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Avian malaria in High-Andean *Polylepis* forests: Interaction network and host responses.

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Avian malaria in High-Andean *Polylepis* forests: Interaction network and host responses.

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DEDICATORIA

To my friends, who walk the same path, may our shared steps toward understanding and conserving life continue to grow stronger with each challenge and discovery.

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RESUMEN

Las interacciones parásito-hospedador son factores clave de las dinámicas ecológicas que influyen en la distribución de especies, el comportamiento y la aptitud biológica. La malaria aviar, que tiene como vector mosquitos, es causada por parásitos haemosporidios, que dañan los glóbulos rojos, afectando frecuentemente la salud de los hospedadores. Aunque la malaria aviar ha sido ampliamente estudiada en zonas bajas, evidencia reciente sugiere que los haemosporidios también persisten en sitios de gran altitud (sobre los 3000 m s.n.m.), indicando adaptaciones complejas a ambientes extremos. Este estudio presenta la primera caracterización de las interacciones hospedador-parásito en bosques de *Polylepis*, un ecosistema alto-andino único y poco estudiado (~4000 m s.n.m.) en los Andes tropicales. Nuestro objetivo fue (1) evaluar si las variables ambientales, fisiológicos o morfológicos de los hospedadores que influyen en la probabilidad de infección, (2) estimar la diversidad de parásitos haemosporidios, (3) describir la estructura de la red de interacciones hospedador-parásito y (4) investigar los efectos fisiológicos de la infección en los hospedadores mediante la medición de hemoglobina, hematocrito y condición corporal. Utilizamos técnicas moleculares dirigidas al gen del citocromo b mitocondrial de los parásitos. Nuestros resultados revelaron que 23 de 37 especies de aves estaban infectadas con parásitos Haemosporida, con una prevalencia general del 40.6%. Encontramos que la probabilidad de infección se asoció significativamente con la edad, el peso, las reservas de grasa y la altura del pico, lo que sugiere que el tamaño corporal, la condición física y el comportamiento de forrajeo modulan el riesgo de exposición. Registramos una alta prevalencia de parásitos del género *Leucocytozoon* y descubrimos 14 linajes previamente no reportados, resaltando la diversidad oculta de parásitos y el papel de los bosques de *Polylepis* como importantes reservorios. La red de interacción fue altamente modular y especializada, moldeada por la especificidad hospedador-parásito y las relaciones evolutivas. No se detectaron efectos fisiológicos significativos entre individuos infectados y no infectados, aunque el tamaño de muestra limitado por especie podría haber restringido la detección de estos efectos. Nuestros hallazgos destacan la complejidad ecológica y evolutiva de los sistemas hospedador-parásito en alta montaña y subrayan la necesidad de ampliar los estudios para comprender plenamente los factores claves de las dinámicas de infección en estos ecosistemas amenazados. Finalmente, nuestro estudio

proporciona una línea base para detectar cambios futuros, incluyendo la posible llegada de linajes de parásitos de tierras bajas que podrían amenazar a esta comunidad de aves.

Palabras clave: Ecología de enfermedades, parasitología aviar, ecosistemas de alta montaña, *Haemoproteus*, *Plasmodium*

ABSTRACT

Parasite-host interactions are key drivers of ecological dynamics, influencing species distribution, behavior, and fitness. Avian malaria, caused by haemosporidian parasites, is transmitted by mosquitoes and damages red blood cells, often impairing host health. Although avian malaria has been extensively studied in lowland areas, recent evidence suggests that haemosporidians also persist at high elevations (up to 3000 m a.s.l.), indicating complex adaptations to harsh environments. This study presents the first characterization of host–parasite interactions in *Polylepis* forests, a unique and understudied high-Andean ecosystem (~4000 m a.s.l.) in the tropical Andes. We aimed to (1) evaluate whether environmental, physiological, or morphological host traits influence infection probability, (2) assess haemosporidian parasite diversity, (3) describe the structure of the host–parasite interaction network, and (4) investigate the physiological effects of infection on hosts by measuring hemoglobin, hematocrit, and body condition. Using molecular techniques targeting the parasite mitochondrial cytochrome b gene. Our results revealed that 23 of 37 bird species were infected with Haemosporida parasites, with an overall sample prevalence of 40.6%. We found that infection probability was significantly associated with host age, weight, fat reserves, and beak height, suggesting that body size, condition, and foraging behavior modulate exposure risk. We recorded a high prevalence of *Leucocytozoon* parasites and discovered 14 previously unreported *Leucocytozoon* lineages, underscoring the hidden parasite diversity and the role of *Polylepis* forests as important reservoirs. The interaction network was highly modular and specialized, shaped by host–parasite specificity and evolutionary relationships. No significant physiological impairments were detected between infected and uninfected individuals, although limited sample sizes per species may have constrained the detection of subtle effects. Our findings highlight the ecological and evolutionary complexity of high-elevation host–parasite systems and underscore the need for expanded studies to fully understand the drivers of infection dynamics in these threatened ecosystems. Finally, our study provides a baseline to detect future changes, including the potential arrival of lowland parasite lineages that may threaten this bird community.

Keywords: Disease ecology, avian parasitology, high-altitude ecosystems, *Haemoproteus, Plasmodium*

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INTRODUCTION

Interactions between parasites, hosts and vectors, are important for understanding ecological processes in natural ecosystems. Parasites contribute to shaping ecological communities by altering ecological interactions, thereby influencing community structure and stability. For instance, parasite interactions can directly affect host abundance and distribution by reducing host fitness and altering behaviors (Marzal et al., 2005; Mouritsen & Poulin, 2005; Wood et al., 2007). Parasites may also influence predator-prey relationships and competition by increasing vulnerability of both parties (Møller & Nielsen, 2007). Additionally, they exert selective pressures on hosts by modifying their genetic diversity and adaptation, potentially driving coevolution across space and time (Bascompte, 2009; Latta & Ricklefs, 2010; Ricklefs et al., 2005, 2014). Thus, disentangling host-parasite interactions offers valuable insights into the ecology and evolution complex species relationships, and can be crucial for understanding broader ecosystem dynamics.

Avian malaria, caused by protozoan parasites of the order Haemosporida (genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), exemplifies complex parasite-host-vector relationships. These parasites rely on bloodsucking flies or mosquitos as vectors to infect avian host blood cells and organs to complete their life cycle (Atkinson et al., 2008; Valkiunas, 2005).The lineage of the parasite transmitted to the avian hosts depend on the vector group involved (Atkinson & Van Riper III, 1991; Valkiunas, 2005). *Plasmodium* is primarily transmitted by *Anopheles* mosquitoes and *Haemoproteus* is transmitted by *Hippoboscidae* and *Ceratopogonidae* flies, while *Leucocytozoon* is transmitted by *Simuliidae* flies (Atkinson & Van Riper III, 1991). Furthermore, the pathological effects of these parasitic infections in avian hosts may be acute, chronic or latent, depending on the parasites development and life cycle within different regions of

the host's body (Asghar et al., 2015; Valkiunas, 2005). Single infection or combinations of parasites (multiple or coinfection) can produce varying effects on avian host fitness (Marzal et al., 2005). For instance, Haemosporida infections may affect physical activity, social interactions, overall health (e.g. reduce hematocrit), reproductive success, and host survival (reviewed in Ellis et al., 2020). Additionally, multiple factors such as genetics, age, nutrition, immunology, etc., can influence the impact of the infection on the host (Atkinson & Van Riper III, 1991; Cornet et al., 2014; Ferraguti et al., 2018).

The evolution of haemosporidian parasites involves varying degrees of specialization towards their avian hosts, reflecting the ecological niches of both hosts and parasites, and it is influenced by host competence, regional factors, and climate conditions, which also shape parasite prevalence and distribution across populations (Doussang et al., 2021; Fecchio et al., 2011, 2019, 2020; Ellis et al., 2020; González et al., 2014; Valkiunas, 2005). Lower elevation regions, warmer temperatures and higher precipitation rates create favorable conditions for the development of blood sucking flies that serve as vectors of the parasites (Cosgrove et al., 2008; Zamora-Vilchis et al., 2012). These conditions accelerate the parasite´s life cycle, thereby increasing the probability of transmission (Santiago-Alarcon et al., 2012). Despite limitations in the presence of vectors in high-elevation environments, studies indicate the presence of avian malaria at these elevations, highlighting the resilience and adaptability of the parasites and their vectors (Fecchio et al., 2013; González et al., 2014; Moens et al., 2016; Theodosopoulos et al., 2023).

Polylepis forests are high-Andean fragmented ecosystems that harbor unique bird species and serves as a focal point for avian diversity (Fjeldså, 1993; Gareca et al.,

2010; Lloyd, 2008). Despite their fragmentation, these forests are connected by bird movement, as many species traverse habitat patches, creating dynamic ecological networks (Astudillo et al., 2020; Astudillo et al., 2019; Lloyd & Marsden, 2011) that may facilitate the dispersal of pathogens including avian malaria (Laurance et al., 2013). The unique ecological conditions of these forests (e.g. water storage, high-elevation) combined with the spatial configuration and fragmentation of patches, likely influence the diversity and distribution of avian hosts, mosquito vectors, and therefore haemosporidian parasites, with implications for transmission dynamics and adaptation strategies that can shape ecological communities and host fitness (Ferraguti et al., 2018; Laurance et al., 2013).

This study provides the first characterization of avian malaria parasites in *Polylepis* forests, focusing on which parasite lineages are present, their prevalence among host species, their interactions with bird hosts, and their impact in avian physiology. Specifically, our objectives are (1) determine if environmental, individual physiological condition, or morphological traits of the hosts constrain infection probability; (2) determine the diversity of Haemosporida parasites infecting the blood cells of bird species within *Polylepis* patches; (3) identify the interactions and levels of specialization among Haemosporida parasites; (4) assess the physiological responses of *Polylepis* associated birds infected with Haemosporida, focusing on variables such as haemoglobin and haematocrit levels, and body condition. Using *Polylepis* forest as a study system, we want to explore the ecological dynamics between hosts and parasites, and understand how these interactions affect avian communities in high-altitude environments.

We hypothesize (1) that infection probability in High-Andean birds is influenced by individual physiological condition, and morphological traits of the host. Specifically, we expect that individual condition traits, including age, fat reserves, and molt intensity affect susceptibility, with juveniles and birds in poorer physiological condition (e.g., low fat or active molt) being more vulnerable to infection due to weaker immune responses (Granthon & Williams, 2017; Schoenle et al., 2017). Furthermore, morphological characteristics (e.g., weight, tarsus, and wing length) may influence infection probability either because larger or heavier individuals offer a greater surface are for vector contact, or because certain morphologies are associated with behaviors that increase exposure to vectors (Jakubas et al., 2011; Yan et al., 2017). Based on these expectations, we predict that birds in poorer condition or with certain morphological traits, exhibit a higher probability of infection.

We also hypothesize that (2) *Leucocytozoon* parasites exhibit higher prevalence among the three genera (Fecchio et al., 2013; Fecchio, Silveira, et al., 2018; González et al., 2014; Lotta et al., 2016; Matta et al., 2014; Valkiunas, 2005; White et al., 1978).

