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ARTICLE

Disease Ecology

Infection intensity shapes specialization and beta diversity of haemosporidian–bird networks across elevations

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Abstract

Host–parasite distribution and interactions depend on geography, evolutionary associations, species composition, and their response to environmental variables. In this sense, host specificity as a key parasite's life history trait may determine parasite community composition but can be difficult to identify in nature. The haemosporidian–avian host communities of tropical mountain systems offer an opportunity to better understand how network interactions influence parasite–host specialization. By using frequency (i.e., prevalence) and interaction intensity (i.e., number of parasites per infected host) data, we calculated two quantitative indices to evaluate haemosporidian host specialization at both community (H_2') and haemosporidian lineages and bird species levels (d'). Additionally, we evaluated alpha (S_T) and beta diversity (β_{WN}) of haemosporidian–bird interactions along four distinct elevations (representing different vegetation types) in Central Veracruz, Mexico. We recorded 607 birds from 88 species in two sampling years, registering 78 haemosporidian cytochrome b lineages by PCR in 125 positive samples from 38 bird species. Haemosporidian–bird interactions showed high specialization and modular network structure for each vegetation type. The degree of specialization and modularity was stronger when considering interaction intensity (i.e., parasitemia). Species-level specialization was higher for birds than for haemosporidians at the two vegetation types with highest interaction richness (i.e., pine–oak forest and tropical deciduous forest). There were high interaction beta diversity values (~ 1) among vegetation types, reflecting turnover in both avian and parasite communities. Our findings suggest that vegetation type (proxy for the local environmental conditions) constrains the distribution of hosts and parasites, and that intraspecific infection intensity plays an important role in the transmission dynamics and interactions of the haemosporidian community.

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KEYWORDS

avian malaria, ecological networks, environmental gradient, *Haemoproteus*, landscape parasitology, *Leucocytozoon*, *Plasmodium*

INTRODUCTION

The distribution and interactions of parasites and their hosts are strongly related to the environment (e.g., temperature and elevation), their evolutionary history, and species ecological traits that drive interaction patterns involving different spatiotemporal processes (Ellis et al., 2020; Morand & Krasnov, 2010). Host specificity involves niche differentiation of parasites, where specialist parasites exploit single host species, while generalist parasites interact with several host species (Runghen et al., 2021; Tylianakis & Morris, 2017). There are multiple facets of host specificity and several descriptors and frameworks that assess the ways in which different host species contribute to a parasite's specificity (Schmid-Hempel, 2021; Wells & Clark, 2019). Host specificity is not a fixed trait since multihost parasites are able to exploit a set of host species in a local context, which depends on abiotic and biotic conditions (realized niche). Alternatively, the range of host species that a parasite is able to infect in the absence of any restrictive condition represents the full range of host species and is considered as an intrinsic species attribute (fundamental niche) (Devictor et al., 2010; Wells & Clark, 2019). The use of approaches that consider species distributions and biotic interactions is critical to understand how host specificity is affected by environmental filters; in addition, it is necessary to unravel the influence of factors such as host susceptibility, parasite virulence, adaptive plasticity, and transmission seasonality as ecological filters on the host community (Devictor et al., 2010; Lopes et al., 2020; Wells & Clark, 2019).

The specificity of a particular parasite in its geographic range could be measured by prevalence data as the presence or absence of infection in a sufficiently large number of individuals and in their diversity of sympatric host species sampled (Bellay et al., 2018; Devictor et al., 2010; Lopes et al., 2020; Wells & Clark, 2019). Prevalence provides valuable information on the contribution of different host species to the realized specificity of the parasites arising from their ability to infect bird species (Doussang et al., 2021; Ventim et al., 2012). In addition to recording prevalence, knowing the parasite infection intensity of infected hosts is of particular interest for understanding parasite specificity (Huang et al., 2018). It has been widely discussed that virulence depends on the intensity of infection (number of parasites per infected host) and the damage that each parasite causes to the

host (Råberg & Stjernman, 2012; Schmid-Hempel, 2021). The success of parasites hinges on completing their life cycle across their different hosts, which depends on the interaction of the parasite with the host immune system and with the environment (Schmid-Hempel, 2021). The virulence transmission trade-off hypothesis considers the benefits of high parasite replication within the host (favoring increased transmission to the next host), the associated reduction in infection life span and the consequences of coinfection on virulence as a competitive advantage for more virulent parasites in a multiple infection (Alizon et al., 2009; Schmid-Hempel, 2021). The infection intensity of a host population could be estimated in the laboratory; however, it is convenient to contrast it with that observed in the field, since variables such as host immune response and parasite interactions can present a significant variation (Alizon et al., 2009). Many studies of antagonistic networks overlook interaction intensity, particularly in studies dealing with a species-level approach (Brian & Aldridge, 2021; Runghen et al., 2021). In these antagonistic networks species are represented by nodes and host–parasite relationships are represented by network links (Dáttilo et al., 2020). When considering interaction intensity, it is expected that specialist parasites within antagonistic networks will infect their preferred host species more often than generalists infecting those same species; at the same time, it would be expected that specialists will show a higher infection intensity in their preferred host species than generalists (Huang et al., 2020; Lima & Pérez-Tris, 2020; Poulin et al., 2011), indicating both a parasite preference and a degree of infection tolerance by the preferred host species. Thus, considering infection intensity may improve our understanding of parasite coexistence, species turnover, and parasite transmission (Dallas et al., 2019; Hellgren et al., 2004; Runghen et al., 2021).

When parasite specialization studies are conducted at different spatial scales, the results may show signs of host phylogenetic conservatism, where specialist parasites infect closely related host species (de Angeli Dutra et al., 2021; Fallon et al., 2005). Additionally, parasite communities may be shaped by the distribution of their hosts, and if parasite–host specialization is common then parasite communities may have similar alpha and beta diversity patterns to those of their vertebrate hosts (e.g., parasite infecting an abundant and widely distributed avian host may be similarly locally abundant and widely distributed)

(Lima & Pérez-Tris, 2020). One of the most consistent predictors of haemosporidian community turnover is host community turnover (Barrow et al., 2021; Clark et al., 2017; Fecchio et al., 2018). Communities are expected to differ in their species composition and interactions, mainly due to species replacement. Thus, the turnover of interactions may be due to species turnover, which may be increased by either geographical or environmental distance (Krasnov et al., 2019; Luna et al., 2020).

The analysis of interaction networks has been widely used to better understand the mechanisms influencing community structure across environmental gradients (Runghen et al., 2021; Tylianakis & Morris, 2017). A high diversity of hosts species may be expected to harboring richer (more diverse) parasite assemblages, with a higher proportion of rare specialists (Hudson et al., 2006; Vázquez et al., 2005). In ecological interaction networks, nodes with few links (specialist parasites) tend to associate with nodes with many links (host species with high parasites richness), which favor coinfections across host communities, resulting in a nested pattern of host–parasite interactions. One explanation is that the number of species with which a given species can interact is determined by host abundance, so rare host species tend to have fewer links (with generalist parasites) than abundant hosts (Bellay et al., 2018; Galen et al., 2019; Vázquez et al., 2005). The second pattern generally observed shows high niche partitioning, with a modular structure characterized by nonoverlapping but densely connected species subsets (i.e., modules). In this case, many interactions occur among species within this subset, and few species interact with other modules (Fortuna et al., 2010; Krasnov et al., 2012). Quantitative descriptors of the degree of specialization reflect properties of interaction networks; two indices of specialization are derived from Shannon entropy, the species-level index d' can be used to analyze variation within networks, while H_2' as a network-level index is useful for comparisons across different interaction networks (Blüthgen et al., 2006; Lopes et al., 2020; Svensson-Coelho et al., 2014). Categorizing parasites as either specialists or generalists within the network approach may be a difficult task since even parasites with host specialization often infect a wide range of hosts albeit with lower infection intensities (Huang et al., 2018; Moens & Pérez-Tris, 2016). Therefore, including infection intensity values in a metaweb (i.e., a representation of a regional pool of potential interactions; Luna et al., 2020) could provide relevant information rather than the interaction frequency, and thus modify specialization values and network structure.

