Based on the number of birds handled, we calculated richness and abundance for both parasite lineages and avian species for our 5 sampling ecoregions in Peru. In this study, we will refer to species richness as the total number of species per ecoregion, and abundance as the number of individuals found in a particular ecoregion (see Mittelbach 2012). Because species richness is highly correlated with sample size, we used the statistical method of rarefaction (Sanders 1968; Heck et al. 1975) to

estimate the number of species expected from a sample in a sub-sample of a given number of individuals, thus, reducing the effect of differential sampling intensity on diversity estimates. The "rarefy" function was used to compute rarefied species diversity and standard error for each ecoregion. We used counts for each parasite lineage as individual-based abundance data regardless of host species identity, which should result in a conservative estimate of species richness. We used Pielou's evenness to compare the actual diversity value (such as the Shannon–Weaver index) to the maximum possible diversity value. In this way, we assess the homogeneity or evenness of a community based on the abundances of its species, which may vary among communities in ways that correlate with biotic or abiotic differences (see Mittelbach 2012). Finally, since not all host and parasite taxa had an equal abundance of individuals, we calculated the effective (true) diversity (also known as diversity numbers), which refers to the number of equally abundant species needed to obtain the same mean proportional species abundance as that observed in our dataset (Jost 2006).

Phylogenetic analyses

Each of the avian phylogenetic trees created in this study relied on 1,000 trees generated by the birdtree.org website (Jetz et al. 2012). A consensus tree was generated with all the bird species detected, as well as a bird phylogenetic tree for each ecoregion by using Geneious v5.4 (Kearse et al. 2012) (see Figure 3A–E). The parasite tree relied on the sequences of the parasite lineages molecularly identified in this study. Then, we created a phylogenetic tree for parasites using Mega7 v 7.0.26 (Kumar et al. 2016) by using a maximum-likelihood method with a bootstrap of 1,000. Since we detected parasites from the *Haemoproteus* and *Plasmodium* genera, we used *Haemoproteus tartakovsky* (Bensch et al. 2016*)* and *Plasmodium gallinaceum* (Böhme et al. 2018) as outgroups for each tree, respectively. Both bird and parasite trees were edited by using the software R studio (3.6.3., 2020) and the libraries ggplot (Wilkinson 2011) and ggtree (Yu et al. 2017).

Statistical analyses

All calculations and computations were conducted in the R language (3.6.3., R Core Team 2020). Scripts developed for all analyses can be obtained from the corresponding authors. Specifically, script for the characterization of specialist or generalist parasite species can be found at https://gist.github.com/Trinity2018/be8465347ced30200828434fd4ac863d. All calculations about parasite lineage and avian diversity indices (richness, abundance, rarefaction, Shannon–Wiener index, evenness and effective diversity) were performed using the vegan package (Oksanen et al. 2017) for R. Additionally, we used generalized linear mixed models (GLMMs, Poisson distribution, R package lme4) (Bates et al. 2015) to analyze if parasite lineage richness was affected by the avian species richness for the 5 sampled ecoregions. In these models on parasite lineage richness, we used avian species richness as an explanatory variable, and the sampling year was included as a random factor.

The characterization of specialist or generalist parasite species may not exclusively rely on the number of infected bird species by the same parasite. In fact, the analyses of phylogenetic distances between bird species infected by the same parasite lineages are also required to determine parasite strategies and how specialist and generalist parasites exploit different host species (Svensson-Coelho et al. 2016; Garcia-Longoria et al. 2019). Thus, with the aim to differentiate between parasites lineages infecting closely and distantly related bird species, we explored phylogenetic distances of bird species infected by the same parasite lineage, while controlling for phylogenetic relationships among bird species from the same ecoregion. We carried out these analyses 5 times (1 per ecoregion); hence, taking into account the specific infection that each parasite lineage could have on each area. Calculations were carried out with the *ses.mpd* function of the R package Picante (Kembel et al. 2010). These analyses provide different values that may determine the phylogenetic mean pairwise distance (MPD). MPDobs defines the observed mean pair-wise phylogenetic distance between all species pairs infected with the same parasite lineage. The mean and standard deviation of MPD in the null distribution were obtained by randomization of species in the phylogenetic distance matrix (*taxa.labels* method in Picante). *Z*-value was calculated following this formula (MPDobs—mean MPD of the null distribution)/SD of the null distribution, which indicates the level of phylogenetic clustering. Negative values of *Z* indicate greater phylogenetic homogeneity (clustering). The significance of this metric was tested by comparison to a null distribution derived from 999 random permutations among the tips of the phylogenetic tree followed by calculation of the MPD. *P* is the probability of drawing an MPD from the null distribution at least as extreme as MPDobs. *P*-values higher than 0.05 denote a specialist parasite lineage, whereas *P*-values equal or lower 0.05 show a generalist parasite lineage.

Results

Parasite lineages detected

A total of 1,336 bird individuals (1,170 from wild populations and 166 from captivity) belonging to 206 bird species were screened for haemosporidian parasites (Supplementary Figure S1 and Supplementary Table S2). We detected 70 different parasite lineages infecting 74 bird species in our 5 selected Peruvian ecoregions (Table 1, Figure 1A,B, and Supplementary Table S2). More specifically, we found a total of 46 *Plasmodium* lineages infecting 41 bird species and 24 *Haemoproteus* lineages infecting 19 bird species, whereas 14 bird species harbored both *Plasmodium* and *Haemoproteus* lineages (Figure 1A,B and Supplementary Table S2 and S3).

By comparison of genetic diversity of haemosporidian parasites, we showed that 25 out of the 70 haplotypes from our study had not been previously recorded in former studies (Supplementary Table S3). Moreover, we found that 21 out of 206 bird species analyzed in this study had not been previously documented as infected by haemosporidian parasites in molecular studies (Supplementary Table S2). Furthermore, we also identified 81 new host–parasite interactions, which represent new bird host records for these haemosporidian parasites (Supplementary Table S2).