Additionally, (3) given the relatively low diversity of avian hosts in high-elevation environments (Terborgh, 1977), we hypothesize that haemosporidian parasites follow a generalist infection strategy rather than exhibiting strict host specialization. Accordingly, we expect parasite diversity to be relatively similar across different bird species, reflecting ecological fitting, where parasites opportunistically exploit available host regardless of phylogenetic distance (Doussang et al., 2021; Fecchio et al., 2013; Moens et al., 2016; Moens & Pérez-Tris, 2016).

Lastly, (4) because Haemosporida parasites utilize haemoglobin as a nutrient source and cause damage to erythrocytes, we hypothesize that infected individuals show a reduced hemoglobin concentration and hematocrit levels (Ellis et al., 2015; Krams et al., 2013;

Palinauskas et al., 2008; Schoenle et al., 2017). However, the magnitude of these reductions may vary depending on the intensity of infection (parasitaemia) and the host's immune system (Ricklefs, 1992; Schoenle et al., 2017). Furthermore, we expect that infected birds exhibit lower body condition, reflecting the potential physiological trade-off between immune investment and energy allocation (Asghar et al., 2015; Coon et al., 2016; Cornet et al., 2014; Schoenle et al., 2017).

MATERIALS AND METHODS

Study area

This study was conducted in Papallacta, northern Ecuador, within Antisana and Cayambe-Coca National Parks. The area has three distinct vegetation types: *Polylepis* forest, páramo, and highland montane forest (Romoleroux et al., 2016). *Polylepis* forests are characterized by abundant water and dense vegetation, including bryophytes, vascular plants, lichens, and shrubs, that often make these forests impenetrable (Kessler, 2006; Romoleroux et al., 2016). Although we focused our sampling on *Polylepis* forests, these forests share several species with páramo and highland montane forest, which means that we sampled a suit of generalist (all vegetation types) and specialist (*Polylepis* forest) birds. We selected three sites across the study area: Laguna del Sucus at 3927 m a.s.l. (-0.3452, -78.1919), Virgen de Papallacta at 3987 m a.s.l. (-0.3421, -78.2012), and Ponce-Paluguillo at 3714 m a.s.l. (-0.3066, -78.2317) (Figure 1). These sites were selected to capture a range of birds' species associated to these high elevation forests.

Sample collection

We captured birds by mist-netting using nets of 6, 9, and 12 m long. Nets were placed inside *Polylepis* forests or in their borders, depending on the accessibility to each site. This sampling took place from 6:00h to 12:00h, peak bird activity time (Bibby et al., 1998). We accumulated a total mist-netting effort of 36,504 meter-hours (m·h) over 52 sampling days. Sampling effort was standardized across the three sites, with an equal number of sampling days allocated to each site between June 2024 and January 2025. Given the naturally lower bird densities in high-elevation Andean forests, sampling intensity was designed to maximize capture probability across sites.

We marked all birds captured to avoid recaptures: non hummingbirds with color leg bands and hummingbirds by cutting the first (left or right) tail feather. We collected blood from the jugular vein using a syringe of 30G for hummingbirds and 25–27G for the other birds. Blood was preserved in a 2-ml tube with absolute alcohol (99%) and a blood slide was made for each sample taken. Blood tubes were stored at room temperature in the field (4-10°C), and then at -20°C in the laboratory until further use (Musa et al., 2024). Blood slides were fixed with methanol (99%) within 24 hours of sampling, and then stained with Giemsa within 60 days of sampling. For physiological measurements of birds, we measured hemoglobin (g/dL) and hematocrit (%) levels with a hemoglobin analysis meter (Lysun, Hangzhou Lysun Biotechnology Co., LTD).

Individual condition variables consisted of age (classified as juvenile or adult), fat score, feather patch, body molt, and wing molt, each scored on a scale of absence, low, medium, or high. Morphological traits measured included wing length, tail length, beak dimensions (length, width, height), commissure length, and tarsus length, all recorded with a digital caliper (±0.02 mm). Co-variables to control the possible small variation

between sites included Julian day, weather conditions (categorized as cloudy, rainy, or sunny), sampling site, and elevation.

To assess potential multicollinearity among continuous variables, we calculated pairwise Pearson correlation coefficients. Variable pairs with high correlation ($r > 0.8$) were excluded from joint analyses to avoid redundancy. As a result, the following highly correlated pairs were excluded from models: tarsus length with beak length, tarsus length with weight, beak length with weight, and commissure with weight.

Molecular analyses

To determine the diversity of Haemosporida parasites infecting blood cells of the birds captured in *Polylepis* forests, we employed PCR amplification and sequencing of the parasites mitochondrial cytochrome b gene. We followed the Hellgren et al. (2004) protocol that enables identification of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasites. This protocol consists in an unspecific PCR with primers NF1/NR3 to amplify parasite mtDNA from genera *Haemoproteus*, *Plasmodium, and Leucocytozoon*. For the identification of the genus, we run specific nested PCRs using the product of the first PCR. The nested PCRs use genus primer pairs tailored for the parasite groups: HaemF/HaemR2 for *Plasmodium* and *Haemoproteus*, and a third PCR with HaemFL/HaemR2L for *Leucocytozoon*. Following amplification, the genus PCR products were visualized using agarose gel electrophoresis to confirm successful amplification of an approximate 478–480 base pairs fragment size. Then, PCR products underwent purification and sequencing using Sanger sequencing technology in an ABI prism 3100 genetic analyzer (Applied Biosystems/ Thermo Fisher Scientific, Carlsbad, CA) using the second PCR primers to obtain nucleotide sequences. Assembly of both DNA strands and consensus sequence editing were performed in Geneious 11.1.2 . In

cases of multiple infections, we were able to identify up to two distinct lineages either by visually resolving sequence ambiguities in the chromatogram (sequences with only one ambiguity) or when the co-infecting lineages clearly belonged to different genera as a result of PCR amplification with specific primers.

Data analyses

To assess whether infection prevalence was associated with co-variables, individual condition, or morphological traits, we conducted generalized linear models (GLMs) with a binomial error distribution, using infection status (infected or not infected) as the response variable. For each variable category, we constructed models including all possible additive combinations of predictors: 16 models for environmental variables, 32 for individual condition variables, and 256 for morphological traits.

All statistical analyses were conducted in R v4.3.2 (R Core Team, 2024). Model selection was performed using the *dredge* function from the *MuMIn* package, and models were ranked based on Akaike's Information Criterion corrected for small sample sizes (AICc; Burnham & Anderson, 2004). The best-fitting models, those with the lowest AICc, were used to generate prediction and distribution plots. To further assess variable importance, we conducted a Random Forest classification using the significant predictors identified in the GLMs (Breiman, 2001). This analysis allowed us to rank the variables most relevant for predicting infection.

For identification of parasite lineages, we compared the obtained sequences with published sequences available in the NCBI database (National Center for Biotechnology Information, 2023) and the MalAvi database (Bensch et al., 2009). Sequences were assigned to known lineages when they showed 100% identity with those in the

194 databases. Infections were classified as single when only one lineage was detected and
195 as multiple when a sample contained more than one parasite lineage. For downstream
196 analyses, we included infections falling into three categories: single infections, double
197 infections with clearly distinguishable lineages, and infections involving different
198 parasite genera, which were impossible to identify to the lineage level due to their
199 sequence ambiguity, which limited our ability to accurately assign parasite identities.
200 These later samples were excluded from further analyses.

201 For *Leucocytozoon* lineages, we constructed a phylogenetic tree including our
202 sequences, all the sequences of MalAvi, and the closest related sequences from NCBI.
203 Sequences were aligned using MUSCLE (Edgar, 2004), and the best-fit nucleotide
204 substitution model (GTR + F + R10) was selected based on the Bayesian Information
205 Criterion in IQ-TREE (Kalyaanamoorthy et al., 2017). Phylogenetic reconstruction was
206 performed with MrBayes v3.2.6 (Ronquist et al., 2012) in Geneious. The Markov Chain
207 Monte Carlo (MCMC) analysis was run for 2,000,000 generations, sampling every 200
208 generations. We used four chains (three heated, one cold) with a temperature parameter
209 of 0.2 to improve chain mixing. The phylogeny was visualized using FigTree v1.4.4
210 (Rambaut, 2018).

211 To analyze host-parasite interactions, we created a binary matrix where rows
212 represented *Polylepis*-associated bird species and columns represented Haemosporida
213 parasite lineages. Interactions were coded as 1 (present) or 0 (absent). This matrix was
214 used to build an interaction network using the *chordDiagram* function from the *circlize*
215 package in R v4.3.2. To assess parasite specialization, we calculated the species-level
216 specialization index d′ using the *specieslevel* function. To evaluate network structure,
217 we calculated metrics such as connectance, nestedness (NODF), and modularity (Q),
218 using the *networklevel* and *specieslevel* functions from the bipartite package, and

modularity (Q) from the *igraph* package. *Connectance* represents the proportion of observed interactions relative to the total number of potential interactions in the network; *Nestedness (NODF)* reflects the extent to which the interaction patterns of specialist species are subsets of those of generalists; and *Modularity (Q)* captures the degree to which the network is organized into distinct modules, clusters of species that interact more frequently among themselves than with species outside their group (Barber, 2007; Fortunato, 2010; Newman, 2006).

To evaluate the physiological responses of *Polylepis* birds infected with Haemosporida parasites, we analyzed the effect of infection on body condition using the Scaled Mass Index (SMI) (Peig & Green, 2009). We fitted generalized linear mixed-effects models (GLMMs) with SMI as the response variable, infection status as the fixed effect, and bird species as a random effect to account for interspecific variation. We used the same model structure to analyze hemoglobin and hematocrit levels. Additionally, we conducted species-specific analyses for the most abundant species: *Catamenia inornata* and *Diglossa humeralis.* For *C. inornata,* we fitted three linear models (LMs), one for each physiological parameter (SMI, hemoglobin, hematocrit), using infection as the predictor. For *D. humeralis*, we fitted three generalized linear models (GLMs) using the same predictor and response variables. All models were fitted using the *glm, lm,* and *glmer* functions from the *lme4* and *stats* packages (Bates et al., 2015) in R v4.3.2. For general data processing, we used the *dplyr* package (Wickham et al., 2019), and all visualizations were generated using the *ggplot* function from the *ggplot2* package (Wickham, 2009).

RESULTS

Malaria prevalence

We analyzed 197 blood samples from 37 bird species across 12 avian families. Of these, 23 species tested positive for haemosporidian parasites. The overall prevalence of Haemosporida infection was 40.6% (80/197), with the following distribution among parasite genera: *Plasmodium* 7.5% (6/80), *Haemoproteus* 10% (8/80), and *Leucocytozoon* 87.5% (70/80). Among infected individuals, 50% (40/80) exhibited single infections, 43.7% (35/80) harbored multiple infections, and 6.3% (5/80) could not be clearly resolved due to poor sequencing quality.