The Haemosporida Order is a multihost, multiparasite system conformed of intracellular protozoans that infect vertebrates via Diptera vectors (Valkiūnas, 2005). The host specificity of haemosporidian parasites is associated with

specific biotic and abiotic factors (Clark et al., 2017; Fecchio et al., 2018; Lima & Pérez-Tris, 2020; Svensson-Coelho et al., 2014). Several studies indicate that temperature and humidity along with their seasonality are the major drivers affecting the distribution of three common haemosporidian genera (i.e., *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) (Clark et al., 2017; Fecchio et al., 2017; Scordato & Kardish, 2014). Those same variables have been found to be reliable environmental predictors at the local scale and have been used as proxies for understanding specialization within haemosporidian and birds in different environmental gradients (Doussang et al., 2021; Pellegrino et al., 2021). In addition, these factors limit the distribution and development of competent dipteran vectors, which affect dispersal capacity and successful transmission (Fecchio et al., 2017, 2019; LaPointe et al., 2012; Lotta et al., 2016). For instance, in the Americas, *Leucocytozoon* transmitted by black flies (Simuliidae) is the most common haemosporidian genus at high elevations with cold climates (Lotta et al., 2016; Santiago-Alarcon & Marzal, 2020). In contrast, *Plasmodium* transmitted by mosquitoes (Culicidae) tends to be more prevalent in warm lowlands (LaPointe et al., 2010; Rodríguez-Hernández et al., 2021), whereas *Haemoproteus* transmitted by biting midges (Ceratopogonidae) and louse flies (Hippoboscidae) is more widely distributed across both warm and cold areas (González et al., 2014, 2015; Harrigan et al., 2014). Hence, the environmental gradient of tropical mountains is an ideal system for examining parasite–host specialization, interaction intensity, and distributional patterns of avian parasites (Chapa-Vargas et al., 2020).

Our study aimed to assess how specialization and network structure of haemosporidian lineages vary across different bird assemblages in four vegetation types along an elevation gradient between 0 and ~2800 m asl in Central Veracruz, Mexico. We calculated all the network indexes for each vegetation type: tropical sub-deciduous forest (TSDF), tropical deciduous forest (TDF), montane cloud forest (MCF), pine–oak forest (POF), and for the metaweb (including all vegetation types). We estimated (1) the specialization degree by means of indices applied to both network-level specialization (H_2') and species-level specialization (d'). Additionally, we determined whether host specialization at the species level differs among haemosporidian lineages and birds. (2) We determined modularity and nestedness of interaction frequency (parasite prevalence within host community) and interaction intensity (number of parasites per host). (3) Finally, we examined α -interactions richness and β -diversity (i.e., dissimilarity of interactions) within and across the four vegetation types. In vegetation types located at the opposite extremes of the elevational gradient, we expected a highly specialized network structure, with high

modularity. This may be a consequence of the species turnover resulting from the specific ecological requirements of each parasite genus (i.e., *Leucocytozoon* at high cold sites, *Plasmodium* at low warm sites) and bird species. We further predicted that communities at mid-elevations would present a network structure with lower specialization and modularity than those at the extremes of the gradient.

MATERIALS AND METHODS

Study area

Our study location was in the State of Veracruz, Mexico, at the junction of the Trans-Mexican Volcanic Belt and the Sierra Madre Oriental. We selected four vegetation types along an elevational gradient between 0 and ~2800 m asl: TSDF, TDF, MCF, and POF (Figure 1; Appendix S1: Table S1).

Field methods

Fieldwork was conducted in June–July 2017 and in June–July 2018, during the avian breeding season to study

only resident communities. We sampled birds using 10 mist nets (12 × 2.5 m) over five consecutive days at each site. We collected blood samples (~50 µL depending on the bird body size) after puncturing the brachial vein with heparinized microcapillary tubes. We stored the blood samples in a cooler, inside empty plastic tubes (1.5 mL), then samples were frozen at –20°C for subsequent analyses. Mist nets were moved within the sampling site after one or two days to obtain a better representation of bird species and high capture rates. We identified all captured species and banded them with unique alphanumeric aluminum rings. Subsequently, all birds were released at the same site. Sampling was carried out from 06:00 a.m. until 12:00 p.m. We operated nets in the afternoons (~14:00–18:00) whenever weather conditions were appropriate. The net sampling effort was 2800 net hours: 350 net hours per site each year.

Microscopy analysis

To quantify infection intensity (i.e., parasitemia), we used microscopy. We stained blood smears with Giemsa staining solution for 90 min (Santiago-Alarcon & Carbó-Ramírez, 2015). Whenever parasites were present, we counted 100 fields at high magnification (×1000) to

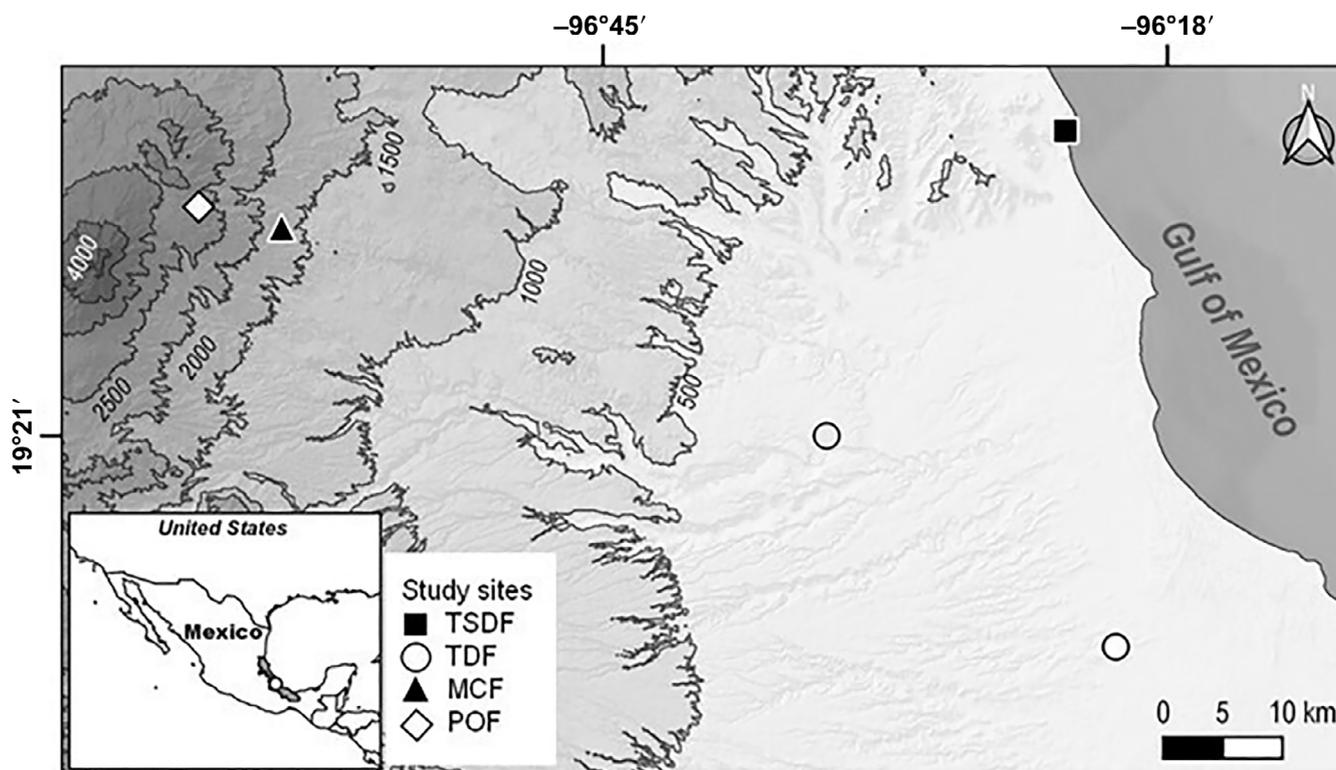


FIGURE 1 Study areas showing the study sites in each vegetation type: POF, pine–oak forest (2793 m asl); MCF, montane cloud forest (1640 m asl); TDF, tropical deciduous forest (262 m asl); TSDF, tropical sub-deciduous forest (9 m asl). Fieldwork was conducted in four vegetation types located at five sites. The darkest zones show the land slope curves according to the elevation increase.

estimate the relative infection intensity (Valkiūnas, 2005). We focused on intraerythrocytic structures of haemosporidians (trophozoites, meronts, and developing or mature gametocytes). We always verified samples by scanning two smears per sample and by molecular diagnosis (see below). We confirmed the identity of the lineages with molecular diagnostics.

Molecular analysis

We extracted DNA using a DNeasy blood and tissue extraction kit (QIAGEN). Then we performed three different PCR protocols (two of them nested) to ensure parasite detection (Beadell et al., 2004; Hellgren et al., 2004; Pacheco et al., 2018). Primers are genus specific for haemosporidian parasites, all protocols amplify a fragment of the mtDNA cytochrome b gene of different lengths. We ran all PCRs with Platinum Green PCR master mix 2× (ThermoFisher) and Top Taq 10× (QIAGEN, Hilden). We added a positive sample (previously confirmed by sequencing and microscopy) and a negative control (ddH₂O). We started running samples with the protocol of Pacheco et al. (2018) because it gets a larger sequence fragment (~1100 bp). Negative samples were subsequently run with Beadell et al. (2004) and Hellgren et al. (2004) protocols. We visualized 5 μL of PCR products in a 1.5% agarose gel stained with GelRed (BIOTUM). Then we cut the required size bands of positive samples from agarose gels and purified them with MinElute PCR purification extraction kit (QIAGEN). Subsequently, we sent samples for bidirectional sequencing to Macrogen (Korea). We assembled, aligned, and edited sequences using Bioedit version 7.0.5 (Hall, 2005). Samples with mixed infections were identified by double peaks in the electropherogram and compared with the microscopy diagnosis. We also used PolyPeakParser online software as an additional aid to identify coinfections (Hill et al., 2014). Length sequences were ~479–1109 bp depending on the protocol by which samples tested positive. Parasite lineages were contrasted with parasite sequences available in the MalAvi database (Bensch et al., 2009). The new lineages were named and uploaded to the GenBank (MZ683438-MZ683475; MZ955363-MZ955367) and MalAvi databases (Appendix S1: Table S2).

