Latitudinal gradients of haemosporidian parasites: Prevalence, diversity and drivers of infection in the Thorn-tailed Rayadito (*Aphrastura spinicauda*)☆

Elfego Cuevas[a,b,*], Juliana A. Vianna[c], Esteban Botero-Delgadillo[d,e,f], Daniela Doussang[g,h], Daniel González-Acuña[g], Omar Barroso[i], Ricardo Rozzi[i], Rodrigo A. Vásquez[d], Verónica Quirici[b,j]

[a] *Programa de Doctorado en Medicina de la Conservación, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile*
[b] *Centro de Investigación para la Sustentabilidad (CIS), Universidad Andres Bello, Santiago, Chile*
[c] *Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile*
[d] *Instituto de Ecología y Biodiversidad, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*
[e] *SELVA: Research for Conservation in the Neotropics, Bogotá, Colombia*
[f] *Department of Behavioral Ecology and Evolutionary Genetics, Max Plank Institute for Ornithology, Seewiesen, Germany*
[g] *Laboratorio de Parásitos y Enfermedades de Fauna Silvestre, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile*
[h] *Facultad de Ciencias de la Vida, Universidad Andres Bello, Viña Del Mar, Chile*
[i] *Instituto de Ecología y Biodiversidad, Parque Etnobotánico Omora, Universidad de Magallanes, Puerto Williams, Chile*
[j] *Departamento de Ecología y Biodiversidad, Universidad Andres Bello, Santiago, Chile*

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ABSTRACT

Latitudinal gradients are well-suited systems that may be helpful explaining distribution of haemosporidian parasites and host susceptibility. We studied the prevalence, diversity and drivers of haemosporidian parasites (*Leucocytozoon*, *Plasmodium* and *Haemoproteus*) along a latitudinal gradient (30°–56° S), that encompass the total distribution (~3,000 km) of the Thorn-tailed Rayadito (*Aphrastura spinicauda*) in the South American temperate forests from Chile. We analyzed 516 individuals from 18 localities between 2010 and 2017 and observed an overall prevalence of 28.3% for haemosporidian parasites. *Leucocytozoon* was the most prevalent parasite (25.8%). We recorded 19 distinct lineages (13 for *Leucocytozoon*, five for *Plasmodium*, and one for *Haemoproteus*). Differences in haemosporidian prevalence and diversity by genus and type of habitat were observed in the latitudinal gradient. Further, we support the existence of a latitudinal associate distribution of Leucocytozoids in South America, where prevalence and diversity increase toward higher latitudes. Distribution of *Leucocytozoon* was associated with sub-antarctic habitat (higher latitude) and explained by cold temperature and high precipitation. On the other hand, we lacked to find a latitudinal associate pattern for *Plasmodium* and *Haemoproteus*, however low prevalence and high diversity were recorded in areas considered as a hotspot of biodiversity in Central Chile. Our findings confirmed the importance of habitat and climatic variables explaining prevalence, diversity and distribution of haemosporidian parasites in a huge latitudinal gradient, belonging the distribution of the Thorn-tailed Rayadito in the world's southernmost forests ecosystems.

1. Introduction

Latitudinal gradients explain global patterns of biodiversity for a wide range of free-living taxa, where diversity is increased to the tropics (Gaston, 2000; Willig et al., 2003). These biogeographical systems usually present spatial heterogeneity in habitat and climatic conditions being helpful explaining host susceptibility for haemosporidian parasites (Valkiūnas, 2005; Merino et al., 2008; Loiseau et al., 2010).

Haemosporidian parasites (*Leucocytozoon, Plasmodium* and *Haemoproteus*) are a diverse group of vector-borne parasites that infect birds worldwide, with Antarctica exception (Valkiūnas, 2005; Bensch et al., 2009; Clark et al., 2014). These protozoa have heteroxenous life cycles, and are transmitted by blood-sucking dipterans (Valkiūnas, 2005; Atkinson et al., 2008; Santiago-Alarcon et al., 2012). Understanding ecological factors driving distribution, prevalence and diversity of vector-borne diseases is a challenge in diseases ecology,