Prevalence by host species is shown in Table I and Figure 2. *Diglossa humeralis* was the most abundant species sampled, with an infection prevalence of 48.1% (13/27). *Aglaeactis cupripennis* was the second most abundant species (n = 19), but no infections were detected in this group. The third most abundant species, *Catamenia inornata*, showed a prevalence of 64.3% (9/14). At the family level, Thraupidae was the most abundant and showed the highest infection prevalence, contributing 51.3% (41/80) of all detected infections. In contrast, Trochilidae was the second most abundant family but was the only one in which no infections were detected.

We found no significant relationship between infection prevalence and co-variables. However, infection was significantly associated with individual condition variables: age (juveniles: $p < 0.01$) and fat reserves (low, medium and high: $p < 0.001$); and a positive relationship with morphological traits: beak height ($p < 0.001$) and weight (body mass) ($p < 0.001$) (Supplementary Table IV-V; Figure 4). A Random Forest classification identified species identity as the most important predictor of infection, followed by beak height, weight, age, and fat score (Table II-III; Figure 3). For details on model selection using the Akaike Information Criterion (AIC), refer to Supplementary Table I-III.

Parasite lineage identification

We were able to confidently identify 52 of the 80 infections (65%), while 27 remained unidentified, primarily due to unresolved co-infections. A total of 29 distinct lineages were detected: 23 belonging to *Leucocytozoon*, 3 to *Plasmodium*, and 3 to *Haemoproteus*, with *Leucocytozoon* representing the most diverse genus. We identified 14 novel lineages that, while showing high similarity to previously reported lineages, exhibited notable genetic divergence based on comparisons with sequences in the NCBI and MalAvi databases. These divergences were supported by our reconstructed phylogenetic tree (Figure 5). New lineages were defined following the established criterion that a single nucleotide substitution is sufficient to designate a novel lineage (Bensch et al., 2009).

Interaction Network

We successfully characterized host–parasite interactions between birds and Haemosporida parasites by constructing an interaction network (Figure 6). The network metrics revealed a high degree of specialization, evidenced by a low connectance value (0.083), indicating few realized interactions relative to all possible ones. Additionally, the network displayed low nestedness (NODF = 4.856), suggesting a lack of hierarchical structure and the presence of discrete interaction patterns. A high modularity value (Q = 0.643) further supported a compartmentalized network architecture, with distinct modules representing subsets of closely interacting host–parasite pairs (Table V; Figure 7).

To assess lineage-level specialization, we calculated the d’ specialization index for each parasite lineage (Table IV). This index revealed a spectrum of interaction strategies,

292 from generalists to specialists. Complete specialization (d' = 1.0) was observed for
293 JQ988715/L_HAPRUS01 and the novel lineage New_L3, indicating exclusive
294 associations with specific hosts. In contrast, H_TROAED15,
295 MN114077/P_CATUST05, KM211346/H_HEMATR01, and
296 MN458861/L_MYORN01 lineages exhibited generalist behavior (d' = 0), infecting
297 hosts that are commonly parasitized by other lineages.

298

299 **Infection effects**

300 Regarding the physiological responses of birds infected with Haemosporida parasites,
301 our results indicate no significant differences in scaled mass index (SMI; p = 0.183),
302 hemoglobin concentration (p = 0.964), or hematocrit levels (p = 0.980) between infected
303 and uninfected individuals (Figure 8). Similarly, in the two most abundant species,
304 *Catamenia inornata* and *Diglossa humeralis*, no significant differences were detected in
305 SMI (p = 0.135; p = 0.474), hemoglobin (p = 0.093; p = 0.113), or hematocrit (p =
306 0.097; p = 0.135) between infected and uninfected individuals (Figure 9). For detailed
307 results, see Supplementary Tables VI-XIV.

308

309 **DISCUSSION**

310 **Malaria prevalence**

311 We documented several novel host–parasite associations, likely due to the limited
312 research previously conducted in high-Andean environments. Studies of avian
313 haemosporidians have traditionally focused on *Plasmodium* and *Haemoproteus*, which
314 are more prevalent in lowland regions (Ranford-Cartwright, 2024; Sehgal, 2015). As a
315 result, the genus *Leucocytozoon* has often been overlooked or excluded from analyses

316 (González et al., 2015). Only recently have high-elevation studies begun to include
317 *Leucocytozoon*, recognizing its higher prevalence in cold environments (González et al.,
318 2014; Lotta et al., 2016; Matta et al., 2014; Rodríguez et al., 2009).

319 We anticipated encountering a variety of *Leucocytozoon* parasites given their
320 association with high-elevation environments and its strict distribution at highlands
321 (González et al., 2014). However, the observed prevalence of 87.5% was high compared
322 to previous reports from similar elevations, for example, 45.4% reported by González-
323 Quevedo et al. (2016) and 6.4% by Lotta et al. (2016). Only Rodríguez et al. (2009)
324 reported a comparable prevalence (76.3%), although within a lower total infection rate
325 (27.9%). This findings suggest that previous studies may have underestimated the
326 prevalence and diversity of *Leucocytozoon*, a parasite well adapted to cooler climates
327 that enhance its development and transmission (Imura et al., 2012; Valkiunas, 2005).
328 While mosquito vectors of *Plasmodium* and *Haemoproteus* are typically less abundant
329 at high elevations, black flies (*Simuliidae*), the primary vectors of *Leucocytozoon*, may
330 be more prevalent under these environmental conditions (Imura et al., 2012; Rooyen et
331 al., 2013; Valkiunas, 2005). Our high prevalence supports this ecological pattern, and
332 with 14 novel lineages within the genus, suggesting active local transmission and a
333 potentially underestimated diversity of parasites in these high-elevation forests.

334 Moreover, multiple infections were common (43.7%, 35/80), including both co-
335 infections between different parasite genera and between lineages within the same
336 genus. Although this pattern is frequently reported in wild bird populations and is
337 considered a common feature of avian haemosporidian infections (Lotta et al., 2016;
338 Pérez-Tris & Bensch, 2005; Rooyen et al., 2013), our observed prevalence still high
339 relative to other high-elevation studies (e.g. 18.2% in Gonzalez-Quevedo et al.; 39.3%
340 in Lotta et al., 2016). While the ecological and physiological consequences of multiple

infections remain poorly understood. Ribeiro et al. (2005) proposed that immune suppression caused by an initial infection could reduce host mobility, thereby increasing exposure to additional infections by prolonging contact with vectors. Conversely, Clark et al. (2016) argue that co-infections are not necessarily more virulent, particularly when they involve chronic, low-intensity infections that persist without eliciting strong immune responses or triggering competitive exclusion among parasite lineages. Clearly, more research is needed to disentangle the effect of multiple infections by Haemosporida parasites, and the *Polylepis* forest emerges as a great study system given the high prevalence of multiple infections unveiled by this analysis.

At the species level, *Diglossa humeralis* was the most frequently captured species and exhibited a relatively high infection prevalence (48.1%, 13/27), while *Catamenia inornata*, the third most abundant species, also showed high prevalence (64.3%, 9/14). These elevated infection rates may be attributed to species specific behavioral and ecological traits, such as foraging strategy, habitat preference, and daily activity patterns, that not only increase their susceptibility to vector-borne parasites but also, make them more likely to be captured in mist nets. These traits could therefore help explain both their high infection prevalence and their abundance in our sampling. Such traits have been shown to influence host-vector interactions and infection risk in avian populations (Chahad-Ehlers et al., 2018; Fecchio et al., 2022; Ferraguti et al., 2018).

At the family level, Thraupidae was the most abundant in captures and accounted for the majority of infections (51.3%, 41/80). This result aligns with previous research indicating that certain families may experience higher infection risks due to shared ecological traits, shared physiologies, and similar interactions with vectors (Loiseau et al., 2010; Ricklefs et al., 2005). Although haemosporidian infections have been

documented in hummingbirds in other regions (e.g., González et al., 2014; Lotta et al., 2016; Matta et al., 2014; Moens et al., 2016), we detected no infections in *Trochilidae* in our study, including *Aglaeactis cupripennis*, the second most abundant species in our sample.

MalAvi database reports only one *Haemoproteus*, one *Plasmodium* and no *Leucocytozoon* in *Aglaeactis cupripennis*. The reduce infection rates in this species may be attributed to ecological and physiological adaptations, including potential resistance to infection (Sorci, 2013). High-elevation environments impose strong selective pressures on metabolic processes, energy regulation, and immune responses, which could drive local adaptations that enhance resistance to parasitic infections (Ishtiaq & Barve, 2018). Moreover, rapid flight, a high metabolic rate, and unique foraging strategies (Buermann et al., 2011) may reduce exposure to vectors and improve the response to parasites. Additionally, black flies (*Simuliidae*), presumed to be the most abundant vectors at these elevations, may have limited feeding success on hummingbirds due to their rapid and erratic flight patterns, and small body mass (Malmqvist et al., 2004; Yan et al., 2017). Therefore, while Trochilidae are not inherently immune to haemosporidian infection, our findings suggest that environmental constraints at high elevations with potential local physiological adaptations, may reduce their risk of exposure, supporting previous suggestions that hummingbirds may be poor reservoirs for malaria parasites (Moens et al., 2016) under certain ecological conditions.

Murdock et al. (2013) emphasize that vector phenology is a critical factor through which seasonal changes most strongly influence disease transmission, particularly for *Leucocytozoon*. In our study, we did not find any significant relationship between

389 infection prevalence and co-variables (e.g. weather, Julian day) likely due to the small
390 variation between sites and the short temporal scale of our sampling. Additionally, in
391 tropical systems where seasonality operates differently than in temperate zones, parasite
392 transmission can be more continuous or driven by alternative ecological cues (e.g.
393 McNew et al., 2019).

394 Infection status was significantly associated with age, weight, fat score, and beak
395 height. Among these variables, age played a role with juvenile birds showing
396 significantly higher susceptibility to haemosporidian infection compared to adults. This
397 pattern may be attributed to the underdeveloped immune systems of juveniles, which
398 can make them less effective at controlling infections (Sol et al., 2003). Alternatively,
399 the increased vulnerability of younger birds might stem from their limited foraging
400 experience, which can elevate stress levels and compromise immune function (Jakubas
401 et al., 2011). Moreover, juveniles could have acquired infections during the nestling
402 stage due to their limited mobility inside the nest and heightened exposure, particularly
403 in open nests (Fecchio et al., 2022; Rodriguez et al., 2021). These explanations are
404 biologically plausible and have been reported in other avian systems. However, it is
405 important to note that in our study, juveniles were underrepresented in the sample
406 relative to adults, which could introduce a bias and affect the strength of the observed
407 association.

408 Weight, interpreted as a proxy for body mass, was another significant predictor of
409 infection probability, with heavier, or generally larger, individuals being more
410 frequently infected. This pattern may be explained by the fact that larger birds offer a
411 greater surface area, increasing their exposure to biting vectors (Yan et al., 2017).
412 Additionally, larger individuals tend to emit greater amounts of CO_2 and body heat, both
413 of which serve as strong attractants for blood-feeding insects (Figuerola et al., 2008;

Takken & Verhulst, 2013). In particular, black flies, the primary vectors of *Leucocytozoon*, are known to prefer larger hosts (Malmqvist et al., 2004). They are also attracted to chemical cues such as the odors secreted by the uropygial gland in birds (Martínez-de la Puente et al., 2011; Russell & Hunter, 2005), further increasing the likelihood of infection in larger individuals.