Statistical analyses

Network completeness: We included in our data analysis all haemosporidian lineages confirmed by molecular methods and the bird species associated to these lineages. We constructed adjacency matrices for haemosporidian lineages, bird species, and haemosporidian–bird interaction

frequency (prevalence) recorded over two sampling years at each vegetation type. The same sampling effort was conducted at each vegetation type, but we recorded different numbers of individuals at each site; therefore, we evaluated the completeness of our network sampling. We elaborated species accumulation curves for haemosporidian lineages, bird species, and haemosporidian–birds interactions with the package iNEXT (Hsieh et al., 2016) in R software version 3.5.1. This package can generate rarefied (interpolated) species accumulation curves based on observed data and can project a rarefied extrapolation of the predicted number of species or species interactions given a larger sampling effort. Those calculations were accompanied by 95% confidence intervals as a function of sampling effort with Hill numbers of species richness ($q = 0$), Shannon diversity ($q = 1$), and Simpson diversity ($q = 2$).

Network indices: The interactions of haemosporidian lineages and birds were assessed through bipartite networks that show the relationships between species from two distinct groups (Dehling, 2018). We made calculations at both the network level (per vegetation type) and species level. We built a total of 10 adjacency matrices, where five of them correspond to interaction frequency. These matrices included all the interaction observed between haemosporidian lineages and bird species, where a_{ij} = interaction frequency (prevalence) between bird species i (rows) and haemosporidian lineages j (columns). The first matrix was a metaweb (containing all interactions observed over the four vegetation types), and the other four matrices were one for each vegetation type. The other five matrices were for the interaction intensity (parasitemia) data, again one matrix was created for the metaweb, and one for each vegetation type. For the interaction intensity matrices, we included the counts of haemosporidian gametocytes, meronts, and trophozoites recorded in each host. This type of counting represented a challenge for hosts coinfecting with the same parasite genus. There were some individual birds ($n = 14$) coinfecting with different lineages of the same parasite genus; in these cases, it was impossible to distinguish the infection intensity count for a specific haemosporidian lineage, so the same infection intensity value was considered in the data matrix for both lineages.

We calculated network-level indices for the metaweb and for each vegetation type in the package bipartite for R (Dormann et al., 2014). We assessed the reciprocal specialization of the community with the index H_2' . This index estimates a whole matrix structure and returns one value per assemblage, which is useful for comparisons across different interaction webs (Blüthgen et al., 2006). The H_2' values range from 0 (total generalist) to 1 (total specialist) (Svensson-Coelho et al., 2014).

This information provides an insight on how intimate species associations are in an assemblage (Lopes et al., 2020). As haemosporidian–bird interaction networks differ in species richness, connectance, and heterogeneity in the interactions, direct comparison between H_2' values could be compromised (Luna et al., 2017). Therefore, we estimated the significance of the observed H_2' values with a Monte Carlo procedure in which 1000 random matrices were generated using a null model based on the Patefield's algorithm (i.e., r2dtable) (Patefield, 1981). This null model fixes the total number of species interactions and distributes them randomly to produce a set of simulated networks where all species are randomly associated. All H_2' values were significant ($p < 0.008$); thus, the simulations of artificially generated random associations allowed us to confirm that the network-level specialization index H_2' is not significantly affected by network size. As proposed by Blüthgen et al. (2006), H_2' values were not affected by network size or sampling intensity after analysis by rarefied sampling effort and null model simulations. Additionally, we included modularity (Q) because ecologically, the formation of modules is related to specialization. A low degree of specialization is equivalent to random interactions without formation of modules (Dormann & Strauss, 2014). This measure was calculated using a function computeModules, applying the DIRTLPAwb+ algorithm to detect modules based on the quantitative matrix of interactions. However, the Q value merely indicates how well links and interactions can be separated into different modules, and it is dependent on network size (i.e., large networks allow more species modules combinations and higher values of Q) (Beckett, 2016; Dormann & Strauss, 2014). Modularity is an evaluation of how nodes are partitioned into separate subsets forming modules; these modules are link-rich clusters of species in a community. This is done by assessing the extent to which interactions in the network occur within rather than between modules, in relation to a null model (Barber, 2007). A significance test for the selected settings was performed by running 100 randomizations with the null model r2d table, and the resulting values were subsequently used to calculate the Z score ($Z \geq 2.5$, high significance) (Dormann & Strauss, 2014). Furthermore, we measured nestedness based on overlap and decreasing fill, nestednodf for weighted networks (WNODF). The WNODF index indicates whether the network has a nonrandom arrangement and there is a central core with highly interacting species that establish many interactions among themselves, and with specialist species interacting with more generalist species (Almeida-Neto et al., 2008). This metric is based on standardized differences in row and column fills and paired matching occurrences ranging from 0 (non-nested)

to 100 (perfectly nested) (Dormann et al., 2014). Significance was tested by simulating 1000 random matrices, using null model (“r2dtable”). This null model keeps row and column sums constant to distribute the interactions and produce a set of networks in which all species are randomly associated (Blüthgen et al., 2008).

We incorporated a species-level index that can be used to determine structural and functional roles of individual species and to describe the diversity and interaction organization of species in a network (Dehling, 2018). The index of specific specialization (d') quantifies the difference between two probability distributions and can be used to analyze variation within networks, measuring “partner diversity” (standardized Kullback–Leibler distance) (Blüthgen et al., 2006). The species-level index d' calculates how strongly species deviate from a random sampling of interacting partners available, which ranges from 0 (no specialization) to 1 (perfect specialist) (Dormann et al., 2008). The d' index incorporates a parasite species frequency on all its hosts, and focal species frequency in relation to other species, which interact with each of its associates. When species have a high d' value, a high functional importance is most likely for a generalist species (Svensson-Coelho et al., 2014). Using t tests, we compared the d' values of both interaction frequency and interaction intensity for haemosporidian and bird species in each vegetation type, aiming to learn whether parasites tend to use fewer hosts on average (indicating potential parasite–host specialization) and whether some bird species tend to harbor fewer parasites (parasite diversity per host) (Svensson-Coelho et al., 2014).

Diversity of species interactions: We evaluated the local diversity of interactions (α -diversity or interaction richness) in each vegetation type with the number of realized interactions (S_I = number of links/interactions between species). We analyzed the turnover of interactions between vegetation types to understand whether variation in host species composition influenced haemosporidian parasites network structure. We evaluated the dissimilarity of species interactions (β -diversity of interactions, β_{WN}). We calculated the two additive elements into which β -diversity is partitioned: the species turnover between communities (β_{ST}) and the interaction rewiring (β_{RW}) (Corro et al., 2020; Poisot et al., 2012). In other words, the communities may differ in two complementary ways: either because they harbor different bird species and haemosporidian lineages (β_{ST}) or because whichever species or lineages they share might interact in different ways (β_{RW}); thus, although the same species and lineages are shared, they are unable to interact due to ecological or environmental constraints (Poisot et al., 2012). We compared the beta-diversity measures of each vegetation type by pairwise comparison, and through the metaweb approach

with β_{os} index, to emphasize the mechanism governing the interactions within the networks. The β_{os} index evaluated how similar a local network is in relation to the pool of potential interactions, ranging from 0 to 1: 0 indicates that all potential interactions are in the local networks, while 1 indicates that few interactions are found in the local networks (Poisot et al., 2012). Additionally, we evaluated the local network uniqueness (LNU) aiming to measure the proportion of unique interactions of a local network in relation to its metaweb, as proposed by Luna et al. (2020). LNU index ranges from 0 (no unique interactions) to 1 (all interactions are unique). It is calculated with the formula $LNU_X = \text{Unique interactions of } L_{Xj} / L_{Xj}$, where L_{Xj} = metaweb without local network of interest. For the beta analysis network, we used the Betalink package (Poisot, 2016) and built the metaweb according to Luna et al. (2020).