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specially under current climate crisis and habitat modification scenarios (Lafferty, 2009; Sehgal, 2010; Pérez-Rodríguez et al., 2013). Multiple factors are described affecting prevalence, distribution and diversity of haemosporidian parasites (see review of Sehgal, 2015). For example, anthropogenic activities (Patz et al., 2000; Chasar et al., 2009; Gonzalez-Quevedo et al., 2014; Sehgal, 2015), host life-history traits (Valkiūnas, 2005; Quillfeldt et al., 2011; Isaksson et al., 2013), and biotic and abiotic variables (Loiseau et al., 2010; Knowles et al., 2011; Zamora-vilchis et al., 2012; Gonzalez-Quevedo et al., 2014; Padilla et al., 2017). Consequently, water availability and temperature affect vector development, distribution (Wood et al., 2007; Knowles et al., 2011), and abundance of vectors for *Plasmodium* and *Haemoproteus* (Balls et al., 2004). Also, it has been observed that higher temperatures and precipitations for tropical areas give suitable conditions for parasite development and transmission of *Plasmodium* and *Haemoproteus* genus (Clark et al., 2014; Durrant et al., 2006), while *Leucocytozoon* genus is most frequent for temperate areas with higher altitudes (Sehgal et al., 2011; Van Rooyen et al., 2013a; Matta et al., 2014; Lotta et al., 2015, 2016) and latitudes (Valkiūnas, 2005; Merino et al., 2008; Oakgrove et al., 2014).

Latitudinal distribution of haemosporidian parasites has been explored at different geographical scales around the world (Merino et al., 2008; Oakgrove et al., 2014; Clark, 2018; Doussang et al., 2019; Fecchio et al., 2019a). However, most studies have been focused on *Plasmodium* and *Haemoproteus* genus, while *Leucocytozoon* genus has been understudied. In South America *Leucocytozoon* was associated with Andean region (Merino et al., 2008; Harrigan et al., 2014; Matta et al., 2014; Lotta et al., 2015, 2016) and was recently reported in lowlands of Amazonas (Fecchio et al., 2018). For South America Merino et al. (2008) have proposed the existence of a positive association of *Leucocytozoon* with latitude (not a classical latitudinal pattern), while *Plasmodium* and *Haemoproteus* support the classical latitudinal gradient in diversity (Merino et al., 2008; Fecchio et al., 2019a). However, in a global revision of Clark (2018), he did not find a latitudinal gradient for diversity of the three most common haemosporidian parasites, hence the existence of latitudinal-associate parasite distribution continue under debate.

The South American temperate forests are distributed along a narrow and huge latitudinal gradient (~3,000 km; 30°–55° S), mostly in Chile and with a narrow strip in Argentina. These forests are interesting due to their strong biogeographical isolation from other forests regions. At the north, they limit with the Atacama Desert, the driest in the world; to the east the Andean Mountains lies, to the west we find the south Pacific Ocean and to the south the sub-antarctic areas (Armesto et al., 1996, 1998). In Chile, this biogeographical isolation makes these forests present contrasting habitats and climatic conditions (i.e. relicts of temperate forests immerse in semiarid matrix at central-north Chile, forests with Mediterranean climatic influences at central Chile, temperate rainy forests at central-south Chile, and Magellanic sub-antarctic forests at south Chile) (Armesto et al., 1996; López-Cortés and López, 2004).

The Thorn-tailed Rayadito (Furnariidae: Passeriformes) is a small (~11 g) endemic insectivorous species resident of the South American temperate forests (Remsen, 2003, Fig. 1). This species are socially monogamous; both mates contribute to nest building, incubation and the feeding of nestlings (Moreno et al., 2005; Espíndola-Hernández et al., 2017). Furthermore, this species has shown latitudinal differences in anti-predatory behavior (Ippi et al., 2011, 2013), clutch size (Quirici et al., 2014), nest architecture (Botero-Delgadillo et al., 2017a), corticosterone levels (Quirici et al., 2014), telomere length (Quirici et al., 2016), and high levels of gene flow (Gonzalez and Wink, 2010) with exception of the northernmost isolate population (Yáñez et al., 2015; Botero-Delgadillo et al., 2017b).