Fat reserves were also a significant predictor of infection status, with individuals exhibiting visible fat deposits being more likely to be infected with haemosporidian parasites. One possible explanation is that birds with higher fat reserves have greater energetic capacity, allowing them to engage more actively in foraging and to explore resource-rich areas habitats that may also support higher densities of haemosporidian vectors such as mosquitoes and black flies, thereby increasing exposure risk. For instance, Enslow et al. (2023) reported a positive association between *Leucocytozoon* prevalence and bird species that forage in environments conducive to black fly proliferation. Similarly, Fecchio et al. (2022) highlighted that habitat use and foraging strategy significantly influence haemosporidian infection patterns. In addition, birds in better body condition with high fat reservoirs may serve as more suitable hosts for parasite development due to enhanced metabolic resources that support both host and parasite survival (Gutiérrez-Ramos & Acevedo, 2024). This suggests that higher fat scores may not only correlate with increased vector exposure but also reflect host suitability for sustaining infections. However, this interpretation should be approached with caution, as parasite infections can also induce metabolic alterations, often termed "metabolic syndrome", through immune activation and inflammatory responses, which may elevate carbohydrate levels in blood and contribute to increased fat accumulation in infected individuals (Gutiérrez-Ramos & Acevedo, 2024; Schilder & Marden, 2006).

In addition, beak height was the strongest morphological predictor of infection status. Although this relationship is less intuitive, it may also relate to foraging behavior (Fecchio et al., 2022). Birds with taller or more robust beaks could be adapted to forage in dense vegetation or on substrates that coincide with vector-rich microhabitats, such as shrubs or bark crevices where mosquitoes or black flies may be more prevalent. This suggests that morphological traits associated with resource acquisition could inadvertently increase exposure to haemosporidian parasites. Still, the precise ecological mechanisms underlying these associations remain unclear and warrant further investigation to better understand how behavioral and morphological traits influence infection dynamics.

Parasite lineage identification

Our phylogenetic analyses revealed that several newly identified haemosporidian lineages exhibit strong host associations, potentially indicating host specificity. For instance, *Turdus fuscater* hosted four novel lineages (New_L9, New_L12, New_L13, and New_L14), none of which were found in any other bird species. Notably, *T. fuscater* was the only representative of the family Turdidae in our study, and this pattern is consistent with previous findings by Lotta et al. (2016), who also reported a high degree of host specificity in Turdidae. Similarly, *Grallaria quitensis* harbored two unique lineages (New_L4 and New_L5), which is notable given the limited record of parasites in the Grallariidae family (Lotta et al., 2015). This further supports the hypothesis that certain haemosporidian lineages may be restricted to specific host genera or families. A similar pattern was observed in *Atlapetes latinuchus* (New_L3) and *Myioborus melanocephalus* which carried the lineage JQ988715/L_HAPRUS01. These exclusive associations may reflect co-evolutionary histories between parasites

and hosts or may be shaped by host-specific physiological or immunological constraints.

While these patterns support potential host specificity, it remains unclear whether haemosporidian diversity is primarily shaped by host phylogenetic relationships or by ecological factors such as habitat overlap and foraging behavior (Chahad-Ehlers et al., 2018; Fecchio et al., 2022; Laurance et al., 2013). Some studies suggest that both host evolutionary history and ecological similarity can structure parasite communities (Fecchio, Bell, et al., 2019; Svensson-Coelho et al., 2013).

Interaction Network

The structure of our host–parasite interaction network in high-Andean *Polylepis* forests, reveals a highly specialized, weakly nested, and strongly modular system. These structural features probably reflect a combination of ecological and evolutionary processes, including host immune variation, parasite host specificity, vector ecology, and environmental filtering (Runghen et al., 2021). Together, they suggest that avian haemosporidian parasites in this ecosystem interact in a compartmentalized manner, with limited overlap in host use. This architecture may restrict parasite spillover across host groups and potentially buffer bird communities against broad-scale outbreaks, while also suggests long-term co-evolutionary associations between particular host and parasite lineages (Bellay et al., 2015; Poulin, 2011; Ricklefs et al., 2014).

Specialization in interaction networks often reflects resource partitioning. While specializing in a limited number of hosts can enhance parasite fitness by allowing fine-tuned exploitation of specific host traits, it also increases vulnerability (Colwell et al., 2012; de Angeli Dutra et al., 2021). Parasites that rely on a narrow range of hosts are

more susceptible to local extinction events or environmental disruptions, which may reduce host availability (Colwell et al., 2012; Fecchio, Wells, et al., 2019). Hence, highly specialized networks like ours tend to be less resilient to disturbances. However, the presence of strong modularity can counterbalance this fragility by localizing perturbations, if one module is disturbed, the effects are less likely to cascade across the network (Grilli et al., 2016).

The low nestedness observed reinforces the idea of a non-hierarchical, compartmentalized network. In nested networks, specialist parasites typically infect subsets of hosts used by generalists. The lack of this pattern in our results keeps affirming distinct host-parasite pairings, possibly shaped by host and vector traits, ecological niches, or transmission barriers. This finding challenges the predictability hypothesis proposed by Svensson-Coelho et al. (2016), which suggests that parasite specialization is driven by host abundance. Contrary to that prediction, we observed cases where parasite lineages were complete specialists on hosts that were not among the most abundant species. For instance, *Myioborus melanocephalus* and *Atlapetes latinuchus* were each associated with complete specialist parasites despite their relatively low abundance in our sample. This pattern implies that specialization in avian malaria is not strictly determined by host abundance and may vary across environments and time, particularly in high-elevation systems with limited host availability (Fecchio, Wells, et al., 2019).

At the species level, our results reflect a typical pattern in avian malaria systems: a mixture of specialist and generalist lineages. Some parasite lineages, such as JQ988715/L_HAPRUS01 and the newly identified New_L3, were complete specialists (d' = 1), each infecting a single, rarely parasitized host. These lineages may face reduced transmission potential due to their host restriction, posing a risk to their persistence, as

suggested by Woolhouse et al. (2001). Nevertheless, over time and space, evolutionary pressures (e.g. vector selection, phylogenetic constrains, local adaptation) may drive these specialists to eventually cross host species barriers, broadening their host range and transforming into generalist pathogens, a dynamic that has been previously documented for avian haemosporidians (Alcala et al., 2017; Fecchio, Bell, et al., 2018; Ricklefs et al., 2014). This potential shift challenges static interpretations of parasite strategies and adds nuance to our understanding of resource predictability and host-use evolution. Interestingly, when the evolutionary cost of host-switching is relatively low, another common mechanism shaping the structure of host-parasite interactions is duplication, where parasites diversify within a single host lineage. In some cases, this process may even occur more frequently than host switching, highlighting the complex and dynamic pathways through which host–parasite associations evolve (Ricklefs et al., 2004).

Conversely, several parasite lineages (e.g., H_TROAED15, H_HEMATR01, MN114077/P_CATUST05) behaved as complete generalists (d' = 0), infecting a wide range of hosts or appearing in hosts frequently parasitized by other lineages. This generalist strategy may stem from greater genetic plasticity or broader vector compatibility, enabling infection across diverse bird species. However, generalist parasites may still face limitations based on host immune defenses, behavior, habitat preferences, and the ecology of vectors that mediate transmission (Chahad-Ehlers et al., 2018; Hellgren et al., 2008; Santiago-Alarcon et al., 2012; Schoenle et al., 2017; Takken & Verhulst, 2013). Furthermore, the remainder of parasite lineages exhibited intermediate levels of specialization (d' = 0.2–0.7), which could reflect either weak host preferences, or ecological filtering based on spatial overlap, vector exposure, host immunology or behavioral compatibility.

One intriguing pattern emerged from field observations: *Anisognathus igniventris* and *Urothraupis stolzmanni*, which were observed foraging in the same mixed-species flock shortly before capture, both carried the same *Leucocytozoon* lineage. This pattern supports the idea that flocking behavior can facilitate shared vector exposure and increase the likelihood of infection by the same parasite lineages (Menzies et al., 2021). Our finding aligns with González et al. (2014), who reported higher haemosporidian prevalence in bird species that participate in mixed-species flocks. Foraging behavior and social interactions, therefore, represent important ecological mechanisms that may shape host-parasite networks by modulating contact rates with insect vectors (Poulin, 2018).

Infection effects

The pathogenicity of parasite lineages may vary and could be influenced not only by the parasite genotype but also by host-specific immune responses. Although better body condition is often associated with a stronger immune response to parasitic infections, our results did not show significant differences in scaled mass index (SMI), hematocrit (HCT), or hemoglobin (HB) levels between infected and uninfected individuals. This lack of variation suggests that the parasites present in our study system may not be exerting a detectable physiological cost on their avian hosts. A possible explanation is that infected birds are able to efficiently control the infection through an effective immune response, resulting in low levels of parasitaemia. This pattern is consistent with chronic infections commonly observed in wild birds (Granthon & Williams, 2017; Santiago-Alarcon & Marzal, 2020) where the parasite burden is minimized and the physiological impact remains low.

Contrary to Garvin et al. (2006) that reported a reduced body condition in passerines infected with blood parasites, the infected individuals in our system may be exhibiting tolerance strategies, maintaining body condition (SMI) despite infection, or experiencing chronic infections with low parasitaemia that do not significantly reduce nutritional status (Dyrcz et al., 2005; Granthon & Williams, 2017; Santiago-Alarcon & Marzal, 2020).

Hematocrit and hemoglobin levels reflect oxygen carrying capacity and the abundance of blood cells, and low levels are indicative of physiological stress resembling anemia induced by parasite infections (Krams et al., 2013; Schoenle et al., 2017). Such effects can potentially impair energy intensive activities such as flight, parental care, thermoregulation and can reduce fitness of the hosts (Dyrcz et al., 2005; Fronstin et al., 2016; Merino et al., 2000). Although we observed several individuals with hemoglobin and hematocrit levels outside the typical avian reference range of 35–55% (Fair et al., 2007), these deviations may reflect adaptive responses to high-elevation hypoxia rather than parasitic stress. In high-elevation environments, reduced atmospheric oxygen pressure can stimulate erythropoietin production, leading to an increase in red blood cell concentration as a compensatory mechanism to enhance oxygen-carrying capacity (Fair et al., 2007). Conversely, we detected some individuals with hematocrit values below 29% who were not infected suggesting that other factors, such as nutritional deficiencies, reproductive effort, age, or environmental stressors, may contribute to reduced hematological parameters in this ecosystem (Brown et al., 2021; Johnstone et al., 2017; Kausar et al., 2025; Williams et al., 2004). Despite growing interest, the physiological consequences of haemosporidian infections in wild birds remain poorly understood, especially in high-elevation ecosystems where environmental pressures may interact with parasite effects in complex ways (Forrester & Greiner, 2008).

