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ORIGINAL ARTICLE

Chilean Elaenias (*Elaenia chilensis*) are tolerant and resistant to haemosporidian parasites during migration

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Abstract

Migratory birds are exposed to a diverse fauna of parasites and vectors throughout their annual cycle, which can potentially facilitate disease transmission across wide geographic ranges. However, few studies have examined how these pathogenic organisms affect the immune profiles and body condition of avian hosts while they are refueling at stopover sites. Here, we used molecular barcoding and microscopic examination to detect, quantify, and identify haemosporidian parasites in Chilean Elaenias (*Elaenia chilensis*) during their migration stopover in the Serra do Mar, Brazil. We then evaluated whether haemosporidian infections were associated with immunological condition (heterophil, lymphocyte, and H/L ratios), body condition, and fat storage. Among the 79 adult Chilean Elaenias captured, 26 were infected with haemosporidian parasites. However, most individuals carried extremely low parasite burdens during migration. Contrary to our expectations, haemosporidian infection did not affect body condition or fat storage in migrating Chilean Elaenias, regardless of host sex. Likewise, the absence of a relationship between immunological parameters and parasite presence suggests that haemosporidian parasitism does not significantly impact the immune response of these migrating birds. Moreover, the condition of chronically infected and uninfected Chilean Elaenias did not differ, suggesting that these parasites may not constrain host arrival at the stopover site in Serra do Mar.

Keywords Avian malaria · Bird migration · Body condition · Immune response · Leucocyte profile · Parasite transmission

Zusammenfassung

Weißbauch-Olivtyrannen (*Elaenia chilensis*) sind während ihrer Wanderung tolerant und resistent gegenüber Haemosporidien-Parasiten

Zugvögel sind während ihres jährlichen Zyklus einer vielfältigen Fauna von Parasiten und Vektoren ausgesetzt, die potenziell die Übertragung von Krankheiten über große geografische Gebiete hinweg begünstigen können. Allerdings gibt es nur wenige Studien, die untersucht haben, wie diese pathogenen Organismen das Immunprofil und die körperliche Verfassung der

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Vogelwirte beeinflussen, während diese an Zwischenstopps neue Energie tanken. Hier haben wir molekulare Barcodes und mikroskopische Untersuchungen verwendet, um Haemosporidien-Parasiten bei Weißbauch-Olivtyrannen (*Elaenia chilensis*) während ihres Zwischenstopps in der Serra do Mar in Brasilien nachzuweisen, zu quantifizieren und zu identifizieren. Anschließend haben wir untersucht, ob Haemosporidien-Infektionen mit dem immunologischen Zustand (Heterophil-, Lymphozyten- und H/L-Verhältnisse), der körperlichen Verfassung und der Fettspeicherung in Zusammenhang stehen. Von den 79 gefangenen erwachsenen Weißbauch-Olivtyrannen waren 26 mit Haemosporidien-Parasiten infiziert. Die meisten Individuen wiesen jedoch während des Zuges eine extrem geringe Parasitenbelastung auf. Entgegen unseren Erwartungen hatte die Haemosporidieninfektion keinen Einfluss auf die Körperkondition oder die Fettspeicherung der wandernden Weißbauch-Olivtyrannen, unabhängig vom Geschlecht des Wirts. Ebenso deutet das Fehlen eines Zusammenhangs zwischen immunologischen Parametern und dem Vorhandensein von Parasiten darauf hin, dass der Haemosporidienparasitismus keinen signifikanten Einfluss auf die Immunantwort dieser Zugvögel hat. Darüber hinaus unterschied sich der Zustand chronisch infizierter und nicht infizierter Weißbauch-Olivtyrannen nicht, was darauf hindeutet, dass diese Parasiten die Ankunft der Wirte am Zwischenstopp in Serra do Mar möglicherweise nicht einschränken.