To our knowledge, haemosporidian parasites had never been explored in a huge latitudinal gradient with contrasting environments covering the total distribution for an only bird-species in a natural isolate biome. These studies help to elucidate the community composition of parasites and the changes in space and time (Bensch et al., 2007; Van Rooyen et al., 2013b). The aims of this study were to assess the prevalence, diversity and drivers of haemosporidian parasites (*Leucocytozoon*, *Plasmodium* and *Haemoproteus*) throughout the latitudinal distribution of a passerine species along the world's southernmost forests.

2. Materials and methods

2.1. Bird sampling and study sites

Between 2010 and 2017, we collected blood samples of the Thorn-tailed Rayadito from 18 localities along a latitudinal gradient in the South American temperate forests from Chile (30°–56° S; Fig. 1). In Bosque Fray Jorge National Park, Manquehue, Chiloé, and Navarino Island birds were captured from their nesting boxes (with a manually triggered metal trap that sealed the entrance hole when adults entered to feed their 12–14 days old nestlings). In the rest of the localities, we captured the birds using mist-nets. All captured birds were ringed with numbered metal rings provided by the Servicio Agrícola y Ganadero de Chile (SAG). Birds were weighed, and tarsus length was measured. A blood sample was obtained from the brachial vein by punction using sterile needles. The volume of blood extracted never exceeded 1% of the bird body mass. Blood was collected in microhematocrit tubes and stored into FTA Classic Cards (Whatman®) for subsequent molecular analysis. Birds were immediately released at the place of capture.