Concluding remarks

Although this work was based on an extensive eight-month field campaign, it still represents an initial step toward understanding host-parasite dynamics in this region. One limitation of our study is the relatively low sample size for certain species, which may have reduced our ability to detect potential physiological responses to infection at the species level, as well as limited the detection of rare parasite lineages. Future studies focusing on single-species analyses with larger sample sizes will be crucial to better understand species-specific responses to infection, uncover the underlying physiological mechanisms, and clarify host–parasite specificity patterns. Additionally, continued efforts incorporating multi-seasonal and multi-site sampling, detection of co-infections, and trait-based network analyses will be essential to uncover the functional drivers of host–parasite associations and inform conservation strategies in these increasingly threatened high-Andean ecosystems.

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TABLES

Table I. Haemosporida lineages obtained from avian hosts.

		NCBI			MalAvi		
Species	Prevalence	Lineage	Identity	Query	Lineage	Identity	Query
Anairetes parulus	1/8	New_L1					
Anisognathus igniventris	3/4	*Leucocytozoon* sp. JQ988120/MN459610	100	98	L_MYFUM01	100	97
		New_L6					
		New_L2					
Arremon assimilis	3/4	*Leucocytozoon* sp. MN459156	99.79	97	L_ATLSCH01	99.791	97
		New_L11					
Asthenes fuliginosa	2/11				L_TROAED06	100	96
		New_L8					
Atlapetes latinuchus	8/8	New_L3					
Atlapetes pallidinucha	3/3	New_L11					
Catamenia homochroa	1/1	*Leucocytozoon* sp. KF717050	100	95	L_CAINO01	100	95
Catamenia inornata	7/14	*Plasmodium lutzi* MN114077	100	100	P_CATUST05	100	96
		Leucocytozoon sp. KF717050	100	95	L_CAINO01	100	95
		New_L7					
		Plasmodium lutzi KF537284/KJ780795/KF537276	100	100	P_DIGLAF01	100	96
		Haemoproteus sp. KC121057	99.60	100	H_TROAED15	100	97
		Plasmodium lutzi MN114077/KX867096/KY653815/KF537312/KY653816	100	100	P_CATUST05	100	97
Cnemathraupis eximia	1/1	?					
Conirostrum cinereum	4/4	New_L7					
		New_L2					
		New_L10					
		Leucocytozoon sp. MN458839/MN459548	99.59	99	L_ZOCAP07	100	96
Diglossa humeralis	13/27	*Plasmodium* sp. KT1936331	99.78	100	P_ZONCAP15	100	97
		Leucocytozoon sp. MN458861	100	99	L_MYORN01	100	98

		Plasmodium sp. EU627827/ON227240/ON227235/ON227221/KX867099	99.79	100	P_ZONCAP15	100	98
		Leucocytozoon sp. MN459610	100	97	L_MYFUM01	100	96
		New_L2					
		Plasmodium lutzi MN114077/KX867096/KY653815/KF537312	100	100	P_CATUST05	100	97
		Leucocytozoon sp. MN458839/MN459548	99.78	100	L_TROAED02	100	98
		Haemoproteus sp. KM211346	100	100	H_HEMATR01	100	96
		Plasmodium lutzi MN114077/KX867096/KY653815/KF537312	100	100	P_CATUST05	100	96
		Haemoproteus sp. KM211346/KM211352	100	100	H_HEMATR01	100	97
		Leucocytozoon sp. KF717050	100	96	L_CAINO01	100	96
		Haemoproteus sp. KC121057	99.60	100	H_TROAED15	100	96
		New_L10					
Diglossa lafresnayii	4/5	*Leucocytozoon* sp. MN459519/MN459531	98.99	100	L_METYR01	100	96
		New_L2					
Geospizopsis unicolor	5/7	New_L1					
		Plasmodium cathemerium MK077679	100	100	P_ZONCAP15	100	100
		New_L10					
Grallaria quitensis	6/6	New_L4					
		New_L5					
Margarornis squamiger	2/8	*Haemoproteus* sp. KY002546	99.78	100	H_PHRATR01	100	99
		Leucocytozoon sp. MN459519/MN459531	99.19	100	L_METYR01	100	96
Mecocerculus leucophrys	2/11	*Leucocytozoon* sp. MN459519/MN459531	99.20	100	L_METYR01	100	96
Myioborus melanocephalus	2/6	JQ988715	100	96	L_HAPRUS01	100	96
Ochthoeca fumicolor	2/11	*Leucocytozoon* sp. MN459548/MN458839	100	99	L_TROAED02	100	98
Scytalopus latrans	1/1	?					
Silvicultrix frontalis	1/3	*Leucocytozoon* sp. MN459548/MN458839	99.38	100	L_TROAED02	99.788	97
Turdus fuscater	5/5	New_L11					
		New_L14					
		New_L13					
		New_L12					

		New_L9					
Urothraupis stolzmanni	3/4	New_L2					
		Leucocytozoon sp. KF717050	100	96	L_CAINO01	100	96
Zonotrichia capensis	1/2	New_L8					

Table II. Variable importance of the Random forest classification of the significant variables reported.

Variable	Mean Decrease Accuracy	Mean Decrease Gini
Species	36.72	30.75
Beak height	23.51	26.54
Weight	18.03	20.76
Age	2.29	2.45
Fat	0.08	6.11

Table III. Summary of random forest results.

Metric	Value / Description
OOB Error Rate	30.32%
	Confusion Matrix
True Class 0 (not infected)	82 correct, 30 incorrect (Error: 26.79%)
True Class 1 (infected)	49 correct, 27 incorrect (Error: 35.53%)

Table IV. Values of the specialization index for parasite lineages.

Lineages /Sequence ID	d’
H_PHRATR01	0.6989700
H_TROAED15	0.0000000
JQ988715/L_HAPRUS01	1.0000000
KF537284/KJ780795/KF537276/P_DIGLAF01	0.3010300
KF717050/L_CAINO01	0.3333333
KM211346/H_HEMATR01	0.0000000
L_METYR01	0.7489262
L_TROAED06	0.6989700
L_ZOCAP07	0.3979400
MN114077/P_CATUST05	0.0000000
MN458861/L_MYORN01	0.0000000
MN459156/L_ATLSCH01	0.6989700
MN459548/MN458839/L_TROAED02	0.5829754
MN459610/L_MYFUM01	0.1305784
New_L1	0.7191703
New_L10	0.1329302
New_L11	0.5829754
New_L12	0.3010300
New_L13	0.3010300
New_L14	0.3010300
New_L2	0.2288905
New_L3	1.0000000
New_L4	0.6989700
New_L5	0.6989700
New_L6	0.5228787
New_L7	0.2342243
New_L8	0.8228162
New_L9	0.3010300
P_ZONCAP15	0.1305784

Table V. Measures at the network level.

Connectance	Nestedness NODF	Modularity (Q)
0.083	4.856	0.643

FIGURES

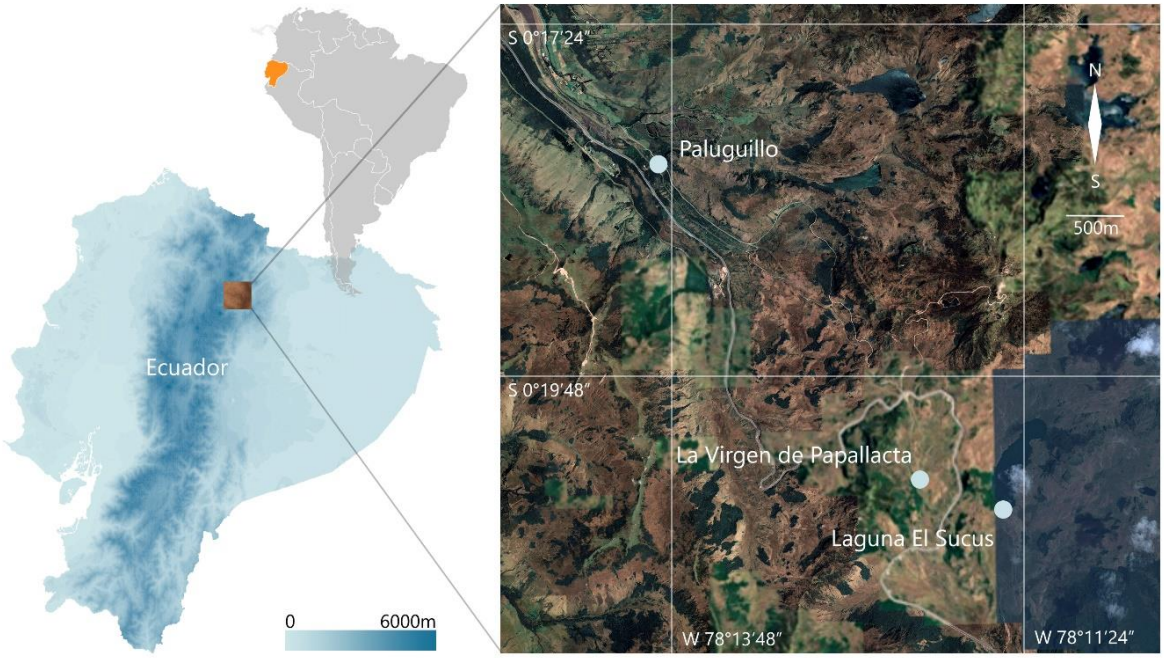

Figure 1: Map showing the location of the three selected study sites in the Papallacta region, Ecuador. Sites are marked with blue circles and include: Laguna El Sucus (0°20′42.7″ S, 78°11′30.8″ W), La Virgen de Papallacta (0°20′31.6″ S, 78°12′4.3″ W), and Paluguillo (0°18′23.8″ S, 78°13′54.1″ W). These High-Andean *Polylepis* forest remnants were surveyed to assess avian malaria prevalence and host–parasite dynamics at similar elevation