Introduction

Long-distance migration is one of the most strenuous and energetically costly activities for birds and has been shown to reduce immune capacity while increasing physiological stress (Owen and Moore 2006). A reduction in lymphocytes (a defense cell generated during the acquired immune response) and an increase in heterophils (a defense cell involved in the innate immune response) were observed in migrating thrushes at stopover sites (Apanius 1998; Owen and Moore 2006). In addition, migrating thrushes exhibited higher heterophil/lymphocyte ratio after crossing long ecological barriers, indicating chronic stress at stopover sites (Owen and Moore 2006). Because the activation and maintenance of immune responses are physiologically demanding and energetically costly activities (Apanius 1998), a trade-off between migration and immunocompetence must exist during long-distance flights (Owen and Moore 2006; Buehler and Piersma 2008). Therefore, it is expected that migrant birds resting and refueling at stopover sites might be in a poor immunological condition (Owen and Moore 2006) leading to increased parasite burden due to relapses of previous infection.

The Chilean Elaenia (*Elaenia chilensis*) is an austral migratory passerine that breeds in Patagonia and overwinters in tropical South American biomes (Bravo et al. 2017). These long-distance Neotropical migrants are infected with avian malaria and related parasites in their temperate breeding grounds in Argentina and Chile (Merino et al. 2008; Fecchio et al. 2022) and transport a great diversity of haemosporidian parasite lineages during their displacement in the Brazilian wintering grounds (Fecchio et al. 2022). In total, 31 lineages have been reported infecting Chilean Elaenias, with different degrees of specificity and prevalence (Merino et al. 2008; Fecchio et al. 2022). While Chilean Elaenias harbor a higher diversity and prevalence of *Haemoproteus* in Chilean grounds (Merino et al. 2008), they are highly infected with *Leucocytozoon* in Argentinean breeding grounds (Fecchio et al. 2022).

Avian haemosporidian parasites from the genera *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* are a diverse group of protozoans that infect blood cells and vertebrate host tissues for reproduction (Valkiūnas 2005). These intracellular parasites undergo complete sexual reproduction in hematophagous female mosquitos (Diptera: Culicidae), black flies (Diptera: Simuliidae), biting midges (Diptera: Ceratopogonidae), and hippoboscid flies (Diptera: Hippoboscidae), which act as vectors of *Plasmodium*, *Leucocytozoon*, subgenus *Parahaemoproteus*, and subgenus *Haemoproteus*, respectively (Valkiūnas 2005). Infection prevalence varies widely among and within avian host families, as well as across zoogeographic regions (Fecchio et al. 2021).

While avian species-level traits that increase vector exposure or host susceptibility, such as migratory behavior and migration distance, have been extensively used to explain haemosporidian prevalence and diversity in South America (e.g., Anjos et al. 2021; de Angeli Dutra et al. 2021; Fecchio et al. 2021), individual-level traits, such as sex, are often overlooked in community level studies to identify the drivers of avian haemosporidian infection. This is particularly problematic in macroecological analyses that include multiple monochromatic species, such as Chilean Elaenias, because sexual dimorphism cannot be inferred solely from plumage coloration during sampling. Sex-biased haemosporidian infection is expected due to the differences in immunosuppressive hormone levels circulating in males and females during the breeding season or prior migration (Zuk and McKean 1996). For example, testosterone treatment increased *Leucocytozoon* abundance threefold in male Dark-Eyed Juncos (*Junco hyemalis*) after arrival at breeding grounds in Alaska (Deviche and Parris 2006).

In this study, we investigated whether haemosporidian parasites affect the body condition and immunological status of Chilean Elaenias at a stopover site in the Brazilian Atlantic Rainforest. We also evaluated the reliability of different

body condition metrics as indicators of immunological status. In addition, we examined whether host sex influences infection probability and whether body condition and immunological status differ between males and females.

Methods

Study site

We conducted our study on the plateau of the Curucutu Nucleus (800 m a.s.l.; 23.993694° S, 46.732806° W), one of the ten subdivisions of Serra do Mar State Park, located in São Paulo State, southeastern Brazil. This region preserves one of the largest remnants of the Atlantic Rainforest biome, which harbors 422 bird species (Schunck et al. 2019). The bird assemblage in the montane area of the Curucutu Nucleus comprises 310 species, 162 of which have been captured in mist nets over the past 18 years as part of a long-term monitoring program for altitudinal and long-distance migrants (F. Schunck unpublished data). Average temperatures are moderate year-round, ranging from 0 °C in winter to 34 °C in summer, and annual rainfall can exceed 4,400 mm (Schunck et al. 2019).