Estimates of parasite lineage and bird diversity

We analyzed all diversity estimators among different ecoregions. We found a similar pattern in richness, abundance, rarefaction, and Shannon–Weaver index for both parasite lineages and bird species. Specifically, these diversity estimators showed lower values in the Sechura desert and Peruvian Yungas ecoregions than in ecoregions from Amazon basin (Ucayali moist forests, Iquitos várzea, and Southwest Amazon moist forests) (Table 2 and Figure 2). Effective diversity of birds and parasite lineages was positively correlated among ecoregions ($R^2 = 0.3658$) (Figure 2). By analyzing the homogeneity of the community of parasite lineages and birds, the Sechura desert and Peruvian Yungas ecoregions showed the highest evenness

Table 1. Number of bird species, infected species, sampled bird individuals, and infected individuals are given for each ecoregion

Ecoregions	*N* bird species	*N* infected bird species	*N* individuals	*N* infected individuals	*N* parasite lineages
Peruvian Yungas	11	7	75	26	3 P + 5 H
Sechura desert	18	10	238	32	4 P + 4 H
Iquitos várzea	102	30	358	53	18 P + 7 H
SW Amazon moist forests	67	12	275	24	11 P + 2 H
Ucayali moist forests	73	29	390	71	21 P + 12 H

Data on the number of parasite lineages per *Haemosporidian* genera are shown (P = *Plasmodium*, H = *Haemoproteus*).

values, thus indicating that these ecoregions had more even communities than the Amazon basin ecoregions, where the parasite lineages and bird species were less equally distributed (Table 2). Similarly to previous diversity estimators, the effective diversity was higher for Ucayali moist forests, Iquitos várzea and Southwest Amazon moist forests than for Sechura desert and Peruvian Yungas ecoregions (Table 2). We also checked if the 5 ecoregions had distinct parasite lineage richness when controlling for the effect of avian species richness, showing that haemosporidian lineage diversity was positively affected by bird richness (estimate ± SE = 296 0.031 ± 0.004, $Z = 6.876$, $P < 0.001$).

Infected bird species and phylogenetic distances

In the 5 sampled ecoregions, we found 50 parasite lineages infecting one bird species, whereas 20 parasite lineages infected 2 or more bird species (Figure 3A–E).

Random effects for parasite lineages showed that there are considerable differences among parasite lineages in their host exploitation strategies. In all, we found that most of the parasite lineages (70% of parasite lineages infecting more than one bird species) followed a generalist strategy when infecting birds from the 5 ecoregions (Table 3A–E). Our results showed that the only parasite lineage infecting more than one bird species in Peruvian Yungas ecoregion, *Plasmodium relictum* (SGS1), was identified as a generalist parasite, infecting 5 phylogenetically distant host species (Table 3A). In Sechura desert ecoregion, 3 parasite lineages (*Haemoproteus* sp. OCHLEU01, *P. relictum* SGS1, *Haemoproteus passeris* PADOM05) were also characterized as generalist parasites because they were found infecting more than one phylogenetically distant host species and the *P*-value supported this characterization (Table 3B). In the Iquitos Várzea ecoregion, our analyses revealed that 2 out of 6 haemosporidian lineages were identified as specialists: *Plasmodium* sp. DENPET03, because it was detected infecting 10 host species, but these bird species were phylogenetically closely related; and *Plasmodium* sp. RAMCAR01, which infected 2 closely related bird species (Table 3C). In Southwest Amazon moist forests, we only found one haemosporidian lineage infecting more than one host species (*Plasmodium* sp. SCHISME02), which was identified as a specialist parasite because it infected 2 closely related species (Table 3D). Finally, in the Ucayali moist forests, we identified 3 out of 8 parasite lineages (*Plasmodium* sp. VOLJAC02, *Plasmodium* sp. RAMCAR01, and *P. relictum* GRW04) as specialist parasite lineages (Table 3E). These haemosporidian parasites were found infecting 2 and 4 phylogenetically closely related bird species, respectively.

Z-scores (Table 3) and effective diversity (Table 2) values were plotted in order to visually identify parasite strategies and abundance of these strategies depending on the ecoregion (Figure 4). Ecoregion with low effective diversity values (Sechura desert and Peruvian Yungas) showed exclusively parasite lineages following a generalist strategy, while ecoregions with high effective diversity values (Ucayali moist forest, SW Amazon moist forest, and Iquitos Várzea) showed both generalist and specialist parasite lineages (Figure 4).

Discussion

Here, we present one of the largest investigations analyzing the genetic diversity of haemosporidian parasites in the Neotropics, showing a great diversity of malaria parasites in birds from 5 ecoregions of Peru. Importantly, we compared our sequences with those in MalAvi database (Version 2.4.5, 11 May 2020; Bensch et al. 2009) and discovered that 35% of the haemosporidian lineages we found had not been obtained previously in other studies. Moreover, 22 of the infected bird species had not been: either previously screened for haemosporidians, or found to harbor malaria parasites in preceding studies. Furthermore, our efforts revealed more than 80 new bird–parasite interactions, thus identifying new host records for these haemosporidian parasites (see Figure 1A,B and Supplementary Tables S2 and S3). However, amplification of parasite DNA by PCR does not distinguish gametocytes from asexual parasite stages (Valkiūnas and Atkinson 2020). Hence, we cannot discard that some of the new records on haemosporidian infections in our study could be abortive infections, as it has been experimentally shown for some haemosporidian parasites (Dimitrov et al. 2015; Valkiūnas et al. 2018). Results from further studies exploring the presence of gametocytes circulating in peripheral blood are needed to complement our findings and determine the competence of these avian hosts of supporting development of infective stages that can reach a new host.