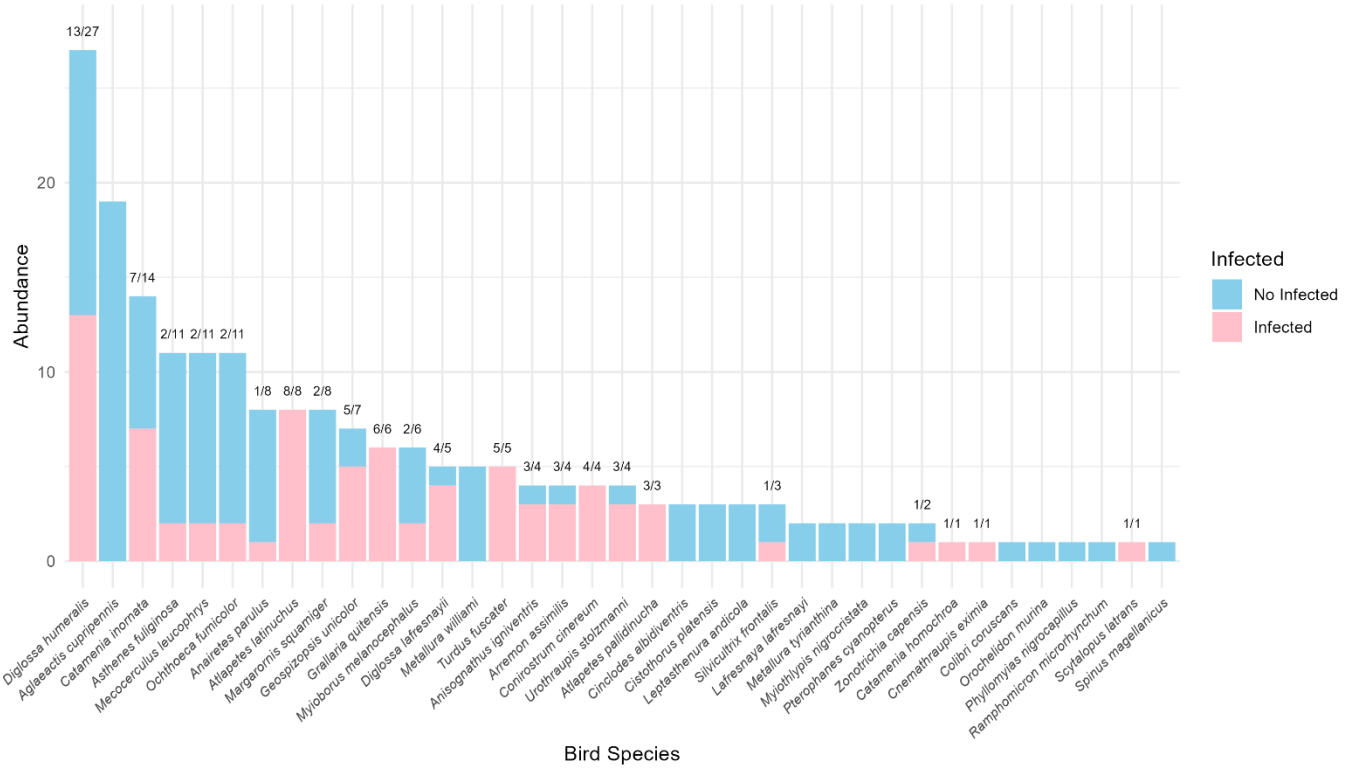

Figure 2. Prevalence of haemosporidian infection across bird species. Bars represent the abundance of the species and bars filled with pink color are the proportion of infected individuals, highlighting variation in susceptibility among host taxa.

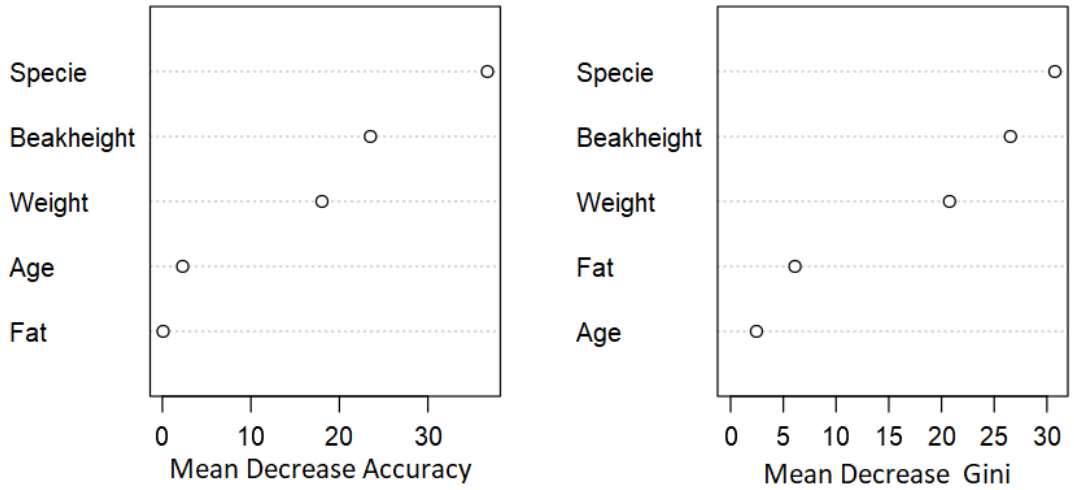

Figure 3. Random forest classification of significant variables.

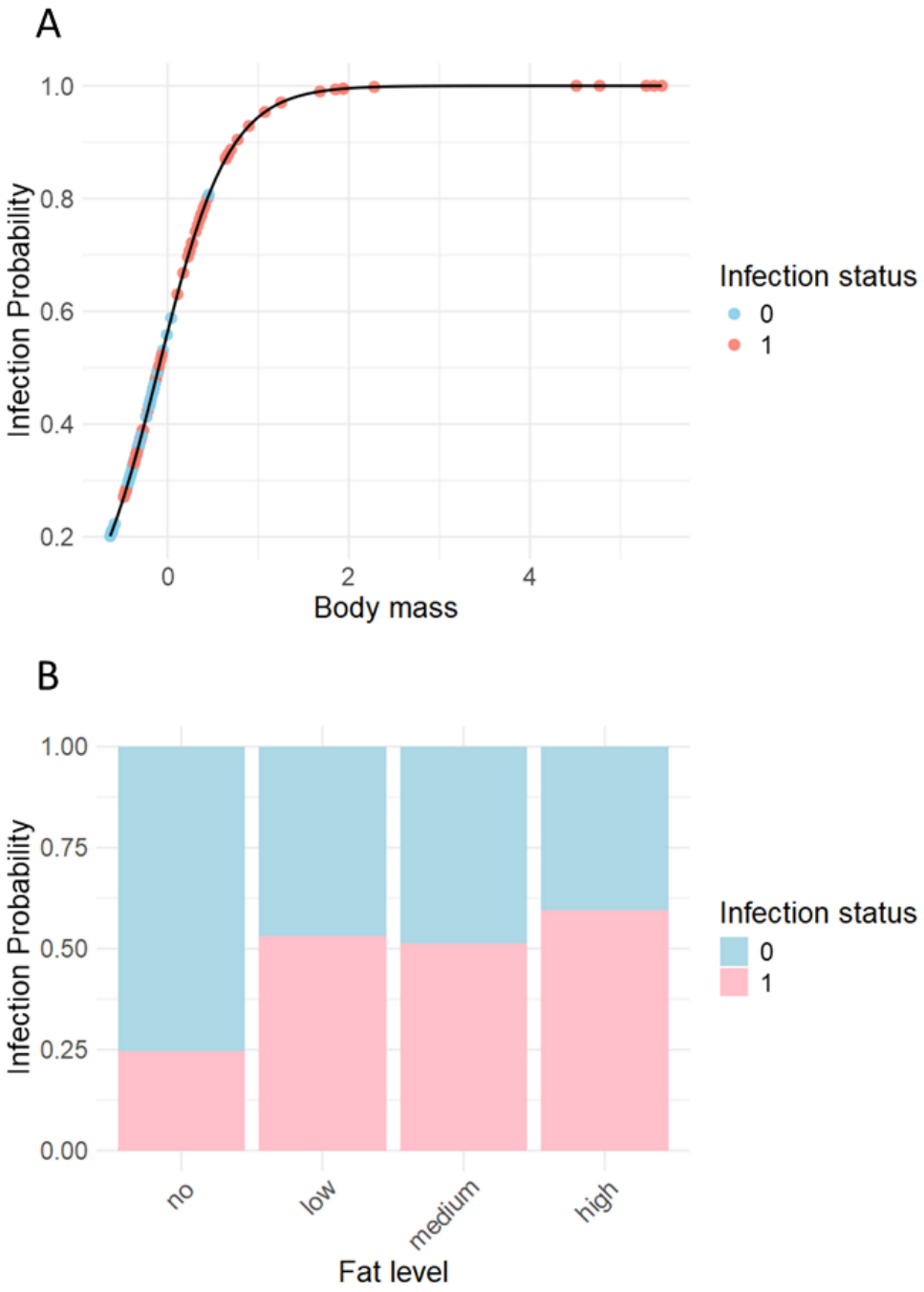

Figure 4. Infection probability based on significant predictors. (A) Body mass (weight) and (B) fat score.

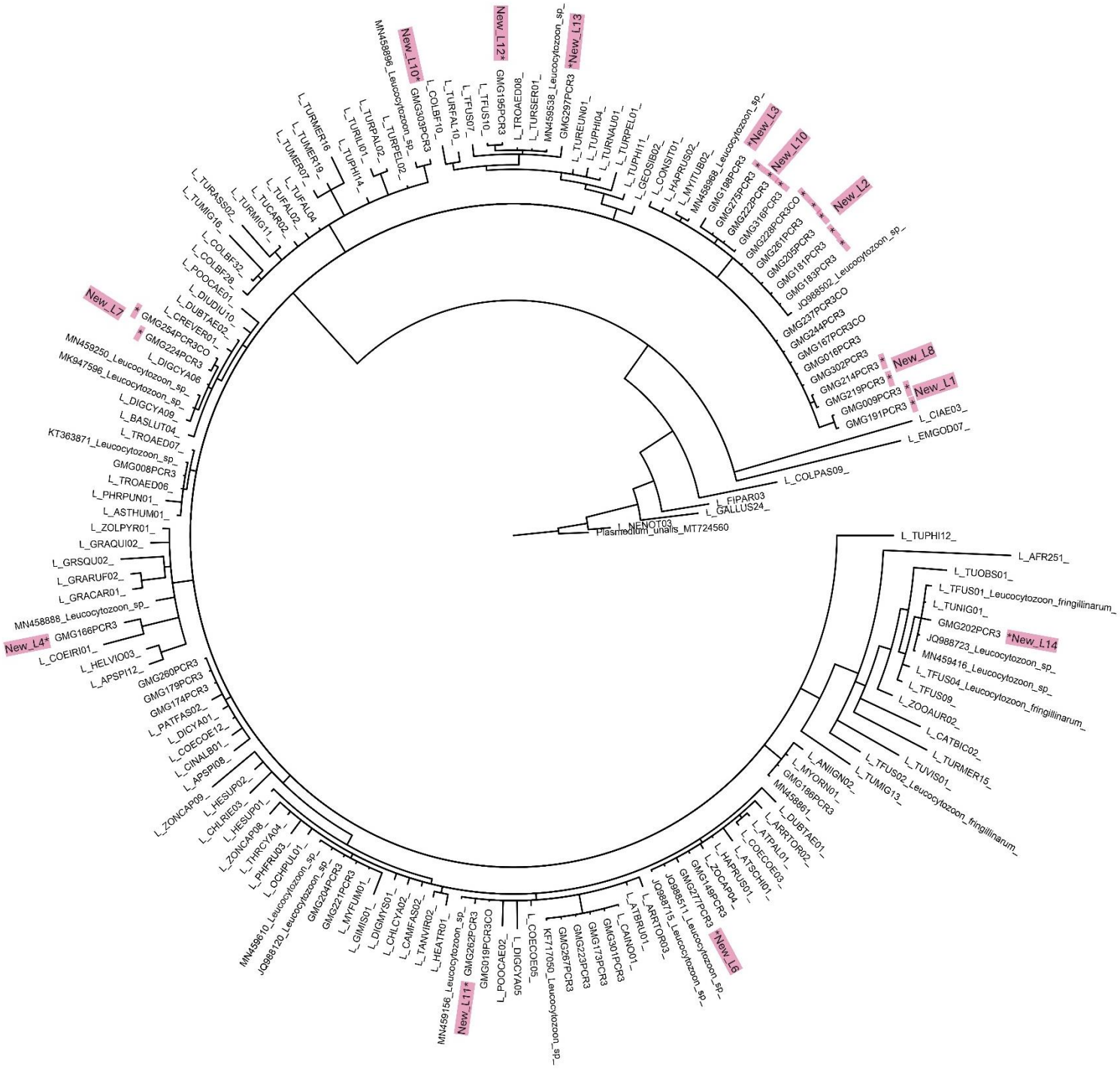

Figure 5. Bayesian phylogeny of *Leucocytozoon* lineages detected in this study and that appeared in MalAvi database. The tree illustrates evolutionary relationships among lineages responsible for avian malaria infections. Newly identified lineages are highlighted with pink color. This phylogeny provides insights into the genetic diversity and evolutionary history of *Leucocytozoon* parasites in high-Andean ecosystems.