Field procedures

We captured Chilean Elaenias using 35 mist nets (12 × 2 m; four shelves; 36 mm mesh) between 4 and 13 April 2019. This sampling period corresponds to the passage of Chilean Elaenias at this stopover site over the last 14 years (F. Schunck unpublished data). For each bird, we measured three morphological traits: body mass (g), tarsus length (mm), and fat deposition. Fat deposition was scored as a categorical variable indicating the amount of fat stored for migration along the pectoral muscle: 0 = no fat, 1 = low fat, 2 = moderate fat, and 3 = high fat. Approximately 30 µL of blood was collected from the brachial vein of each bird and stored on FTA cards (Whatman™) for molecular sexing and parasite diagnosis. In addition, we prepared one or two thin blood slides per bird. Slides were air-dried and fixed in absolute methanol during field collection. Captured birds were banded with metal rings provided by CEMAVE/ICMBio (National Center for Research and Conservation of Wild Birds) and released after biometric measurements and blood sampling. All procedures were conducted under the appropriate permits in Brazil (licenses issued by Instituto Chico Mendes de Conservação da Biodiversidade ICMBio number: SISBIO 59198-11 and Instituto de Pesquisas Ambientais—IPA number: 40059/2006).

Parasite detection and lineage identification

Total DNA was extracted from avian blood using the Wizard® SV 96 Genomic DNA Purification System (Promega, Madison, Wisconsin, USA) with modifications as described in Anjos et al. (2021). Successful DNA extraction was assessed by sexing the individuals using the primers SEX F2 [3'-GTC GTR GAC AGY TTG GT-5'] and SEX R1 [3'-ATY GTT TYT GGT TRA TTA TTG-5'] (Bantock et al. 2008).

All parasite lineages in this study were identified using PCR-based detection, targeting a 479 bp fragment of the cytochrome-b (*cyt-b*) gene of the haemosporidian mitochondrial genome (Hellgren et al. 2004). Briefly, genomic DNA underwent an initial PCR using a set of primers (HaemNF1 and HaemNR3) designed to target a conserved region of the *cyt-b* gene of *Plasmodium*, *Haemoproteus,* and *Leucocytozoon* parasites. In the first nested PCR, primers (HaemF and HaemR2) were used to amplify only *Plasmodium* and *Haemoproteus* mitochondrial DNA. In the second nested PCR, primers (HaemFL/HaemR2L) were used to amplify only *Leucocytozoon* mitochondrial DNA using the product of the first PCR. All PCR amplifications included positive control. Due to the high sensitivity of nested PCR, negative controls were also included in runs to check for possible contamination and false positives, although none were found in any PCR run. Information on detailed reactions, reagents, and cycling conditions can be found in Hellgren et al. (2004).

PCR products from infected samples were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). Forward and reverse sequences were visualized, assembled, and aligned with reference sequences from the MalAvi database (Bensch et al. 2009). Parasite lineages were assigned to the genera *Plasmodium* and *Leucocytozoon*, and to the subgenus *Haemoproteus* (*Parahaemoproteus*) (hereafter *Parahaemoproteus*). Newly detected lineages were named following the standard protocol for avian haemosporidian parasites (Bensch et al. 2009). All sequences have been deposited in GenBank, and their accession numbers are provided in the raw dataset (Table S1).

To assure that Chilean Elaenias are competent hosts and that haemosporidian parasites can reproduce within avian hosts during their migration, we screened blood slides from all individuals using traditional light microscopy. Blood slides were stained with 10% Giemsa solution for 1 h and examined for 20–25 min under an Olympus BX51 light microscope, scanning 100 fields at low magnification (400×) and 100 fields at high magnification (1000×). Parasites and their infective stages were morphologically identified and quantified following Valkiūnas (2005).