RESULTS

Network completeness

A total of 607 birds from 88 species belonging to 21 families were recorded during the two sampling years. We recorded 78 haemosporidian lineages belonging to three genera by molecular diagnosis in 125 positive samples of 38 bird species (Figure 2A,B; Appendix S1: Table S2) for the metaweb (total prevalence of 20.5%). Our estimated interactions frequency (prevalence of all individual birds sampled) was larger than those observed (Appendix S1: Table S3). We recorded 23% of the parasite–host interactions in TSDF (observed: 10; estimated: 43.23), 27% in TDF (observed: 40; estimated: 146.67), 20% in MCF (observed: 13; estimated: 66.16), and 44% in POF (observed: 41; estimated: 100.02) (Appendix S1: Figure S1). We estimated high asymmetry in network structure between the interaction frequency and interaction intensity data for both the metaweb (Figure 2A,B) and each vegetation type (Appendix S1: Figures S2–S5). The high interaction intensity of some lineages modified the number of links per species. (Table 1, Figure 2B; Appendix S1: Figures S2–S5). The highest number of interaction frequencies (prevalence) occurred in POF (58) (Appendix S1: Figure S5), followed by TDF (49) (Appendix S1: Figure S3), MCF (30) (Appendix S1: Figure S4), and TSDF (13) (Appendix S1: Figure S2).

Network indices

The values of network-level specialization (H_2') for interaction intensity (i.e., parasitemia) were closer to 1 in

all vegetation types (Table 1), suggesting that the interactions are specialization cases. The lowest value for interaction frequency was found in TDF (0.520), while the highest was (0.863) in MCF (Table 1). The overall interaction networks exhibited high modularity in all vegetation types ($Q_{\text{mean}} = 0.175$; $Q_{\text{SD}} = 0.218$; all Z -values > 2.5 ; Table 1, Figures 3–6). The observed values of nestedness for frequency interaction and interaction intensity revealed a non-nested network structure pattern with values close to 0 in each vegetation type (Table 2). Only the observed values of interaction frequency were closer to those expected by the null model, while interaction intensity values were lower than those expected by the null model (Table 2; Appendix S1: Figure S6).

Average species-level specialization (d') of the metaweb was significantly higher for birds than for parasites, both for interaction frequency ($t = 6.36$, $df = 102$, $p < 0.001$) and interaction intensity ($t = 4.22$, $df = 81.47$, $p < 0.001$) (Figure 7A,B). When we analyzed the average species-level d' for each vegetation type separately, we recorded significant specialization for TDF ($p < 0.001$) and for POF ($p = 0.001$; Figure 7E,F,I,J) for both interaction frequency and interaction intensity, where birds showed more specialized d' values than parasites (Figure 7).

Diversity of species interactions

We recorded a variable richness of interactions (S_I) between vegetation types. POF had the highest interaction richness value, while TSDF showed the lowest (Table 3). We recorded different numbers of lineages for each haemosporidian genus among vegetation types: TSDF (*Plasmodium* = 5 and *Haemoproteus* = 2), TDF = (*Plasmodium* = 18, *Haemoproteus* = 13, and *Leucocytozoon* = 3), MCF = (*Plasmodium* = 3, *Haemoproteus* = 6, and *Leucocytozoon* = 2), and POF = (*Plasmodium* = 3, *Haemoproteus* = 10, and *Leucocytozoon* = 22) (Appendix S1: Table S2). We recorded coinfections in 23 individuals, of these, 14 belonged to the same parasite genus and 12 belonged to different genera. Three individuals had triple coinfections in the POF. This was also the site with the highest number of coinfections ($n = 13$), followed by TDF ($n = 9$) and finally MCF ($n = 1$). When estimating the beta diversity of interactions (β_{WN}), we found that the species turnover between vegetation types was high (> 0.95 ; Figure 8). The paired comparison by site yielded a value of 1 for all combinations of vegetation types except for TSDF-TDF and MCF-POF (Figure 8), which are adjacent vegetation types in the elevation gradient. This indicates that species were almost entirely different between vegetation types. The additive partitioning of beta diversity of

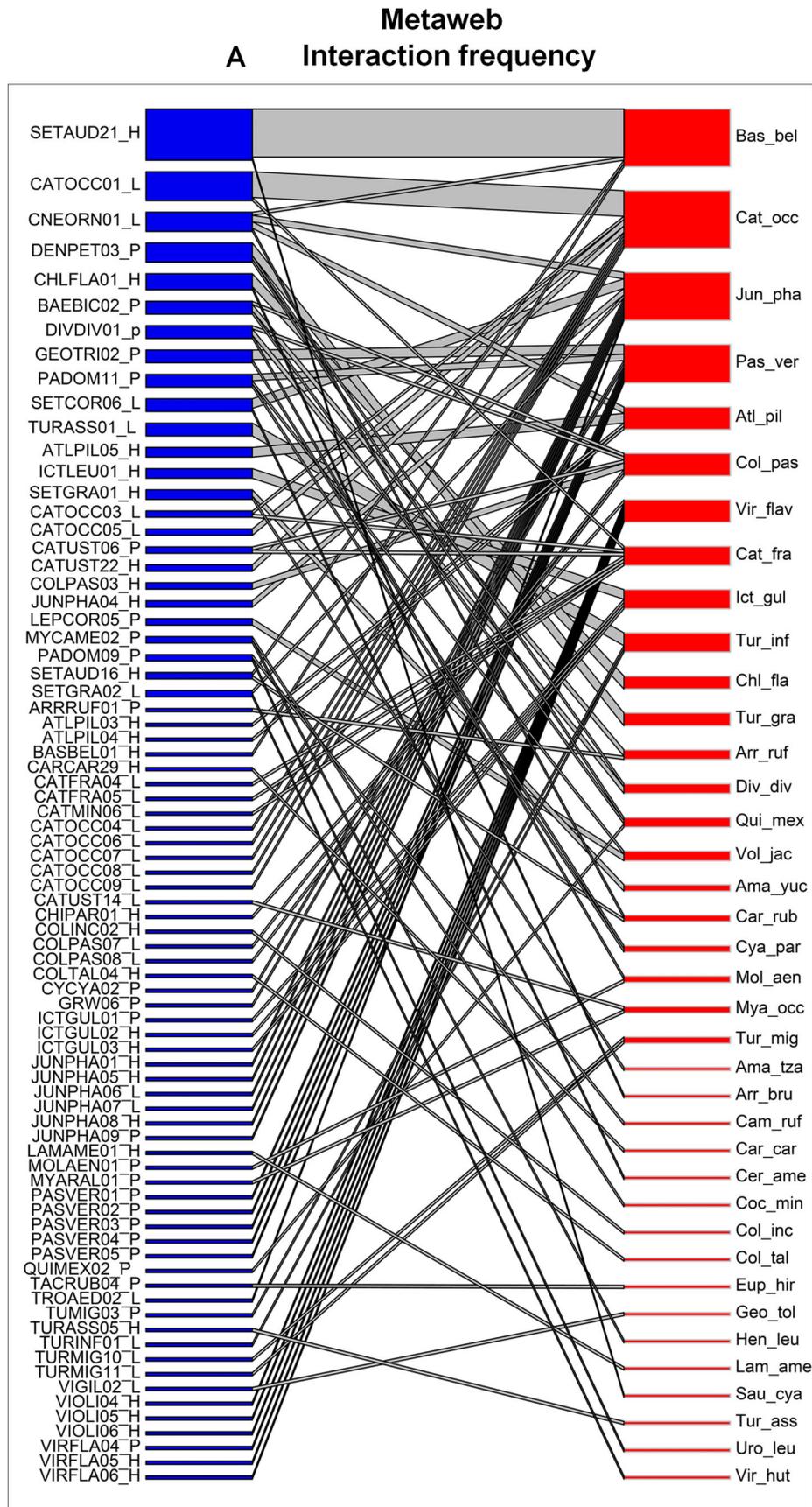


FIGURE 2 Haemosporidian–bird interaction metawebs. Haemosporidian lineages are on the left (blue boxes), and bird species are on the right (red boxes). The bird species codes correspond to those presented in Appendix S1: Table S2. The thickness of the lines corresponds to the number of interactions between bird species and parasite lineages.