Though the lack of replicates in our sampling design does not allow us to carry out statistical analyses to compare diversity estimators between ecoregions, our results clearly show that the effective diversity (as well as the richness, abundance, and Shannon–Weaver index) for both birds and parasite lineages in the Sechura desert and Peruvian Yungas, presented lower values than those found in ecoregions from the Amazon basin (Iquitos várzea, Southwest Amazon moist forests, and Ucayali moist forests) (Table 2 and Figure 2). Biotic and abiotic factors can determine the variation of parasitic infections in space and time (Higgs and Beaty 2005), and complex environmental factors limit the diversity and abundance of haemosporidians, their hosts, and vectors (Renner et al. 2016). Hence, the observed differences in diversity could be due to the fact that these Amazon basin ecoregions are mostly dominated by evergreen rainforests with consistent temperatures (ranging 26–28°C) and rainfall (2,000–4,000 mm over at least a 9-month period), yielding greater vegetation cover that provides high humidity (Mouchet et al. 2008). The Peruvian Yungas and Sechura desert, on the other hand, are drier and exhibit more variable temperatures, showing completely different biotic and abiotic characteristics (Brack 1986; Rundel et al.

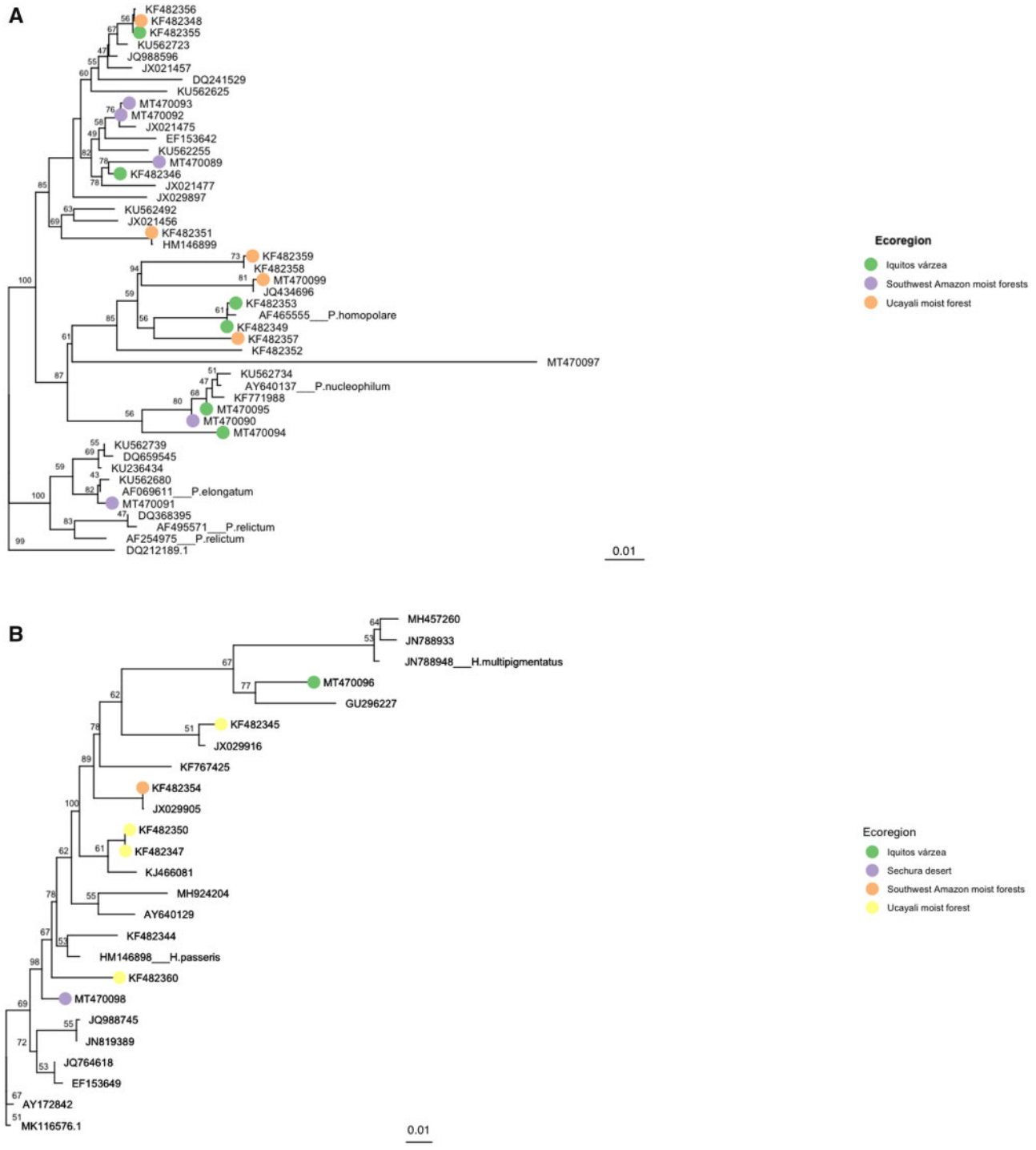

Figure 1. Phylogenetic trees from (**A**) *Plasmodium* and (**B**) *Haemoproteus* lineages detected in the present study. Node numbers indicate bootstrap values. New parasite lineages detected in our study are colored marked depending on the ecoregion they were found.