We grouped the sampling areas into four types of habitats (relict-semiarid, mediterranean, rainy, and sub-antarctic) according to biogeographical regionalization (Morrone, 2014), environmental and climatic conditions (Table 1; Fig. 1), and using the map of terrestrial ecoregions of the world (Olson et al., 2006). (i) *Relicts of temperate forests immerse in a semi-arid matrix (hereafter relict-semiarid)*: located between 30° and 32° S (Fig. 1; Table 1) and are composed by relicts of forests from Pleistocene period. Here, we found the northernmost population (Bosque Fray Jorge National Park, lowest latitude) of the Thorn-tailed Rayadito. These forests are composed mainly of Olivillo (*Aextoxicon punctatum*) and Petrillo (*Myrceugenia correifolia*), distributed in patches at the top of the coastal mountain range (Villagrán et al., 2004) where the fog from the ocean induces microclimatic conditions that allow the forest to exist in this semi-arid matrix (López-Cortés and López, 2004). This fog-induced microclimatic is reflected on temperature and precipitation, observing low variation into the forest, while the climate in the semi-arid matrix is mediterranean-arid, with dry hot summers and cold winters. (ii) *Forests in the mediterranean climate (hereafter mediterranean)*: located between 33° and 35° S (Fig. 1; Table 1) and are composed mainly by xeric forests of Peumo (*Cryptocaria alba*), Hualle (*Nothofagus obliqua*), Quillay (*Quillaja saponaria*) and Litre (*Lithrea caustica*), which are characteristic of the Mediterranean climate semi-arid region of central Chile, where precipitation occurs only during winter and temperature varies greatly during and between days (Rundel and Weisser, 1975). (iii) *Temperate rainforests (hereafter rainy)*: located between 37° and 43° S (Fig. 1; Table 1), are composed by the most important native forest in the evergreen temperate forest with highest biodiversity; the Valdivian and Nordpatagonian forests and with predominant species of Olivillo, Maqui (*Aristotelia chilensis*), Chilca (*Fuchsia magellanica*), Coigüe (*Nothofagus betuloides*). The climate is rainy temperate with high precipitation over all the year (Carmona et al., 2010). (iv) *Sub-antarctic Magellanic forests (hereafter sub-antarctic)*: located between 53° and 56° S (Fig. 1; Table 1), represent the southernmost distribution limits (highest latitude) of the Thorn-tailed Rayadito. The vegetation is characterized by deciduous Magallenic forest, composed mainly by Lenga (*Nothofagus pumilio*), Canelo (*Drimys winteri*), Ñirre (*Nothofagus antarctica*) and Coigüe. The climate is oceanic, with a low annual thermic fluctuation (Rozzi et al., 2007).
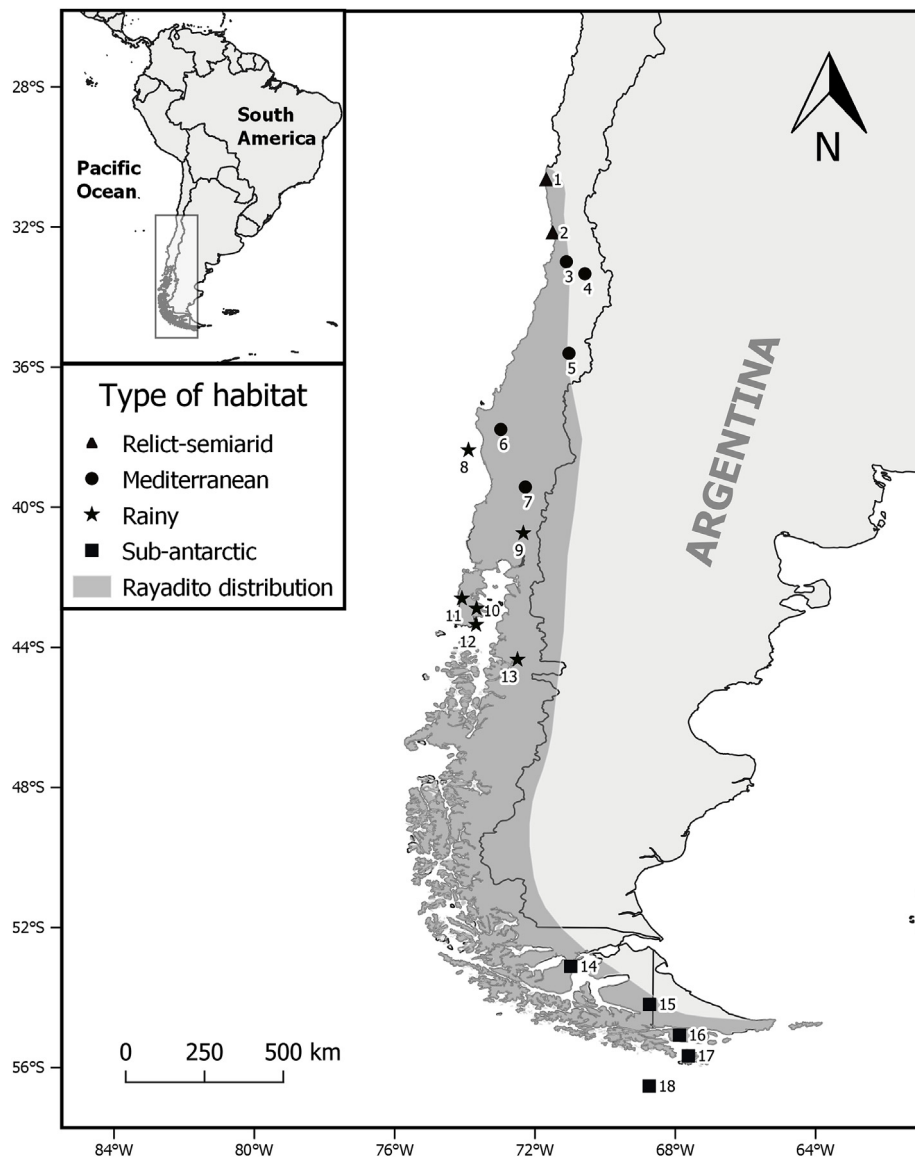
Fig. 1. Map of sampling areas covering all latitudinal distribution of the Thorn-tailed Rayadito along the South American temperate forests.

2.2. Molecular sexing and screening for haemosporidian parasites

Genomic DNA was extracted using the salting-out procedure (Aljanabi and Martinez, 1997). The sex of birds was determined by using a molecular method (Fridolfsson and Ellegren, 1999). Polymerase Chain Reaction (PCR) products were run in 1% agarose gels, stained with Syber Safe®, and visualized using the system for the documentation and analysis of fluorescently stained gels GBOX F3 (Syngene, MD, USA). Birds were sexed as females (heterogametic: WZ) and males (homogametic: ZZ).