Metaweb B Interaction intensity

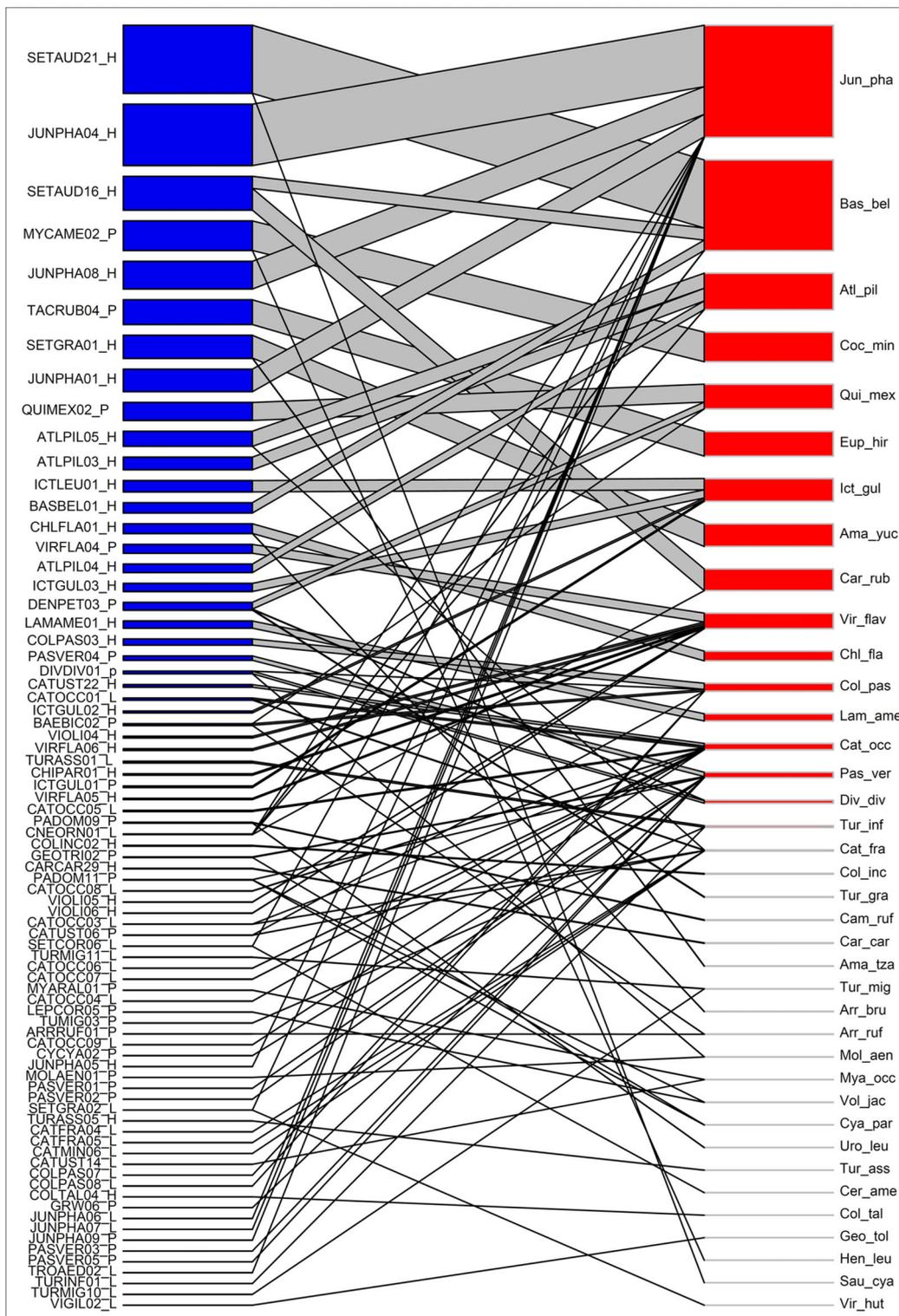


FIGURE 2 (Continued)

interactions (β_{WN}) showed that species turnover between communities (β_{ST}) contributed a larger proportion (0.8–1.0) than interaction rewiring (β_{RW}) (Figure 8). This was

confirmed by the values of the β_{os} and LNU, both indices showed that the communities are unique in their interacting species (Table 3).

TABLE 1 Network-level index for interaction frequency (Ifq) and interaction intensity (IIn) of four vegetation types located at different elevations in Central Veracruz, Mexico.

Vegetation types	Bird species	No. individuals	Unique bird species	Haemosporidian lineages	Unique haemosporidian lineages	No. interactions	Specialization (H_2')	Modularity (Q)	Z
Metaweb									
Ifq	38	607	...	78	...	150	0.672	0.280	15.308
IIn	38	607	...	78	...	9723	0.975	0.296	373.435
Tropical sub-deciduous forest (TSDF)									
Ifq	8	88	5 (62.50%)	7	4 (57.14%)	13	0.800	0.668	2.674
IIn	8	88	5 (62.50%)	7	4 (57.14%)	633	0.992	0.232	27.356
Tropical deciduous forest (TDF)									
Ifq	15	205	12 (80%)	34	29 (85.29%)	49	0.520	0.142	3.902
IIn	15	205	12 (80%)	34	29 (85.29%)	2312	0.994	0.112	138.613
Montane cloud forest (MCF)									
Ifq	10	196	8 (80%)	11	8 (66.66%)	30	0.863	0.012	7.524
IIn	10	196	8 (80%)	11	8 (66.66%)	2875	0.999	0.0002	111.346
Pine-oak forest (POF)									
Ifq	10	118	8 (80%)	33	30 (88.23%)	58	0.655	0.222	6.439
IIn	10	118	8 (80%)	33	30 (88.23%)	3903	1.000	0.017	152.189

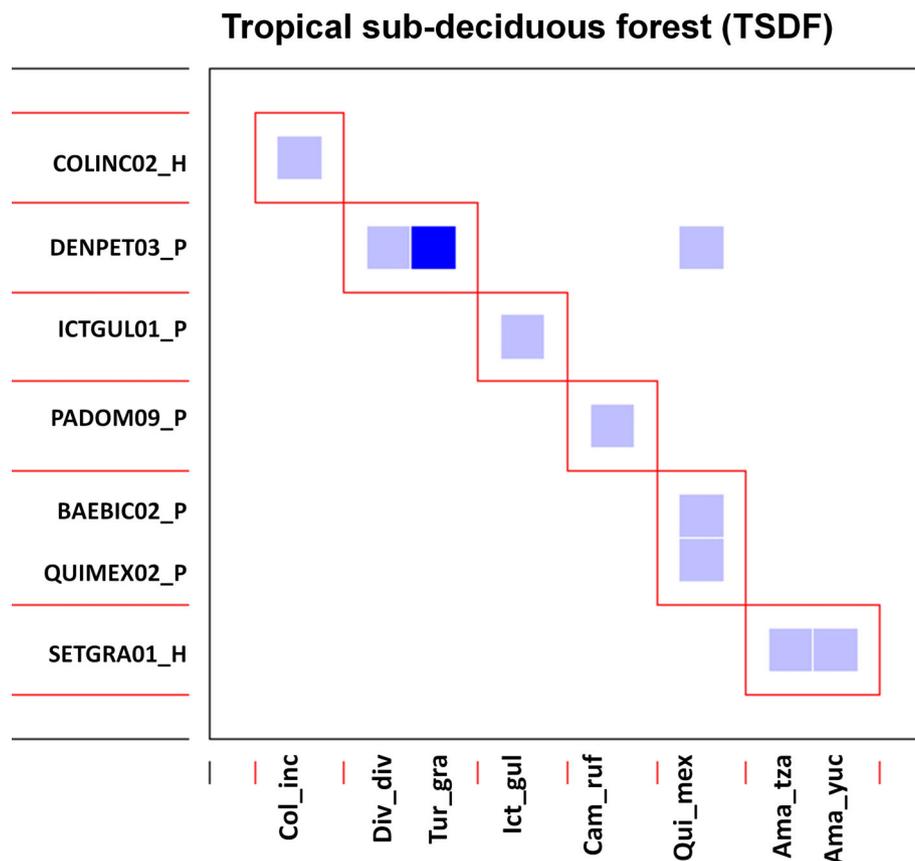


FIGURE 3 Visual comparison of the modular structures of haemosporidian–bird interaction matrices for tropical sub-deciduous forest. Darker colors indicate high occurrence of interactions. The red squares delimit the modules.

DISCUSSION

We analyzed haemosporidian–bird interactions in four vegetation types across different elevations using network

indices at the network level (H_2') and species level (d'). All networks were highly specialized (e.g., showing constrained host–parasite interactions and values of H_2' close to 1) and we observed that interaction intensity