2007; Dinerstein et al. 2017). Those ecological factors linked to higher vegetation cover in Amazon basin are positively related to the availability of breeding habitats for blood-sucking vectors (Rubio-Palis and Zimmerman 1997; Roiz et al. 2015), which would favor a higher diversity of malaria parasite lineages in these ecoregions. Moreover, an increase in vector abundance and diversity could be related to a higher prevalence of vector-borne parasites in birds (Sol et al. 2000; Martínez-De La Puente et al. 2013). This heterogeneous distribution of avian haemosporidians has also been described across various Brazilian biomes (Lacorte et al. 2013; Villar et al. 2013; Roos et al. 2015). Evenness of abundance in bird communities is associated with biological features of the component species (e.g., communities with more species, with smaller species, and with a greater variation in body mass have more even abundance distributions) and with the environment (e.g., communities occurring in more complex habitats, such as tropical forests, being more even in

Table 2. Estimates of A) parasite lineage diversity and B) avian diversity based on the analyzed birds on each of the 5 ecoregions sampled in Peru

	Ecoregions	Richness	Abundance	Rarefaction ± SE	Shannon–Weaver index	Evenness	Effective diversity
A) Parasite lineages	Peruvian Yungas	8	27	7.68 ± 0.50	1.721	0.374	5.591
	Sechura desert	8	33	6.94 ± 0.84	1.492	0.322	4.448
	Iquitos várzea	25	54	14.63 ± 1.64	2.788	0.279	16.244
	Southwest Amazon moist forests	13	25	13.00 ± 0.00	2.361	0.343	10.609
	Ucayali moist forests	33	72	15.85 ± 1.75	3.035	0.259	20.805
B) Bird species	Peruvian Yungas	11	75	11.00 ± 0.00	1.942	0.340	6.976
	Sechura desert	18	238	12.84 ± 1.48	1.825	0.242	6.205
	Iquitos várzea	102	358	42.03 ± 3.23	4.014	0.209	55.617
	SW Amazon moist forests	67	275	35.71 ± 2.87	3.594	0.226	36.408
	Ucayali moist forests	73	390	31.02 ± 2.97	3.123	0.201	22.721

All ecoregions were rarefied to $N = 25$ lineages and 75 host individuals.

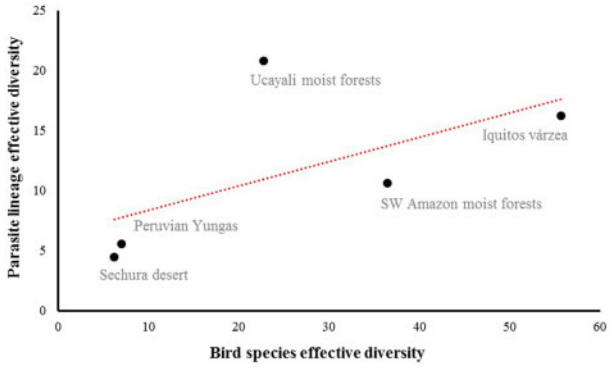

Figure 2. Scatterplot showing the relationship between the effective (true) diversity index for bird species (*x*-axis) and parasite lineages (*y*-axis) in our 5 Peruvian sampling ecoregions.

their abundance distributions) (Cotgreave and Harvey 1994). In this sense, our results suggest that individuals are more evenly distributed across the different bird species in the Sechura desert and Peruvian Yungas than in the other 3 ecoregions located east of the Peruvian Andes, possibly owing to the higher diversity of birds found in Amazon basin ecoregions. Evenness is, therefore, an important factor to consider when analyzing communities since it may give a general sense about how species are distributed among ecoregions (Bulla 1994). Our outcomes also revealed that the bird evenness index is important in bird–haemosporidian studies since it could be directly related with the diversity and abundance of malaria parasite lineages (Fecchio et al. 2018a). However, regarding malaria parasite evenness, we found that different lineages were more evenly distributed in Southwest Amazon moist forests, in addition to the Sechura desert and Peruvian Yungas. On the contrary, Iquitos várzea and Ucayali moist forests revealed a less homogeneous parasite community, which could be related to the parasite strategies (specialists–generalists) in heterogeneous host communities (Jones et al. 2018). Regarding the composition and richness of parasite communities, we have obtained similar results to those shown by Lacorte et al. (2013), who found the same pattern for both bird and parasite communities. These outcomes suggest that, probably due to co-evolutionary processes, the composition of a particular bird community may determine both the composition and diversity of haemosporidian lineages in a certain area (Fecchio et al. 2018b), which also are different from each other, as we observed. In addition, our GLMM analysis had also revealed that ecoregions with a greater diversity of bird species also had higher parasite richness, which is in agreement with previous studies showing that the vertebrate host community is crucial in explaining parasite richness (Keesing et al. 2006; Lacorte et al. 2013; Illera et al. 2017; Ferraguti et al. 2018).

Nevertheless, haemosporidian prevalence and richness may not be exclusively driven by climate factors and host richness, but may also be shaped by host-switching and dispersal limitations (Ellis et al. 2015; Fecchio et al. 2018a), and by factors related to vector ecology (Pérez-Rodríguez et al. 2013; Gutiérrez et al. 2019), such as feeding patterns of vectors (Ferraguti et al. 2013) and host compatibility (Medeiros et al. 2013). Moreover, we should also be aware that, despite the lack of marked seasonality in the tropics (implying favorable conditions for a year-round vector availability, and hence parasite transmission), some environmental factors associated with dry and wet seasons (e.g., temperature and precipitation) could govern the presence, distribution, and host specificity of avian hemoparasites (Fecchio et al. 2019; Chapa-Vargas et al. 2020). In addition, hormonal changes during breeding season or other type of stressors may trigger relapses of haemosporidian chronic infections during the bird-breeding season (Valkiūnas 2005; Santiago-Alarcon et al. 2020). Because in our study we did not control for seasonality in all localities, our outcomes hence may not represent the true diversity of the ecoregions. Further studies exploring both prevalence and diversity of vector and haemosporidians across seasons in these ecoregions would be desirable to provide new insights on this topic.