Screening for parasites were performed with a parasite genus-specific primers in a nested-PCR protocol that amplifies a fragment of 480 bp (excluding PCR primers) of the mitochondrial cytochrome b (cyt b) gene of haemosporidian parasites (Hellgren et al., 2004). Two positive controls for parasites and two negative controls (ddH2O) for each 48 samples were included, no contamination was detected. We screened each sampled at least twice, to avoid false negatives. Positive PCR products were purified and sequenced using the Macrogen sequencing service (Macrogen Inc., South Korea).

2.3. Prevalence and genetic diversity

We used the software Quantitative Parasitology v.3.0 (Rózsa et al., 2000; Reiczigel et al., 2019) to calculate unbiased prevalence and its 95% confidence intervals (CI) with Sterne's exact method (Reiczigel, 2003). Prevalence and CI take in account the sample size, avoiding the problems that normal theory faces with skewed distributions, especially for small sample sizes (Rózsa et al., 2000; Reiczigel et al., 2019). In order to avoid pseudo-replication, only individuals at the first-time capture, were considerate for statistical analyses. The effect of latitude on prevalence and diversity of haemosporidian parasites was assessed indirectly by using the type of habitat as a dependent variable in statistical analyses (because of the latitudinal distribution of type of habitat and to avoid pseudo-replication). We used a bivariate Pearson's Chi-square test to compare infection prevalence of haemosporidian parasites between genus, sex and type of habitat.

Genetic diversity was assessed for each type of habitat, and for the total distribution of the Thorn-tailed Rayadito, using number of polymorphic sites (S), haplotype number (h), gene diversity (Hd), and nucleotide diversity (π) from mtDNA *cytb* for each haemosporidian parasite genus, using the software DNAsp v.5.10.1 (Rozas, 2009).

Table 1
Study areas indicating localization, environment and climatic characteristics related to years of sampling and the Thorn-tailed Rayadito captured and recaptured in each locality.

Locality	Coordinates	Temperature (mean annual)	Precipitation (mean annual)	Habitat	Year of capture	Birds captured/recaptured
1) Bosque Fray Jorge National Park	30°38′ S, 71°40′ W	11.37 °C	132.62 mm	Relict-semiarid	2010–2016	153/207
2) Nature sanctuary Santa Inés Hill	32°10′ S, 71°30′ W	13.45 °C	170 mm	Relict-semiarid	2016	9
3) La Campana National Park	33°01′ S, 71°09′ W	ND	ND	Mediterranean	2013–2014	3
4) Manquehue Hill	33°21′ S, 70°34′ W	16.44 °C	342.9 mm	Mediterranean	2010–2011, 2016	61/68
5) Altos of Lircay National Reserve	35°37′ S, 71°03′ W	12.92 °C	492.4 mm	Mediterranean	2013–2014	4
6) Nahuelbuta National Park	37°47′ S, 72°59′ W	11.8 °C	1185.6 mm	Mediterranean	2014	4
7) KodKod Private Reserve	39°25′ S, 71°76′ W	11.59 °C	936.4 mm	Mediterranean	2012,2014	4
8) Mocha Island National Reserve	38°23′ S, 73°52′ W	11.82 °C	1168 mm	Rainy	2015	39
9) Puyehue National Park	40°47′ S, 72°12′ W	10.95 °C	1373 mm	Rainy	2014–2016	35
10) Senda of Darwin Biological Station	42°53′ S, 73°40′ W	9.14 °C	1754.5 mm	Rainy	2010	7
11) Chiloé National Park	42°07′ S, 74°09′ W	10 °C	2448 mm	Rainy	2014	10
12) San Pedro Island Private Reserve	43°21′ S, 73°44′ W	9.99 °C	2489.7 mm	Rainy	2017	10
13) Queulat National Park	44°24′ S, 72°24′ W	9.88 °C	4825.2 mm	Rainy	2015	2
14) Punta Arenas	53°09′ S, 70°54′ W	6.85 °C	189 mm	Sub-antarctic	2016	3
15) Karukinka Natural Reserve	53°42′ S, 69°18′ W	6.26 °C	515.4 mm	Sub-antarctic	2016	20
16) Navarino Island	55°40′S 67°40′W	5.9 °C	446.24 mm	Sub-antarctic	2010–2014	145/181
17) Cape Horn National Park	55°48′ S, 67°17′ W	6.5 °C	486.2 mm	Sub-antarctic	2016	4
18) Diego Ramírez Island	56°31′ S 68°44′ W	6.5 °C	486.2 mm	Sub-antarctic	2016	9