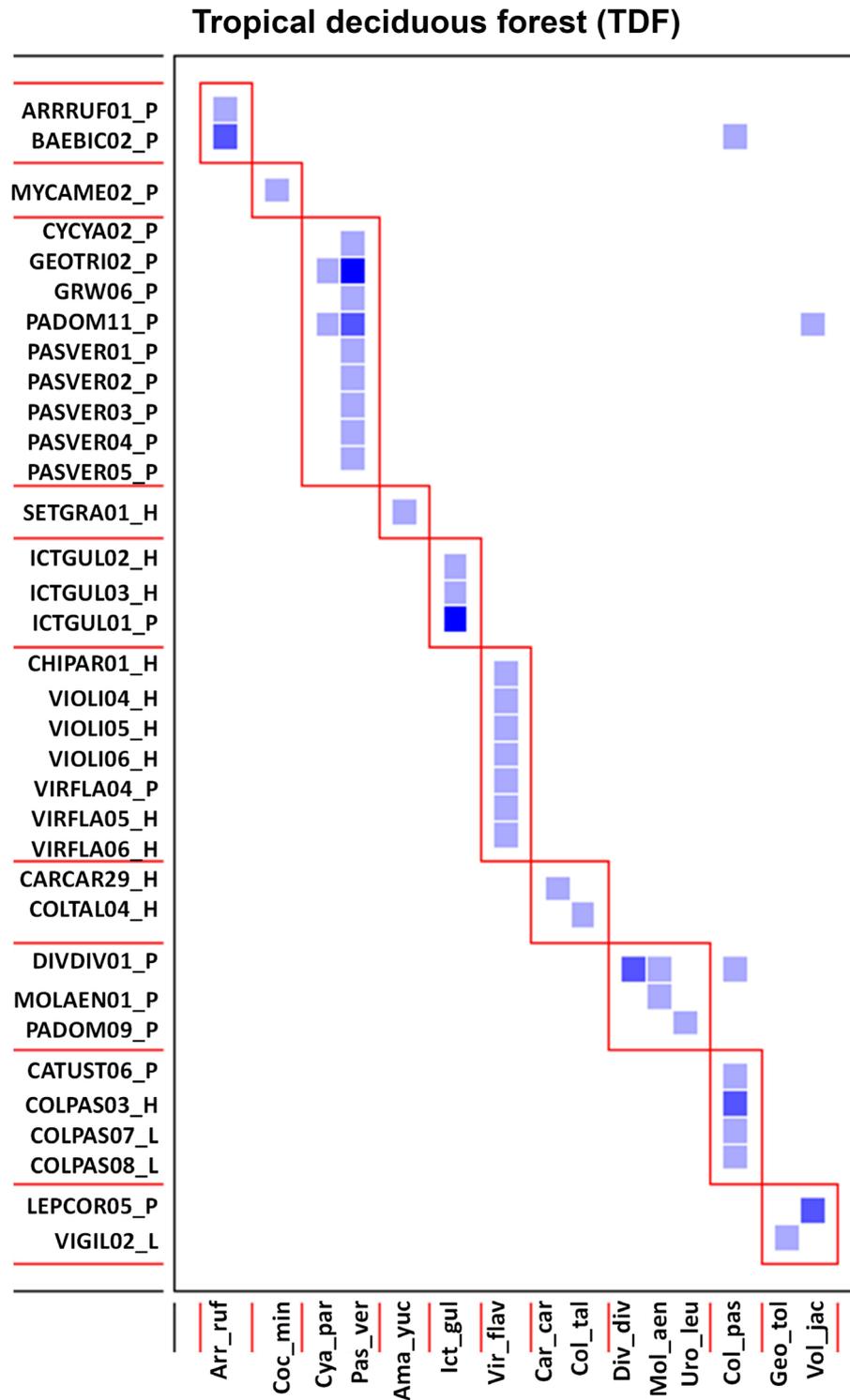


FIGURE 4 Visual comparison of the modular structures of haemosporidian–bird interaction matrices for tropical deciduous forest. Darker colors indicate high occurrence of interactions. The red squares delimit the modules.

(number of parasites per infected host) values showed a more specialized network structure (H_2') than those observed for the interaction frequency values. When we analyzed species-level interactions (d'), we found significantly high values of bird specializations in TDF and POF, which were mainly determined by high unique

haemosporidian–bird interactions and a modular network structure in each vegetation type. Moreover, we expected a nested structure at intermediate elevation sites, however all networks had a modular structure. Finally, beta diversity of interactions (β_{WN}) was close to 1 for all our paired comparisons of vegetation types,

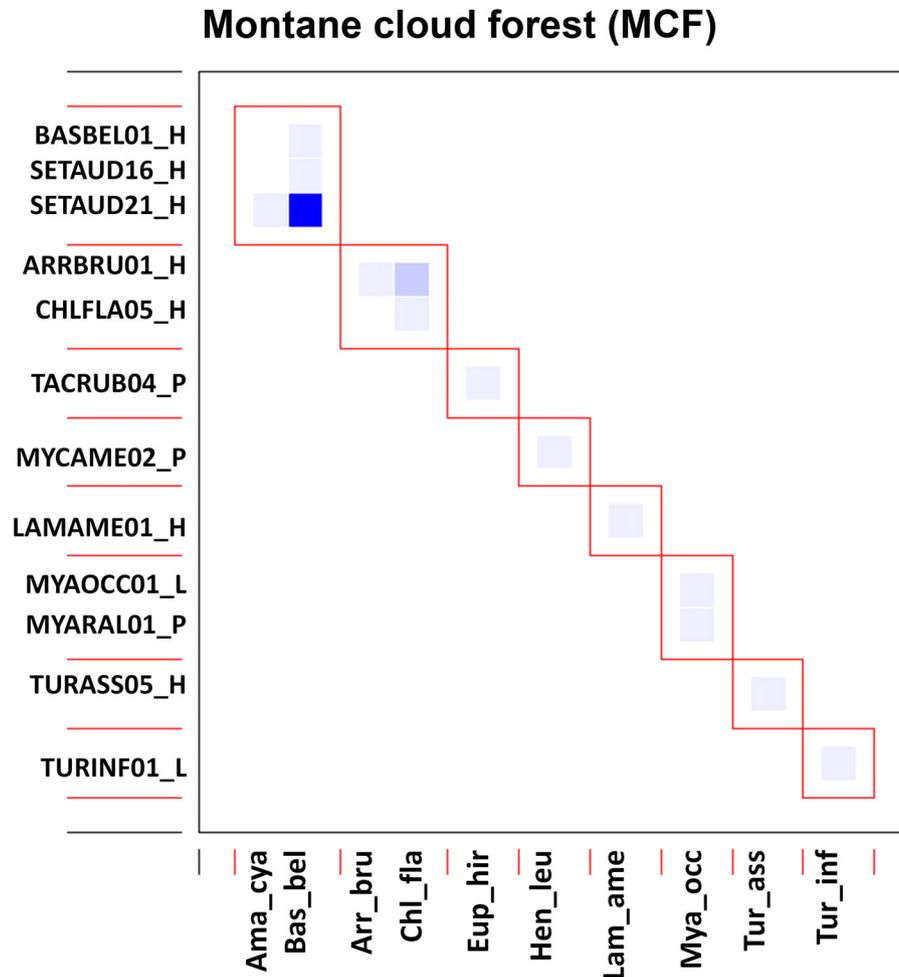


FIGURE 5 Visual comparison of the modular structures of haemosporidian–bird interaction matrices for montane cloud forest. Darker colors indicate high occurrence of interactions. The red squares delimit the modules.

which is a result of the few shared species and interactions between sites.

The high values of H_2' indicated a high strength or specialization of haemosporidian–bird interactions. These results were more evident for the interaction intensity data in TDF and POF, which generally had high infection intensity, higher parasite lineage diversity, and a higher number of coinfections than the other sites. Some network studies dealing with parasitic systems have pointed to infection intensity as an important aspect for structuring networks, predicting parasite transmission dynamics, and assessing the effects of parasites on hosts (Brian & Aldridge, 2021; Campião & Dáttilo, 2020). We found evidence for lower specialization in the parasite community (with parasites less constrained to specific bird species and values of d' closer to 0 respect to those presented by the birds). Such lower parasite specialization may be a consequence of a generalist host-feeding preference of vectors (Santiago-Alarcon et al., 2012), which would facilitate parasite dispersion among host species (Carlson

et al., 2015; Njabo et al., 2011) and promote parasite movements within the geographic distribution of their hosts (Ellis et al., 2015). Alternatively, from the bird perspective, the d' values we recorded in hosts may exhibit higher specialization than parasites due to the different barriers that prevent infection. For example, constraints could be imposed by the lack of spatiotemporal co-occurrence, caused by bird life history traits (i.e., nest type, foraging stratum, trophic guild), which could limit the rate of encounter with vectors and, thus, reduce parasite transmission, generating a spatiotemporal filter between hosts, vectors, and parasite genus (González et al., 2014; Lutz et al., 2015; Rodríguez-Hernández et al., 2021; Tchoumbou et al., 2020). Another explanation suggests physiological and phylogenetic constraints since the immune system of birds may prevent successful parasite infections when they are not phylogenetically closely related, where even generalist parasites mostly infect closely related host species (e.g., Ellis et al., 2020). Consequently, encounter probability and immune system

Pine–oak forest (POF)