Our outcomes in phylogenetic analyses revealed a compelling pattern regarding bird diversity indices and exploitation parasite strategies. In the Sechura desert and Peruvian Yungas ecoregions, where the lowest diversity of birds were found, we also showed that all the parasite lineages infecting more than one host species were classified as generalist parasites (able to infect phylogenetic distantly related host species). On the contrary, in Amazon basin ecoregions (Iquitos várzea, Southwest Amazon moist forests, and Ucayali moist forests), where higher richness and abundance of bird species were found, we showed both generalist and specialist parasite strategies (Table 3 and Figure 4). The degree of specialization is a key aspect with profound ecological and evolutionary consequences in host–parasite interactions (Moens et al. 2016; Fecchio et al. 2019; Ellis et al. 2020). Because predictability may increase parasite transmission and survival (Šimková et al. 2006), the resource predictability hypothesis suggests that parasites should specialize on hosts that are predictable (e.g., abundant and/or permanent) in space and time (Combes 2001; Krasnov et al. 2006). Since avian haemosporidians are transmitted by dipteran vectors, adopting a generalist strategy will enhance parasite transmission because the likelihood of

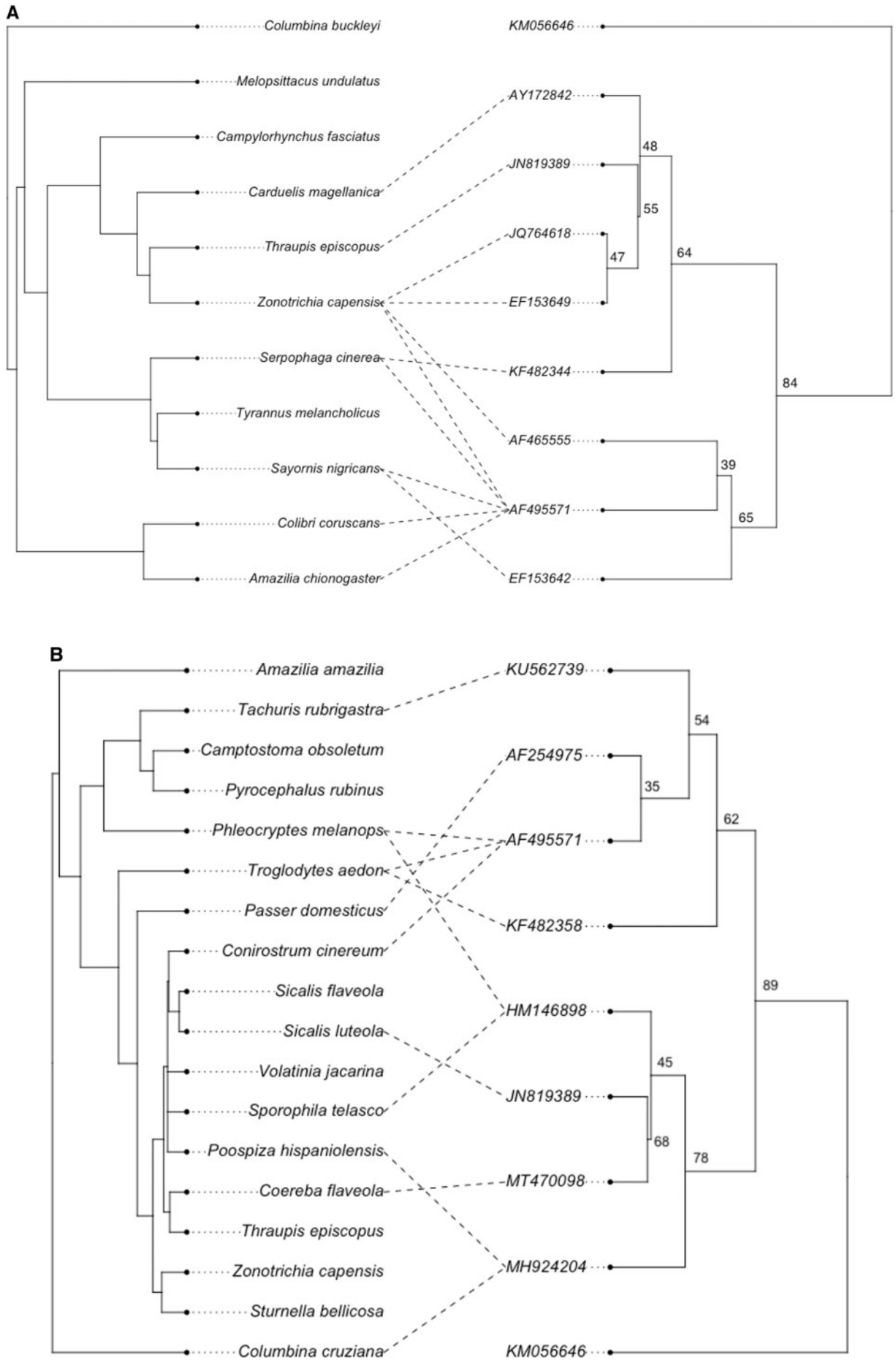

Figure 3 (continued)