2.4. Drivers of haemosporidian infection

Generalizer Linear Mixed Models (GLMMs) were performed to study the influence of ecological (habitat, temperature and precipitation) and a host factor (sex) over the probability of infection by haemosporidian parasites. GLMMs combine the properties of two statistical frameworks, linear mixed models and generalizer lineal models providing a more flexible approach for analyzing non-normal data when random effects are present (Bolker et al., 2009). In order to avoid statistical problems, the data was explored following recommendations of Zuur et al. (2010). We used GLMMs fitted by maximum likelihood with binomial distribution, including pooled haemosporidian (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) and *Leucocytozoon*, which were analyzed separated as a dependent variable. Due the fact of low prevalence for *Plasmodium* and *Haemoproteus*, were not evaluate as dependent variable.

Climatic variables were selected due the relevance for haemosporidian parasites, and the most likely associate mechanism reported in literature. Daily rainfall (mm) and daily temperature (°C) for all the years of study (2010–2017) were collected from local meteorological stations; CEAZA (Centro de Estudios Avanzados en Zonas Áridas; http://www.ceazamet.cl) and the Chilean Meteorological Office (http://www.meteochile.gob.cl). Then, these data were used to construct mean temperature of the coldest month (COLDTEMP), mean temperature of the warmest month (WARMTEMP), mean annual temperature (MEANTEMP), mean precipitation of the less rainy month (MINPREC), mean precipitation of the rainiest month (MAXPREC) and mean annual precipitation (PRECMEAN).

In the models, the environment-related fixed effects were

COLDTEMP, WARMTEMP, MEANTEMP, MINPREC, MAXPREC, MEANPREC, HABITAT (relict-semiarid, mediterranean, rainy, and sub-antarctic), and as host-related fixed effect the SEX (female and male). The random effects were years (2010–2017) and localities. Prior to the running full models, we explored the variables to know collinearity for each pair of predictor variables, using pairwise bivariate correlation. COLDTEMP was highly correlated with MEANTEMP (Pearson's $r = 0.88$, $P < 0.001$). MINPREC was correlated with MAXPREC (Pearson's $r = 0.66$, $P < 0.001$), and with MEANPREC (Pearson's $r = 0.70$, $P < 0.001$). MAXPREC was highly correlated with MEANPREC (Pearson's $r = 0.94$, $P < 0.001$). In this sense, and because of the importance of the predictors to explain haemosporidian infection, MEANTEMP, MINPREC and MEANPREC were removed from the full models. Statistical analysis was conducted with the software R v.3.4.0 (Team, 2013) using the "lme4" package (Bates et al., 2014).

2.5. Phylogenetic analysis

Sequences were aligned and edited using the software Sequencher™ v.5.4.5 (GeneCodes Corporations, Ann Arbor, Michigan, USA). Polymorphic sites were evaluated using Clustal X2 (Larkin et al., 2007). Lineages were identified using the software DNAsp v.5.10.1 (Rozas, 2009). The lineages obtained were compared with parasite lineages recorded in MalAvi database (Bensch et al., 2009) and Genbank. Novel lineages were classified using a code for the host (*A. spinicauda*: Asp), the country (Chile: Ch), the genus (i.e., L for *Leucocytozoon*), and a number. Then were deposited in the MalAvi database and GenBank (accession numbers: **MN083254-MN083268**).