Estimation of immunological status

We performed differential white blood cell (WBC) counts by examining 100 leukocytes per slide (Dein 1984) under a Nikon E100® optical microscope at 1000× magnification with immersion oil. Leukocytes were classified into five types: lymphocytes, heterophils, eosinophils, basophils, and monocytes. Because basophils (median = 0; range = 0–3), eosinophils (median = 0; range = 0–9), and monocytes (median = 3; range 0–19) were rare, our analyses focused on the two most prevalent leukocytes: lymphocytes (median = 79; range = 18–96) and heterophils (median = 14; range = 2–78). Consequently, immunological status was assessed using the relative proportions of leukocyte types rather than a measure of white blood cell concentrations, thus providing a standardized and comparable metric to previous studies evaluating immune response in birds infected with haemosporidians. We also calculated the heterophil-to-lymphocyte (H/L) ratio, a widely used indicator of stress in birds (Gross and Siegel 1983; Maxwell 1993). It is worth noting that, although the use of BCI is common and widely reported in literature, the absence of a direct measure of physiological state may limit the interpretability of this index (Green 2001).

Statistical analysis

We investigated the effects of haemosporidian infection and sex on two indicators of individual condition—body mass and body condition index (BCI)—using linear regression models with the lm function in the *stats* package. The BCI was calculated as the residuals of a linear regression between log-transformed body mass and log-transformed tarsus length, with negative residuals indicating poorer condition and positive residuals indicating better condition (Jakob et al. 1996; Schulte-Hostedde et al. 2001). Body mass was also analyzed separately due to its reliability as a condition measure (Labocha and Hayes 2012). One female with an erroneously recorded body mass was assigned the mean body mass of the other sampled females.

To examine the effects of haemosporidian infection and sex on bird fat content, we fitted a generalized linear model (GLM) with a Poisson distribution using the glm function from the *stats* package. We also evaluated the effects of these predictors, along with different condition metrics, on immunological status. To avoid collinearity, body mass, BCI, and fat content were not included in the same model. Immunological status was assessed using lymphocyte and heterophil counts, as well as the heterophil-to-lymphocyte (H/L) ratio. We used linear models for the H/L ratio and GLMs with a negative binomial distribution for lymphocyte and heterophil counts due to overdispersion. Negative binomial models were fitted with the glm.nb function from the *MASS* package (Venables and Ripley 2002). In addition, we tested whether haemosporidian infection status (infected/uninfected) was associated with sex using a binomial GLM. Model diagnostics were performed with the DHARMa package (Hartig 2022). All models with multiples explanatory variables (e.g., sex, haemosporidian infection, condition metrics) were simplified using likelihood ratio tests, and only significant terms were retained in the final (minimal) models (Crawley 2007). Reported statistics refer to these minimal models, as no additional terms significantly improved model fit.

Haemosporidian parasite prevalence and 95% confidence intervals (CI) were calculated using Sterne's exact method (Reiczigel et al. 2010) with the epi.prev function from the *epiR* package (Stevenson et al. 2021). Descriptive statistics are reported as mean ± standard deviation. All analyses were performed in R (version 4.3.2, R Development Core Team 2023).

Results

We analyzed 79 adult Chilean Elaenias, including 46 males and 33 females. The general prevalence of haemosporidian infection (pooling together all parasite taxa) was 32.9% (95% CI: 23.3–44.3%), with 10 females and 16 males infected. Neither host sex nor arrival date significantly influenced infection probability (LRT; $\chi^2 < 0.45$, $P > 0.502$). *Leucocytozoon* was the most prevalent haemosporidian taxon (17.7%, 95% CI: 10.6–27.8%, $n = 14$ infected birds), followed by *Plasmodium* (8.9%, 95% CI: 4.2–17.5%, $n = 7$) and *Parahaemoproteus* (7.6%, 95% CI: 3.4–15.7%, $n = 6$). We detected ten parasite lineages in Chilean Elaenias: five assigned to the genus *Plasmodium* (COLL4, DENPET03, LEPCOR05, PADOM09, and PHPAT01), four to the genus *Leucocytozoon* (DIUDIU11, ELAALB02, ELAALB05, and TROAED02), and only one to the subgenus *Parahaemoproteus* (ELAALB01). Blood slides showed very low parasitemias for *Plasmodium* (mean = 0.66, SD = 4.44, range = 0–38 parasites in 100 microscopic fields ≈ 10^4 red blood cells) and *Parahaemoproteus* (mean = 0.33, SD = 1.54, range = 0–10). No transmissive stages of *Leucocytozoon* were detected in any of the examined slides.