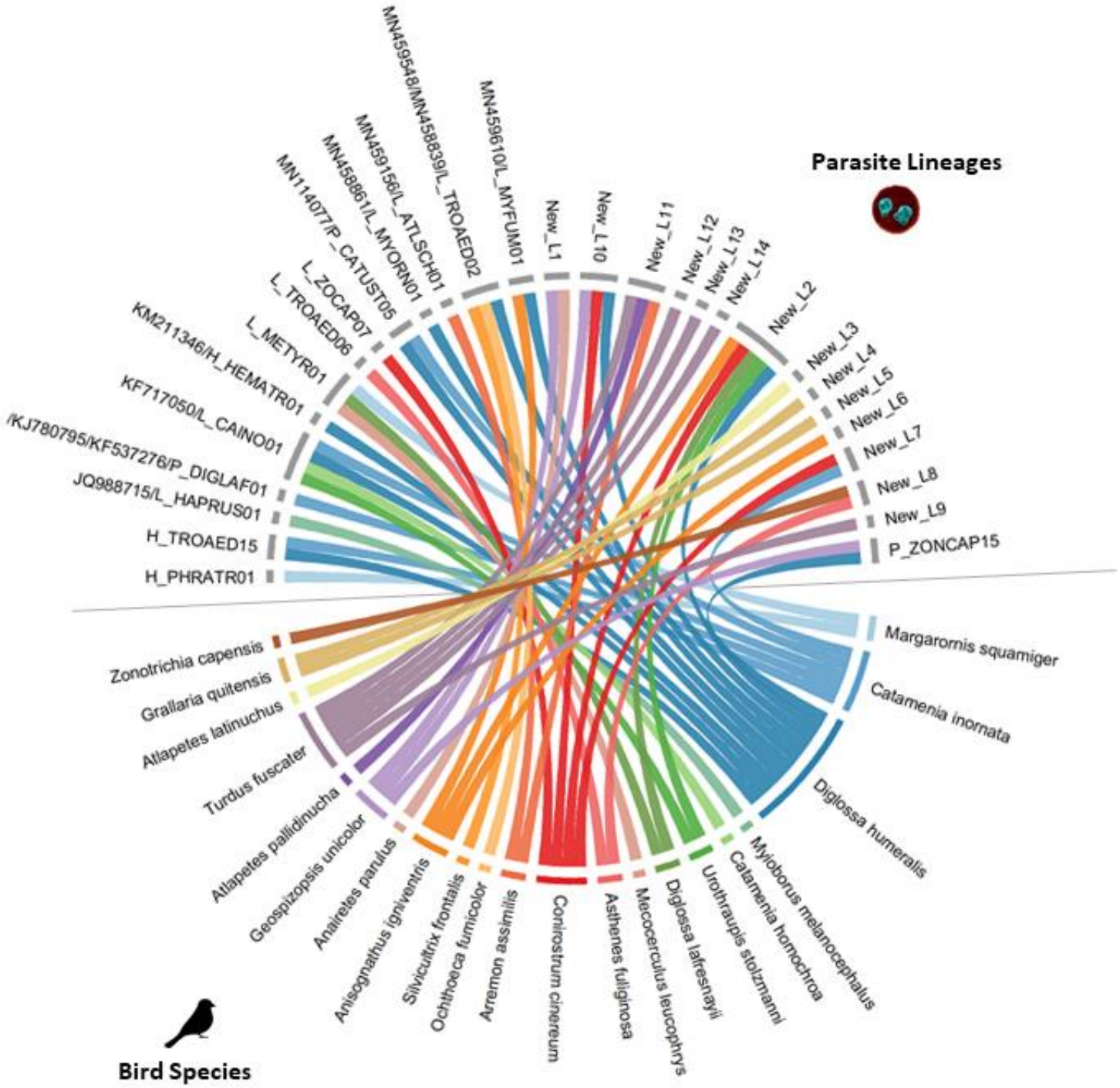

Figure 6. Circular representation of the host-parasite interaction network based on identified infections. Parasite lineages are displayed on the upper half of the circle, while bird species are shown on the lower half. Links represent observed infections, and colors indicate parasite lineages associated with each host species. The structure highlights the specificity and diversity of associations within the community.

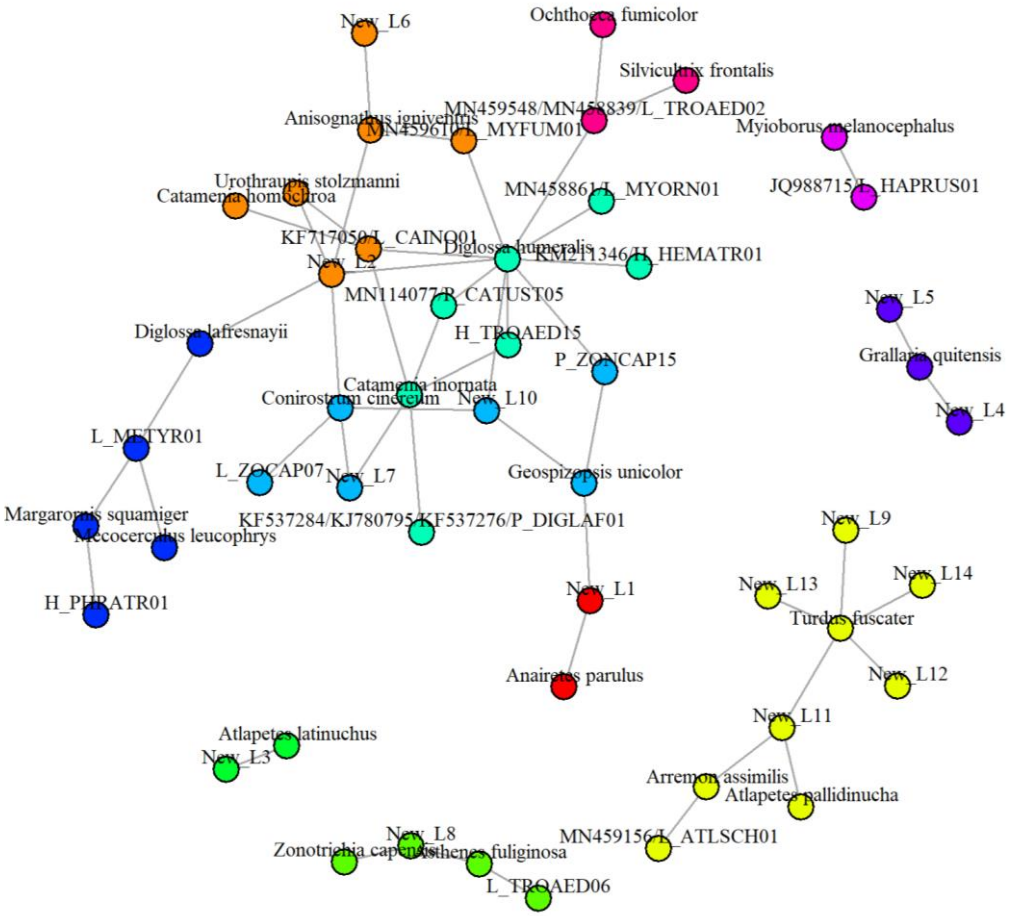

Figure 7. Modular structure of the avian host-parasite interaction network. Each color represents a distinct module, highlighting groups of host and parasite lineages that interact more frequently with each other than with those outside the module. This modularity suggests ecological or evolutionary specialization within the network.

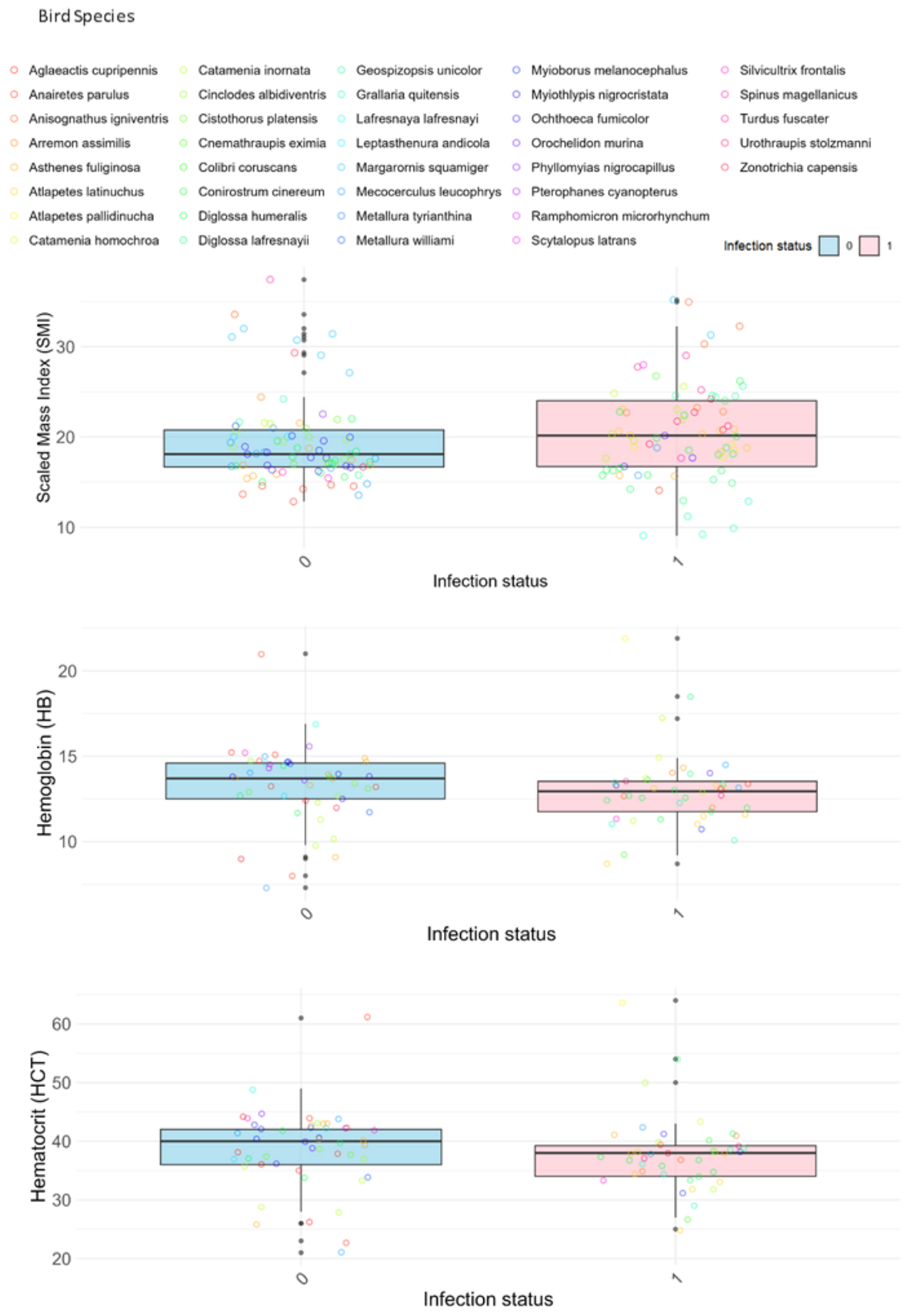

Figure 8. Comparison of body condition (Scaled Mass Index, SMI), hemoglobin (HB), and hematocrit (HCT) levels between infected and uninfected birds. Jittered dots represent individual birds, with each color indicating a different bird species.