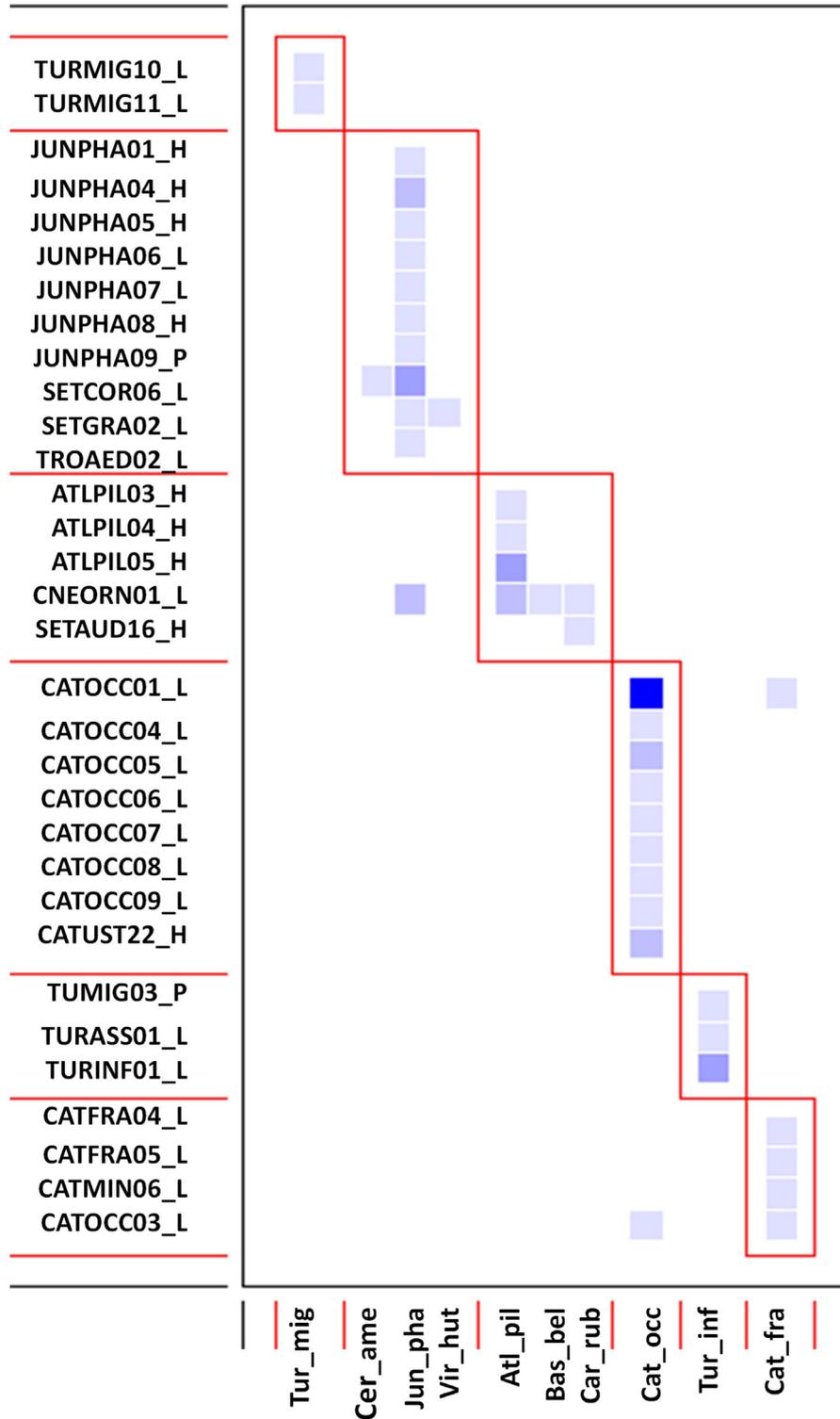


FIGURE 6 Visual comparison of the modular structures of haemosporidian–bird interaction matrices for pine–oak forest. Darker colors indicate high occurrence of interactions. The red squares delimit the modules.

susceptibility of hosts may determine the interaction intensity, parasite identity, and the composition of the parasite community (Barrow et al., 2019; Medeiros et al., 2013; Silva-Iturriza et al., 2012).

We found parasite communities with high modularity, indicating that parasite lineages had higher frequency and intensity of interaction in specific bird hosts within each vegetation type (see also Svensson-Coelho et al., 2014;

TABLE 2 Observed, null-nestedness, confidence intervals (CIs), mean, and SD of null NODF.

Vegetation types	WNODF observed	Null mean	Lower CI	Upper CI	Mean	SD	<i>t</i>	<i>p</i>
Metaweb								
Ifq	2.635	9.739	9.690	9.787	9.703	0.751	287.042	0
IIn	2.766	74.580	74.484	74.675	74.581	1.551	1478.125	0
Tropical sub-deciduous forest (TSDF)								
Ifq	8.163	19.367	18.968	19.766	19.093	6.888	55.135	<0.001
IIn	8.163	80.390	80.006	80.773	79.860	6.225	363.649	0
Tropical deciduous forest (TDF)								
Ifq	4.654	10.130	10.046	10.214	10.101	1.354	127.724	0
IIn	4.654	78.031	77.891	78.172	77.963	2.290	1024.726	0
Montane cloud forest (MCF)								
Ifq	3.000	25.595	25.249	25.941	25.330	5.654	128.256	0
IIn	3.000	82.131	81.875	82.387	82.128	3.939	607.196	0
Pine–oak forest (POF)								
Ifq	10.061	22.038	21.861	22.215	21.966	2.926	132.760	0
IIn	10.878	78.267	78.110	78.425	78.315	2.577	840.995	0

Abbreviations: NODF, nestedness based on overlap and decreasing fill; WNODF, weighted NODF.

Ventim et al., 2012). Recent studies that have analyzed the structure of antagonistic interaction networks have found that the organization of parasite networks have certain similarities (e.g., modularity and specialization level) even among different host–parasite systems. This suggests that the specialization that occurs in these organisms allows them to have composite network structures, with highly connected parasite submodules (Felix et al., 2022; Pinheiro et al., 2016). The high specialization of all communities contributes to the emergence of modules; this is expected due to a high niche partitioning that leads to the formation of species subsets highly connected to each other, forming modules of interactions (Fortuna et al., 2010; Vázquez et al., 2005). The modules were exclusive to each vegetation type, showing that haemosporidians and birds from different elevations are less likely to coexist, perhaps as a consequence of geographic and environmental barriers that restrict species distributions (Álvarez-Mendizábal et al., 2021; Pellegrino et al., 2021). High modularity pointed to a high number of peripheral species that increased the compartmentalization system and underlined the role of host specificity (Bellay et al., 2011; Lopes et al., 2020; Ventim et al., 2012). Also, interaction intensity values emphasized the aggregated pattern of parasites. For example, in TSDF (Appendix S1: Figure S2), the QUIMEX02_P and DENPET03_P lineages infected only *Quiscalus mexicanus*. Therefore, interaction intensity (i.e., parasitemia) data were especially important to record the tolerance of birds to a specific parasite lineage, and to compare how the

network structure is modified with individual- and species-level data.

We also found more interaction richness (S_I) in POF and TDF. This may result from the higher number of bird species recorded and the complex intra- and inter-lineage interactions within coinfections. This result is expected because interactions tend to be influenced primarily by species relative abundances, where communities with abundant hosts tend to harbor richer parasite faunas, and rare species with fewer links compared with abundant species result in specialists interacting mostly with abundant generalists (Vázquez et al., 2005). We observed this pattern in bird species such as *Vireo flavoviridis* and *Catharus occidentalis*, which harbored several lineages of *Haemoproteus* and *Leucocytozoon* respectively (Appendix S1: Figure S7). Several co-occurring parasites were closely related, so they may face minor spatiotemporal and physiological barriers within the host (Lopes et al., 2020; Pinheiro et al., 2016). Alternatively, distantly related parasites may have co-occurred because they did not face a process of competitive exclusion (Galen et al., 2019).

We recorded few shared interactions between vegetation types through the elevational gradient, as well as a high turnover of hosts and parasites. This pattern may indicate the effect of the environmental variation as a constraint on the parasite lineages and host distribution (birds and vectors) due to their specialized habitat requirements (Clark et al., 2017; Ellis et al., 2015). The effect of temperature on the elevational distribution has been