Male Chilean Elaenias (16.2 ± 1.24 g) were heavier than females (14.9 ± 0.87 g; LRT; $F = 26.71$; $P < 0.001$), exhibited higher body condition indices (males: 0.03 ± 0.08; females: − 0.04 ± 0.06; LRT; $F = 18.63$; $P < 0.001$), and had greater fat reserves (males: 1.87 ± 0.83; females: 1.06 ± 0.83; LRT; $\chi^2 = 8.78$; $P = 0.003$; Table S1). However, none of the condition metrics were correlated with general haemosporidian infection (Fig. 1, Table S2). Analyses performed separately for each haemosporidian taxon yielded similar results (Supplementary Material 2, Tables S3–S5).

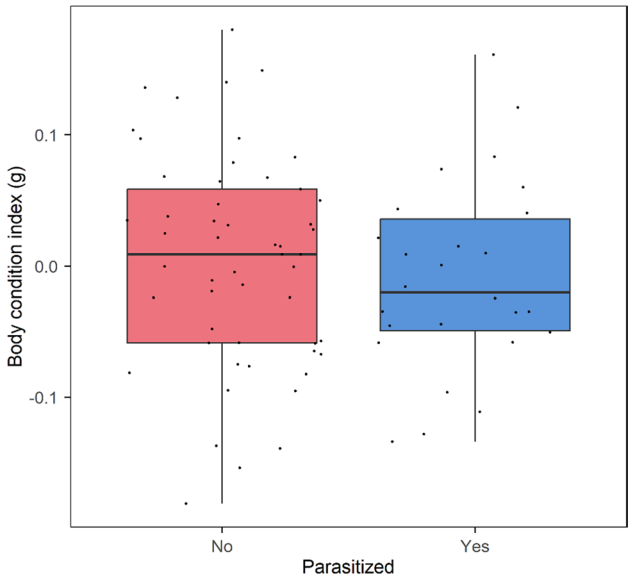

Fig. 1 Variation in the body condition index between parasitized and non-parasitized Chilean Elaenias

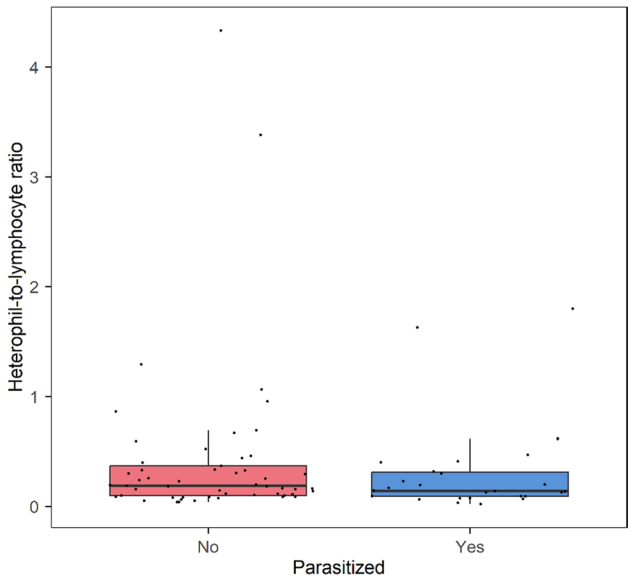

Fig. 2 Variation in the heterophil-to-lymphocyte ratio between parasitized and non-parasitized Chilean Elaenias