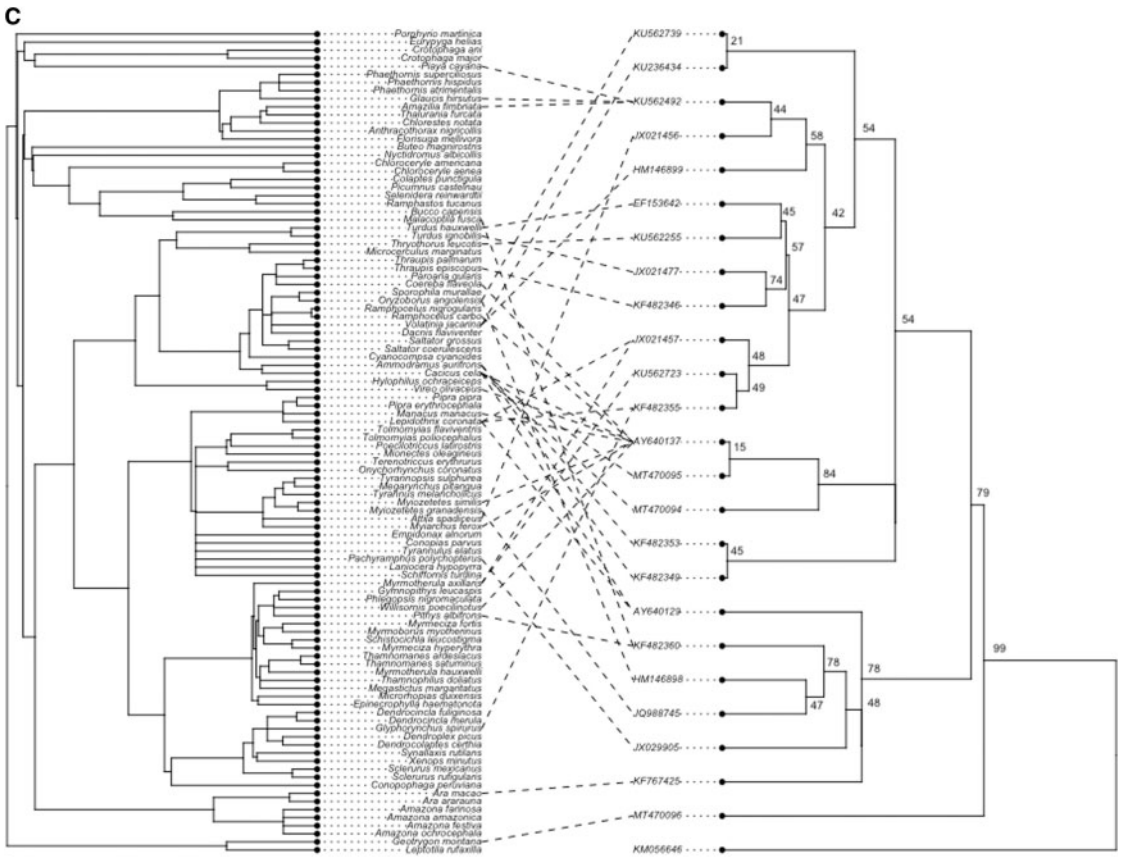

Figure 3 (continued)