The best substitution model (GTR + I + G) suitable for phylogenetic reconstruction was determined using the software JModelTest v.2.1.4 (Posada, 2008), selected with both Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC). Phylogenetic reconstruction was performed using the software MrBayes v.3.2.6 (Ronquist et al., 2012). We used a sequence of *Plasmodium falciparum* as outgroup to root the consensus phylogram, the 19 sequences (478 bp) belonging to the lineages found in this study were aligned with 32 lineages (409 bp) previously recorded for Andean region (Colombia, Perú, Ecuador, Chile). Two independent Markov Chain Monte Carlo (MCMC) simulations were run for 5 million generations with sampling every 200 generations to create a consensus tree, the convergence of the analysis was corroborated by observing the standard deviation split criterion (< 0.01). Phylogeny was visualized using the software FigTree v.1.4.3. (Rambaut, 2009). Additionally, we estimated genetic distances (gd) between lineages of *Leucocytozoon* found in this study and other lineages recorded in Andean region using a Kimura two-parameter model of substitution, implemented in MEGA v. X (Kumar et al., 2018).

3. Results

3.1. Prevalence and distribution of haemosporidian parasites

Haemosporidian parasites were detected in 146 of 516 individuals with an overall prevalence of 28.3% (95% CI: 24.5–32.3%). The most frequent parasite genus was *Leucocytozoon*, infecting 133 individuals with 25.8% (95% CI: 22.1–29.7%) of prevalence. *Plasmodium* and *Haemoproteus* showed low prevalence 3.5% (95% CI: 2.2–5.4%), infecting only 18 individuals (nine individuals 1.7% with *Plasmodium*, four individuals 0.8% with *Haemoproteus* and five individuals were unsolved sequences; for details see Table 2). Additionally, 28 parasite co-infections (infections by two genera or simultaneous infections of two parasite lineages) were observed 5.4% (95% CI: 3.7–7.7%) (Table 2). There were no sex differences in prevalence ($\chi^2 = 0.047$, df = 1, p = 0.82) among females (30.59%) and males (31.55%).

The distribution of haemosporidian parasites in the latitudinal gradient showed 2.5% (95% CI: 0.8–6.4%) of prevalence for relict-semiarid habitat (lower latitude, 30°–32° S), 13.2% (95% CI: 7–22.8%) of prevalence for mediterranean habitat (33°–39° S), 33% (95% CI: 24.3–42.6%) of prevalence for rainy habitat (38°–44° S), and 55.4% (95% CI: 47.7–62.7%) of prevalence for sub-antarctic habitat (higher latitude, 53°–56° S) (Table 2). The most frequent parasite genus was *Leucocytozoon* with the higher prevalence for all the latitudinal distribution (Table 2). Prevalence was significantly different between the four types of habitats in the latitudinal gradient ($\chi^2 = 64.28$, df = 3, p < 0.001).

3.2. Diversity of haemosporidian parasites

Genetic characterization identified a total of 19 distinct lineages of 478 pb covering all the latitudinal distribution of the Thorn-tailed Rayadito, 13 lineages belonging to *Leucocytozoon*, five for *Plasmodium* and one for *Haemoproteus* (Table 3). New lineages documented in this study include 12 for *Leucocytozoon* (AspChL1-AspChL12), two for *Plasmodium* (AspChP1, AspChP2), and one for *Haemoproteus* (AspChH1). One lineage of *Leucocytozoon* was previously recorded in Andes mountains of Perú (Galen and Witt, 2014), and lineages of *Plasmodium* were previously recorded in different countries around the world (Bensch et al., 2009). For example, we found the widely distributed GRW04 lineage (morphological species of *Plasmodium relictum*).

The distribution of lineages in four types of habitats along the latitudinal gradient showed three *Leucocytozoon* lineages for relict-semiarid habitat (lower latitude); three *Leucocytozoon*, four *Plasmodium*, and one *Haemoproteus* lineages for mediterranean habitat (central latitude); six *Leucocytozoon*, two *Plasmodium*, and one *Haemoproteus* lineages for rainy habitat (south central latitude); and five *Leucocytozoon* lineages for sub-antarctic habitat (Table 3). The two most common parasite lineages (AspChL1, AspChL4) were associated mainly with sub-antarctic habitat, but AspChL1 was found in all types of habitats and both were found at lower and higher latitudes (Table 2).