Parasitism also did not correlate with the immunological status of Chilean Elaenias, whether assessed by lymphocyte counts (LRT; $\chi^2 = 0.10$; $P = 0.750$), heterophil counts (LRT; $\chi^2 = 0.58$; $P = 0.446$), or the H/L ratio (LRT; $F = 0.47$; $P = 0.496$; Fig. 2). Analyses performed separately for each haemosporidian taxon yielded similar results (Tables S8-S10). Likewise, body condition was not associated with lymphocyte or heterophil counts (LRT; $\chi^2 < 0.15$; $P > 0.694$) nor with the H/L ratio (LRT; $F = 0.22$; $P = 0.642$). Using other measures of individual condition yielded similar results. Both fat content and body mass were not associated with immunological measures (Tables S7–S10). In contrast, males exhibited significantly lower heterophil counts (16.3 ± 13.0) than females (23.7 ± 18.3; $\chi^2 = 5.50$; $P = 0.019$; Fig. S1, Table S6).

Discussion

Haemosporidians do not affect the body condition and immunological status in Chilean Elaenias during migration

The lack of relationship between body condition, fat deposition, and haemosporidian infection in Chilean Elaenias at a stopover site in Brazil might indicate a certain degree of tolerance to these parasites during migration. This is in accordance with previous studies reporting negligible effects of haemosporidian infection on the physiological condition of long-distance migratory birds (Santiago-Alarcon et al. 2013; Emmenegger et al. 2018; Ágh et al. 2022; Orfanides and Pagano 2024). We detected no increase in H/L ratio and heterophil counts between uninfected and infected individuals, even when considering parasite genus-specific responses. This suggests that haemosporidian infection did not induce detectable immune-related changes prior to migration in Chilean Elaenias. Several studies have also reported inconsistent or absent effects of natural haemosporidian infections on avian immunological parameters (Ricklefs and Sheldon 2007; Lebeau and Dunn 2024), including analyses at the parasite lineage level rather than genus level (Ellis et al. 2014). Collectively, these correlational findings support the hypothesis that long-term coevolution between haemosporidians and their avian hosts can reduce parasite virulence, leading to a weaker immune response (Ricklefs and Sheldon 2007; Lebeau and Dunn 2024).

However, bird immune-function activity may be triggered even in chronically infected hosts, as parasites have already successfully invaded the avian host tissue and few gametocytes are circulating in the peripheral blood (Rimša et al. 2024). Heterophils are the first line of bird immune defense to both detect and destroy pathogenic and parasitic organisms (Kogut et al. 2005). Consequently, the lack of association between haemosporidian infection and heterophil counts in migrating Chilean Elaenias may also indicate a certain degree of tolerance to these parasites during migration. Moreover, the extremely low number or lack of gametocytes in most blood slides from infected individuals (infection confirmed by molecular barcoding) suggests that Chilean Elaenias reduce their parasite burden prior to or during migration, indicating a degree of resistance to haemosporidian parasites.

Parasite culling may provide an alternative explanation for the absence of heavily infected Chilean Elaenias arriving at the Brazilian stopover site. Two non-exclusive mechanisms could contribute to this pattern. First, birds harboring a high haemosporidian load may be unable to complete migratory flights and die before reaching stopover areas (Bradley and Altizer 2005). This effect could be especially pronounced near the end of migration. Thus, Chilean Elaenias with high parasitemia may fail to reach Serra do Mar, which is geographically closer to their Brazilian wintering grounds than to their Patagonian breeding grounds. Second, naïve hosts in the acute phase of infection (e.g., hatch-year birds) may be unable to depart from their Patagonian breeding grounds. During the acute phase, characterized by high parasitemia, anemia, lethargy, and reduced food intake, parasites can severely impair host body condition and leukocyte profiles (Valkiūnas 2005; Ellis et al. 2015). If Chilean Elaenias survive this acute stage on the breeding grounds, they may depart carrying only low levels of parasites circulating in their blood. In such cases, even in the absence of detectable gametocytes in peripheral blood, *Leucocytozoon* may persist in host tissues during migration, with relapses occurring in the following breeding season, when competent vectors are available to complete the parasite life cycle (Anjos et al. 2021). Latent infection could, therefore, account for both the absence of *Leucocytozoon* transmissive stages and the lack of immune response detected at the Brazilian stopover site. However, because chronic infection (i.e., low parasite intensity) may not impair migration ability (Hahn et al. 2018) or migration distance (Sorensen et al. 2019), the culling of Chilean Elaenias carrying chronic or latent infections may be less likely to occur.