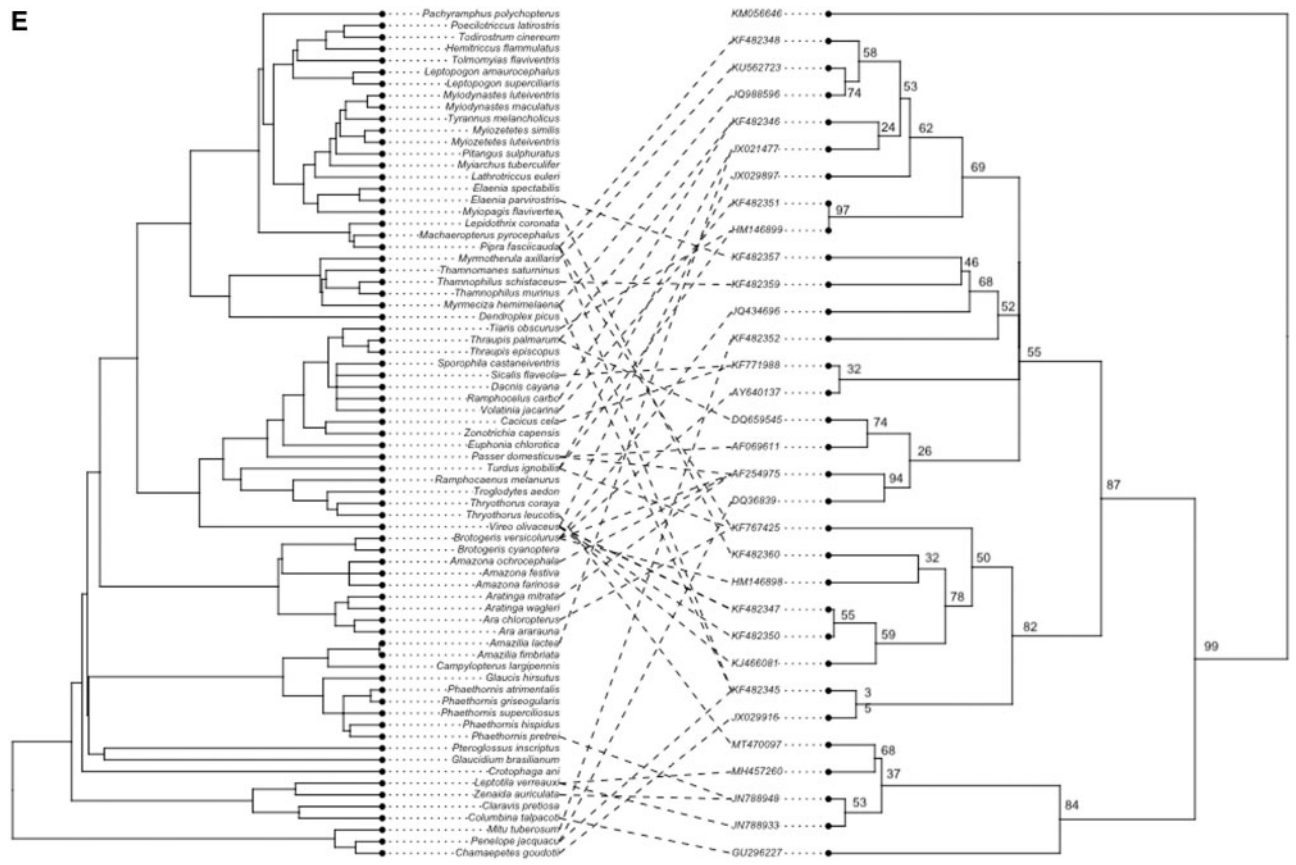

Figure 3. Phylogenetic associations between *Plasmodium* and *Haemoproteus* parasites and their avian hosts in the different areas of study. (**A**) Peruvian Yungas; (**B**) Sechura desert; (**C**) Iquitos várzea; (**D**) Southwest Amazon moist forests; (**E**) Ucayali moist forests. Node numbers indicate bootstrap values.

Table 3. Phylogenetic dispersion of parasite lineages infecting more than one host species in sampled ecoregions

Ecoregion	*N*	MPD_{obs}	Mean ± SD	*Z*	*P*
A) Peruvian Yungas					
Plasmodium relictum SGS1	5	137.043	141.274 ± 14.056	−0.295	0.387
B) Sechura desert					
Haemoproteus passeris PADOM05	2	138.891	102.019 ± 54.960	0.670	0.675
Haemoproteus sp. OCHLEU01	2	175.574	104.080 ± 54.506	1.311	0.917
Plasmodium relictum SGS1	3	122.344	105.162 ± 39.752	0.432	0.656
C) Iquitos várzea					
Plasmodium relictum THCAE01	2	107.028	137.947 ± 39.625	−0.780	0.237
***Plasmodium* sp. DENPET03**	**14**	**110.571**	**137.278 ± 11.656**	**−2.291**	**0.024**
Plasmodium sp. DENPET01	2	137.929	137.573 ± 40.732	0.008	0.369
Plasmodium sp. LEPIDO01	2	107.028	137.133 ± 41.058	−0.733	0.170
Plasmodium sp. MONNIG01	3	131.792	137.988 ± 26.120	−0.237	0.392
Haemoproteus sp. MYIFLA01	2	166.839	136.300 ± 40.292	0.757	0.698
***Plasmodium* sp. RAMCAR01**	**2**	**30.976**	**138.682 ± 38.842**	**−2.772**	**0.019**
D) SW Amazon moist forests					
***Plasmodium* sp. SCHISME02**	**2**	**31.359**	**132.175 ± 43.695**	**−2.352**	**0.044**
E) Ucayali moist forests					
Plasmodium sp. ZEMAC15	2	172.905	140.771 ± 46.993	0.747	0.921
***Plasmodium* sp. VOLJAC02**	**2**	**44.430**	**142.994 ± 47.022**	**−2.096**	**0.050**
Plasmodium sp. TUAMA01	2	167.000	141.639 ± 46.269	0.548	0.609
Haemoproteus sp. TROAED19	3	157.796	144.084 ± 30.044	0.289	0.521
***Plasmodium* sp. RAMCAR01**	**2**	**30.596**	**142.628 ± 45.457**	**−2.463**	**0.025**
Haemoproteus sp. PENJAC01	3	175.686	142.757 ± 30.635	1.074	0.903
Plasmodium sp. PADOM11	2	90.024	143.406 ± 43.774	−1.219	0.142
***Plasmodium relictum* GRW04**	**4**	**109.009**	**144.300 ± 23.269**	**−1.516**	**0.049**

N = number of bird species infected. Values in bold represent parasite lineages that were detected in close phylogenetic bird species and hence considered as specialist parasite lineages.