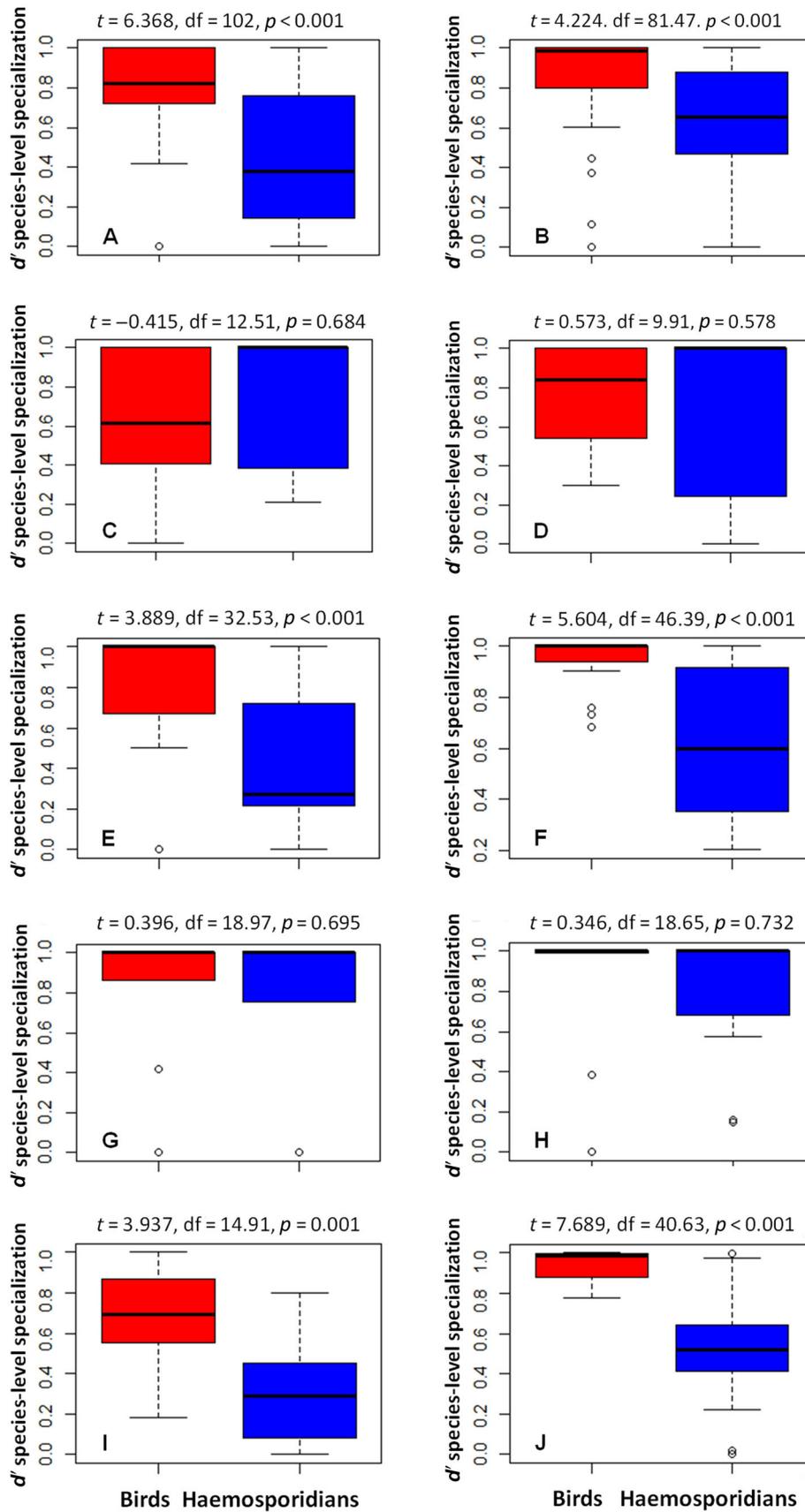


FIGURE 7 Comparison boxplot of specialization (d') for interaction frequency and interaction intensity of metaweb (A, B), TSDF (C, D), TDF (E, F), MCF (G, H), and POF (I, J) (see Figure 1 for an explanation of vegetation type abbreviations). A value of 0 indicates no interaction specialization, while a value of 1 indicates a perfect interaction specialist.

reported for both *Leucocytozoon* (located in sites with low temperatures, high elevation, and high humidity) and *Plasmodium* genus (which occurs in warm and low elevation sites) (Álvarez-Mendizábal et al., 2021; Barrow et al., 2021; González et al., 2015; Pellegrino et al., 2021). Furthermore, haemosporidian–bird interactions have been studied on islands and continental systems, and in both cases, they have been described as highly specialized. Yet, several studies have analyzed different variables that may influence network structure. For example, a study conducted on an island system recorded a differential distribution of haemosporidian parasite genera due to differences in temperature and humidity along the elevational gradient (Pellegrino et al., 2021), which is similar to the pattern recorded in our geographic area (Álvarez-Mendizábal et al., 2021). At the continental scale, there are variations in interactions due to the turnover of host species derived from bird migration movements (Lopes et al.,

2020); another study comparing temperate and tropical communities observed an effect on network structure due to the identity and phylogeny of the host species (Svensson-Coelho et al., 2014). Hence, the importance of different variables on the avian haemosporidian interaction networks will change depending on the scale of the study.

Some studies have suggested that generalist parasites are better adapted to a subset of host species across their host range (e.g., Hellgren et al., 2009), particularly those that are more abundant and that represent a stable reservoir for persistence and transmission, which via coevolution may result in parasite specialization (Huang et al., 2018; Moens et al., 2016; Svensson-Coelho et al., 2016). Detection of parasite specialization patterns through infection intensity (i.e., parasitemia) is a challenge that requires highly specific and accurate results that consider factors such as season, age, and infection phase (acute or chronic), aided by laboratory studies (Palinauskas et al., 2016). A strategy would be to conduct temporally continuous field monitoring to understand parasite intensity variations in wildlife, given that infection intensity values or parasitemia are dynamic and dependent on species interactions and habitat characteristics, which would modify the interaction network structure in a seasonal fashion (Huang et al., 2020).

TABLE 3 Measures of local interaction diversity (α -diversity) and turnover of species interactions (β -diversity) for four vegetation types at different elevations in Central Veracruz, Mexico.

Vegetation types	Interaction richness (S_j)	β_{os} index	Local network uniqueness (LNU)
TSDF	13	0.000	0.900
TDF	49	0.100	0.975
MCF	30	0.000	0.923
POF	58	0.086	0.975

Abbreviations: MCF, montane cloud forest; POF, pine–oak forest; TDF, tropical deciduous forest; TSDF, tropical sub-deciduous forest.

CONCLUSIONS

We found a highly specialized and modular network structure pattern in haemosporidian–bird interactions in

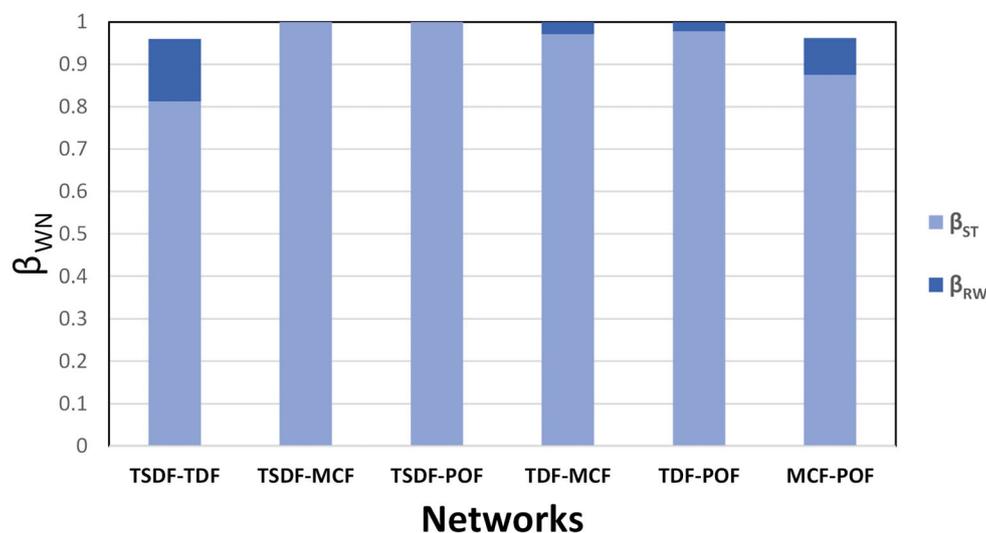


FIGURE 8 Additive partitioning proportion of the interactions turnover (β_{WN}) recorded in each vegetation type (TSDF, TDF, MCF, and POF; see Figure 1 for an explanation of vegetation type abbreviations). Each bar represents the value of the interaction turnover, divided into two colors: light blue represents the species turnover between communities (β_{ST}), dark blue denotes the interaction rewiring (β_{RW}), and bars represent the dissimilarity between the paired comparison of sites.

different types of vegetation across an elevational gradient in Central Veracruz, Mexico. This indicates the formation of highly interconnected haemosporidian–bird modules that are poorly connected across sites. Moreover, the pattern of specialization gets stronger when considering the intensity of haemosporidian–bird interactions, suggesting then that infection intensity networks provide valuable information to understand the structure of interactions. Finally, the distribution and dissimilarity of haemosporidian–bird interactions among vegetation types seem to be linked to environmental constraints, vector distribution and their feeding patterns, and host-specific requirements.

AUTHOR CONTRIBUTIONS

Karla Rodríguez-Hernández and Diego Santiago-Alarcon formulated the idea. Karla Rodríguez-Hernández, Paulina Álvarez-Mendizábal, and Diego Santiago-Alarcon conducted fieldwork. Karla Rodríguez-Hernández and Paulina Álvarez-Mendizábal generated sequencing data and molecular analyses. Karla Rodríguez-Hernández and Diego Santiago-Alarcon analyzed the data. Karla Rodríguez-Hernández and Diego Santiago-Alarcon wrote the manuscript. Paulina Álvarez-Mendizábal, Leonardo Chapa-Vargas, Federico Escobar, and Wesley Dáttilo provided editorial advice.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files. Data and R code (Rodríguez-Hernández et al., 2023) are available from Figshare: <https://doi.org/10.6084/m9.figshare.19126976>.

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

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