Sex bias in Chilean Elaenias

We found that male Chilean Elaenias migrating with better body condition and greater fat reserves had the same probability of being infected as individuals in poorer condition with reduced fat storage. The superior condition of males during migration may be linked to the female-biased nestling provisioning observed in this species (Gorosito et al. 2022). Consequently, males likely allocate more resources for fat deposition at the end of the breeding season in Patagonia. This sex bias in food provisioning may also explain the higher heterophil counts observed in migrating females, as food intake can influence heterophil production and function (Zuidhof et al. 1995). For instance, nutritional stress caused by feed restriction can increase circulating heterophils (Zuidhof et al. 1995). Alternatively, the elevated heterophil numbers in females may reflect ongoing bacterial or parasitic infections not assessed in this study.

We detected no sex differences in haemosporidian prevalence among adult Chilean Elaenias at the Brazilian stopover site. There is currently no consensus on sex bias in avian haemosporidian infections. Some studies report male-biased prevalence (e.g., Calero-Riestra and García 2016; Rodriguez et al. 2021) or female-biased infection rates (e.g., Jones et al. 2024), and many studies have showed that males and females are equally susceptible to haemosporidian infection (Emmenegger et al. 2018; Bosholn et al. 2020; Huang et al. 2020). This variability highlights the need for a broader synthesis to identify the ecological and evolutionary factors driving sex-specific infection patterns in avian hosts.

Role of Chilean Elaenias as competent hosts during migration

Although large-scale movements of infected birds can introduce new parasite lineages into new regions or naïve host populations, transmission is ultimately shaped by local factors such as host–parasite compatibility, vector availability, and climatic conditions. Migrating Chilean Elaenias spend a few hours during the day at the Serra do Mar for feeding and resting, which may not be sufficient for exposure to potential vectors. Moreover, the absence of transmissive stages in blood smears during stopover suggests that Chilean Elaenias migrate carrying very low intensities of *Leucocytozoon* parasites. Because we detected gametocytes only of *Plasmodium* and *Parahaemoproteus,* we cannot conclusively state that the four *Leucocytozoon* lineages identified through molecular barcoding complete their life cycle and produce gametocytes in this migratory species at either stopover or wintering sites in Brazil. The absence of *Leucocytozoon* gametocytes in infected Chilean Elaenias may instead indicate abortive infections.

Conclusion

Comparative studies assessing immune response of naturally infected birds through both molecular screening and microscopic examination of blood smears inform us about the ability of hosts to control infections. Here, we show that chronic or latent infection by *Leucocytozoon*, *Plasmodium*, and *Parahaemoproteus* did not induce increases in the two most common white blood cell in Chilean Elaenia during migration. Because these parasites also did not affect body condition or fat reserves, we suggest that migrating Chilean Elaenias may be both tolerant and resistant to these parasites. Whether individuals shift investment between resistance and tolerance to reduce haemosporidian loads throughout their annual cycle remains unknown. Nonetheless, such strategies could influence not only host fitness but also parasite transmission across the species' migratory range. This is particularly relevant for *Leucocytozoon*, which infected more than half of the parasitized individuals, yet showed no

transmissive stages in blood slides. This pattern suggests that *Leucocytozoon* may not be transmitted at Brazilian stopover sites despite being transported by Chilean Elaenias.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10336-025-02354-5.

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Data availability The raw data used in statistical analyses and edited DNA sequences are available as supplementary materials. All DNA sequences are available in GenBank.

Declarations

Ethical approval All animal procedures (capture, handling, and blood collection) were approved by the Brazilian authority: Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio number: SISBIO 59198–11 and Instituto de Pesquisas Ambientais—IPA number: 40059/2006.

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