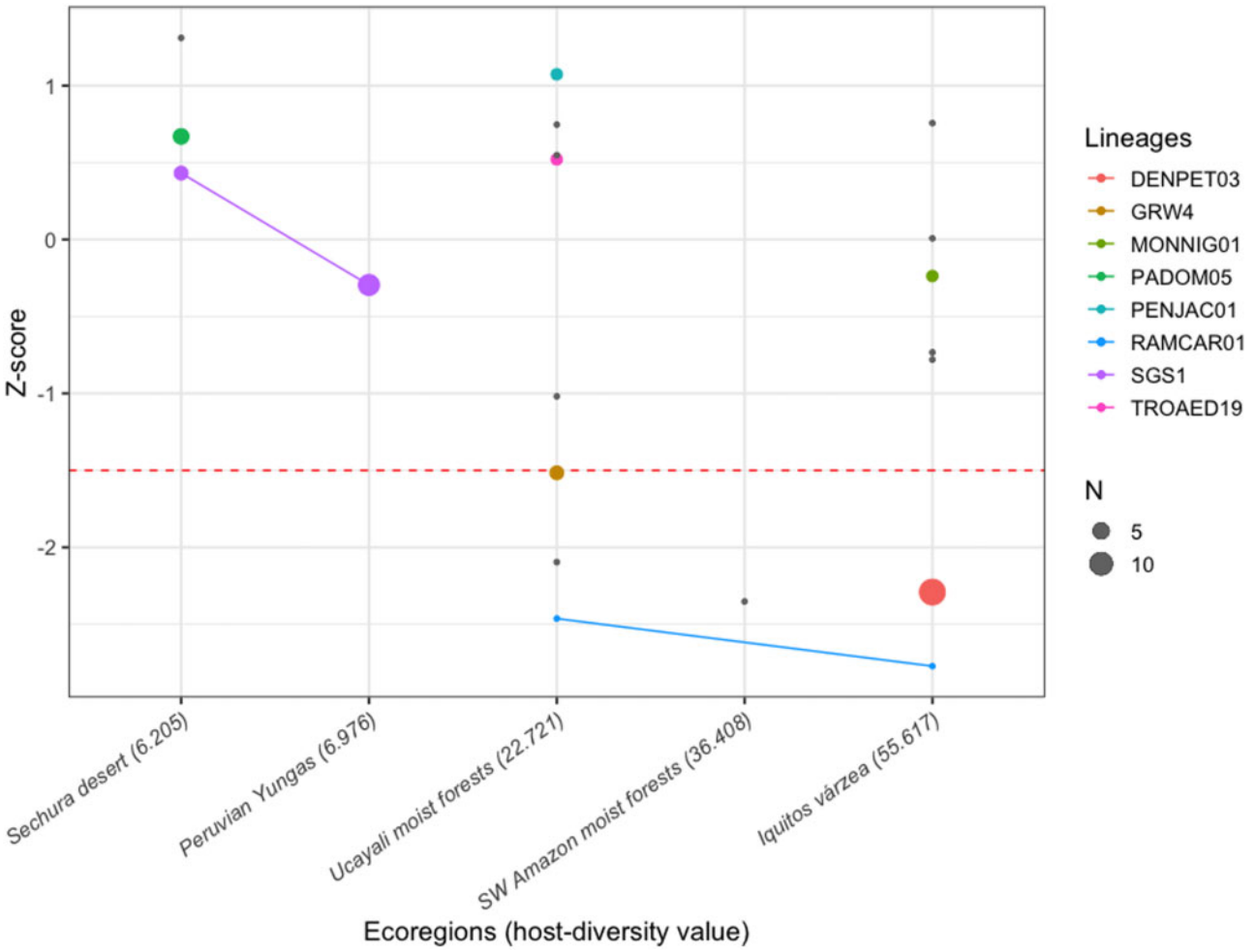

Figure 4. *Z*-score values for each parasite lineage per ecoregion. Lineages infecting more than 2 bird species, or present in more than one ecoregion, have been colored marked, while parasite lineages infecting only 2 host species remain in grey. The size of the dots is proportional to the number of bird species infected (*N*). Dots representing parasites lineages detected in more than one ecoregion were connected with a colored line. Red dashed line indicates the *z*-score value to categorize exploitation strategy followed by parasite lineages (generalist: *z*-score > -1.5; specialist: *z*-score < -1.5). Host-diversity values per ecoregion are shown.

encountering a variety of host species is higher than encountering a specific host species alone (Hellgren et al. 2009). In consequence, a parasite with low host specificity (i.e., generalist parasite) can ensure its transmission between host individuals in bird communities with low diversity of hosts. Because the transmission of specialized parasites should require repeated encounters with preferred hosts, specialist parasites should use hosts that are predictable in space and time (Loiseau et al. 2012; Svensson-Coelho et al. 2016). Therefore, specialist parasites would be expected in bird communities when the frequency of encounters of common and rare hosts increased (e.g., communities with higher bird diversity). Alternatively, these associations could also due to environmental factors affecting host–parasite interactions. In this sense, it has been proposed that abiotic factors could crucially influence the distribution of vectors, so that they could be especially sensitive under variable climatic conditions, exerting a direct effect on the abundance and diversity of parasites (Paaijmans et al. 2009; Lapointe et al. 2012; Pérez-Rodríguez et al. 2013). Following this idea, Loiseau et al. (2012) reported a higher number and a greater parasite diversity of specialist parasites in African moist rainforests than in other 2 regions that exhibited more variable environmental conditions in terms of temperature and rainfall. In agreement with these results, we found a higher number of specialist parasites in ecoregions with stable climatic conditions (i.e., Amazon basis ecoregion), whereas we could only identify generalist parasites in ecoregions with more variable climate conditions (i.e., Sechura desert and Peruvian Yungas). Therefore, our overall findings suggest that the exploitation strategy followed by haemosporidian parasites may be evolutionary shaped by environmental conditions and/or the abundance and richness of bird species. In this sense, Ellis et al. (2020) have recently analyzed a community of 67 locally transmitted bird haemosporidian parasite lineages in southern Sweden for testing 10 hypotheses to explain host–parasite associations, concluding that the prevalence and diversity of avian haemosporidians are likely to result of historical host–parasite interactions.

A particular case worth mentioning is *P. relictum* GRW4. Our outcomes suggest that it follows a specialist strategy (Table 3 and Figure 4), but this haemosporidian lineage has been historically described as generalist parasite (Hellgren et al. 2009, 2015). These divergences could be attributed to different strategies followed by parasites to maximize fitness. In this sense, it has been suggested that parasite lineages might modify their strategies depending on the

selection pressures (Svensson-Coelho et al. 2016), being more specialist or generalist depending on the degree of host specialization associated to habitat type and host geographical range (Loiseau et al. 2012; Garcia-Longoria et al. 2019).

In conclusion, we explored the genetic diversity and host assemblage of bird haemosporidian parasites from 5 ecoregions in Peru, confirming the suspected high diversity of avian malaria parasites in the Neotropics. We also found that the effective diversity (as well as the richness, abundance, and Shannon–Weaver index) for both bird species and parasite lineages was higher in Amazon basin ecoregions, thus suggesting that an increase in the parasite richness could be a consequence of higher bird richness in a given area. Our results also showed that generalist parasites were found in ecoregions with lower bird diversity, and presumably also lower vector diversity, implying that biotic and abiotic factors and/or the abundance and richness of hosts may shape the exploitation strategy followed by haemosporidian parasites. Further investigations linking these molecular data with morphology, host distribution, and vector specificity of these parasite lineages will provide important knowledge about phylogenetic relationships, phylogeography, and patterns of evolution and distribution of haemosporidian parasites.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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