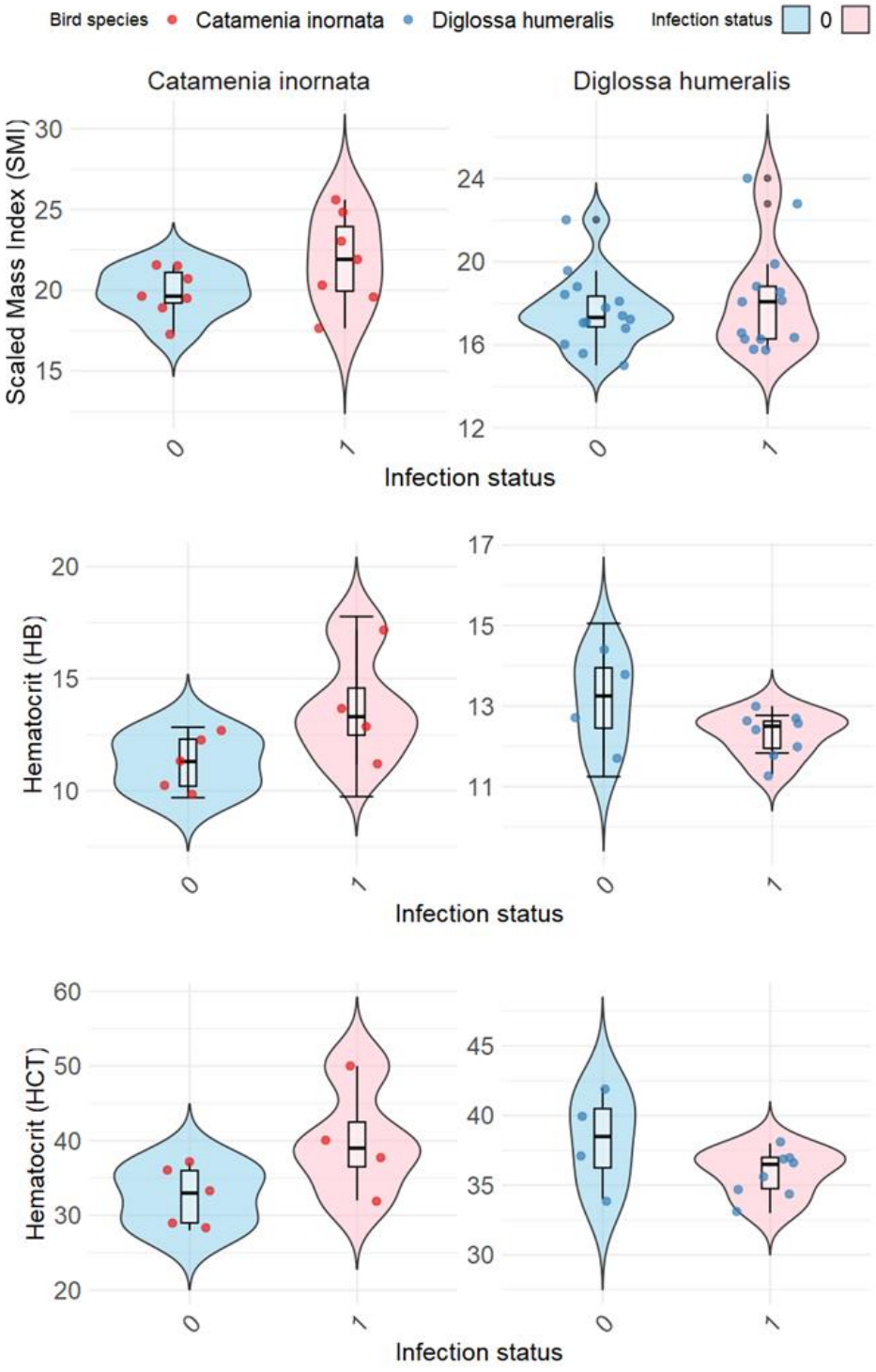

Figure 9. Comparison of body condition (Scaled Mass Index, SMI), hemoglobin (HB), and hematocrit (HCT) levels between infected and uninfected individuals of the two most abundant species: *Catamenia inornata* and *Diglossa humeralis*. Jittered dots represent individual birds included in the analysis

SUPPLEMENTARY INFORMATION

Supplementary Table I. Models with the lowest delta AICc (5 out of 16). Co-variables explaining infection prevalence. Predictors of infection include julian day, weather, altitude and site.

Model	df	LL	AICc	Delta AICc	AICc Wt
Null model	1.00	-134.602	271.224	0.000	0.189
Julian day	2.00	-134.208	272.477	1.252	0.101
Weather	3.00	-133.225	272.573	1.348	0.096
Elevation	3.00	-133.379	272.880	1.655	0.082
Site	3.00	-133.379	272.880	1.655	0.082

Supplementary Table II. Models with the lowest delta AICc (5 out of 32). Individual condition variables explaining infection prevalence. Predictors of infection include age, body molt, wing molt, patch and fat.

Model	df	LL	AICc	Delta AICc	AICc Wt
Age + Fat	5	-110.046	230.450	0	0.268
Age + Fat + Wing molt	6	-109.277	231.058	0.607	0.197
Age + Fat + Patch	6	-109.623	231.750	1.299	0.139
Age + Fat + Patch + Wing molt	7	-108.951	232.577	2.126	0.0925
Fat	4	-112.621	233.479	3.028	0.0589

Supplementary Table III. Models with the lowest delta AICc (5 out of 256). Morphological variables explaining infection prevalence. Predictors of infection include beak length, beak height, beak width, commissure, tail length, weight, wing length and tarsus.

Model	df	LL	AICc	Delta AICc	AICc Wt
Beak height + Beak width + Commissure + Weight	6	-79.821	172.214	0	0.063
Beak height + Beak width + Weight	4	-82.325	172.919	0.705	0.044
Beak height + Commissure + Weight	4	-82.337	172.943	0.729	0.043
Beak height + Weight	5	-81.306	173.017	0.803	0.042
Beak height + Beak width + Commissure + Weight	5	-81.398	173.202	0.988	0.038

Supplementary Table IV. Summary table of the best model (lowest AICc) explaining infection prevalence by individual condition.

Coefficients	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	0.03686	0.17835	0.207	0.83627	
Age Juvenile	-0.94254	0.42899	-2.197	0.02801	*
Fat	1.03950	0.33176	3.133	0.00173	**

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table V. Summary table of the best model (lowest AICc) explaining infection prevalence by morphological variables.

Coefficients	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-0.2927	0.3810	-0.768	0.44241	
Beak height	1.1457	0.3721	3.079	0.00208	**
Beak length	-1.8150	1.0435	-1.739	0.08198	
Beak width	-0.7908	0.4446	-1.779	0.07528	
Commissure	-1.0459	0.6233	-1.678	0.09335	
Weight	5.1555	1.6240	3.175	0.00150	**

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table VI. Summary of the model proposing Scaled Mass Index as a response variable of the infection.

Random effects:				
Group	**Effect**	**Variance**	**Std. Dev.**	
Species	Intercept	0.02600	0.1612	
Residual	—	0.01445	0.1202	
Fixed effects:				
Term	**Estimate**	**Std. Error**	**t value**	**p-value**
(Intercept)	305.085	0.06855	44.508	< 2e-16**
Infected (1)	0.02600	0.01951	1.333	0.183

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table VII. Summary of the model proposing Scaled Mass Index as a response variable of the infection in *Catamenia inornata*.

	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	19.871	0.871	22.814	2.98e-11	***
Infected (1)	1.976	1.232	1.604	0.135	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table VIII. Model of the Scaled Mass Index as a response variable of the infection in *Diglossa humeralis*.

Coefficient	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	286.992	0.03259	88.054	<2e-16	***
Infected (1)	0.03447	0.04740	0.727	0.474	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table IX. Summary of the model proposing hemoglobin (HB) as a response variable of the infection.

Random effects:					
Group	**Effect**	**Variance**	**Std. Dev.**		
Species	(Intercept)	0.005161	0.07184		
Residual	—	0.024338	0.15601		
Fixed effects:					
Coefficient	**Estimate**	**Std. Error**	**t value**	**p-value**	**Significance**
(Intercept)	2.596.023	0.034066	76.207	< 2e-16	***
Infected (1)	-0.001759	0.038968	-0.045	0.964	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table X. Summary of the model proposing hemoglobin (HB) as a response variable of the infection in *Catamenia inornata*.

Coefficient	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	11.2600	0.8543	13.181	3.38e-06	***
Infected (1)	2.4900	1.2814	1.943	0.0931	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table XI. Summary of the model proposing hemoglobin (HB) as a response variable of the infection in *Diglossa humeralis*.

Coefficient	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	2.57642	0.03171	81.26	1.95e-15	***
Infected (1)	-0.06682	0.03841	-1.74	0.113	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table XII. Summary of the model proposing hematocrit (HCT) as response variable of the infection.

Random effects:					
Group	**Effect**	**Variance**	**Std. Dev.**		
Species	Intercept	0.004881	0.06986		
Residual		0.025015	0.15816		
Fixed effects:					
Coefficient	**Estimate**	**Std. Error**	**t value**	**p-value**	**Significance**
(Intercept)	3.662.208	0.033897	108.039	< 2e-16	***
Infected (1)	-0.001001	0.039336	-0.025	0.98	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table XIII. Summary of the model proposing hematocrit (HCT) as a response variable of the infection in *Catamenia inornata*.

	Estimate	Std. Error	t value	p-value	Significance
(Intercept)	32.600	2.581	12.630	4.51e-06	***
Infected (1)	7.400	3.872	1.911	0.0976	

Signif.codes: 0 *** 0.001** 0.01*

Supplementary Table XIV. Summary of the model proposing hematocrit (HCT) as a response variable of the infection in *Diglossa humeralis*.

	Estimate	**Std. Error**	**t value**	**p-value**	**Significance**
(Intercept)	364.414	0.03256	111.912	<2e-16	***
Infected (1)	-0.06410	0.03947	-1.624	0.135	

Signif.codes: 0 *** 0.001** 0.01*