Contrary to prevalence results, *Leucocytozoon* showed similar high genetic diversity (Hd = 0.69, π = 0.025) with *Plasmodium* (Hd = 0.75, π = 0.036), and higher than *Haemoproteus* (Hd = 0, π = 0). Higher genetic diversity was associated with mediterranean and rainy habitats (33°–44° S) for *Plasmodium* and *Haemoproteus*, and rainy y sub-antarctic habitat (38°–56° S) for *Leucocytozoon* (Table 3).

3.3. Drivers of haemosporidian infection

Results of GLMMs explaining haemosporidian infection are summarized in Table 4. The best predictors for both, pooled haemosporidian and *Leucocytozoon* were the HABITAT (sub-antarctic), MAXPREC, and COLDTEMP. However, results for pooled haemosporidian were influenced by the high prevalence of *Leucocytozoon* throughout the latitudinal distribution of the Thorn-tailed Rayadito.

Table 2

Prevalence and lineages of haemosporidian parasites by type of habitat in the Thorn-tailed Rayadito.

Habitat/Latitudinal gradient	Individuals/infected Prevalence [95% CI]	Infected birds by each genus, unsolved infections, and coinfections					Lineages
		L	P	H	Unsolv	Coinf	
Relict-semiarid (30°–32° S)	160/4 2.5% [0.8–6.4]	4	0	0	0	0	AspChL1; AspChL4; *Leucocytozoon sp*. L-T006.
Mediterranean (33°–39° S)	76/10 13.2% [7–22.8%]	4	4	3	7	2	AspChL1; AspChL7; AspChL12; *Plasmodium lutzi*; *Plasmodium relictum*; *Plasmodium sp*.; AspChP2; AspChH1.
Rainy (38°–44° S)	103/34 33% [24.3–42.6%]	28	5	1	10	10	AspChL1; AspChL2; AspChL5; AspChL6; AspChL9; AspChL10; *Plasmodium lutzi*; AspChP1; AspChP2; AspChH1.
Sub-antarctic (53°–56° S)	177/98 55.4% [47.7–62.7%]	97	0	0	1	16	AspChL1; AspChL3; AspChL4; AspChL8; AspChL11.
Total	516/146 28.3% [24.5–32.3%]	133	9	4	18	28	

Infected birds by each haemosporidian genus (P: *Plasmodium*, H: *Haemoproteus*, L: *Leucocytozoon*, Unsolv: Unsolved PH sequences; Coinf: coinfections), and lineages found in theThorn-tailed Rayadito.

Table 3
Genetic diversity of haemosporidian parasites by type of habitat in the Thorn-tailed Rayadito.

Habitat type/Latitudinal gradient	N	Leucocytozoon					Plasmodium					Haemoproteus				
		N+	nH	S	Hd	π	N+	nH	S	Hd	π	N+	nH	S	Hd	π
Relict-semiarid (30°–32° S)	160	4	3	4	0.83	0.004	0	–	–	–	–	0	–	–	–	–
Mediterranean (33°–39° S)	76	4	3	56	1	0.077	4	4	33	0.90	0.032	3	1	0	0	0
Rainy (38°–44° S)	103	28	6	53	0.65	0.039	5	2	27	0.40	0.022	1	1	0	0	0
Sub-antarctic (53°–56° S)	177	97	5	45	0.55	0.007	0	–	–	–	–	0	–	–	–	–
Total	516	133	13	68	0.69	0.025	9	5	48	0.75	0.036	4	1	0	0	0

N = number of samples; N+ = number of positive samples; nH = number of haplotypes; S = number of polymorphic sites; Hd = haplotype diversity; π = nucleotide diversity.

Table 4
Summary of GLMMs predicting presence of haemosporidian infection and *Leucocytozoon* in the Thorn-tailed Rayadito along a latitudinal gradient in Chile.

Dependent variable	Predictor variables	Estimate ± SE	z value	p
Pooled haemosporidian	(Intercept)	−4.9195 ± 1.07	−4.573	***
	COLDTEMP	0.1700 ± 0.06	2.454	***
	MAXPREC	0.0154 ± 0.00	3.681	***
	HABITAT	−0.7292 ± 0.64	−1.127	***
	-Rainy	−0.4552 ± 0.77	−0.590	
	-Relict-Semiarid	5.4989 ± 1.26	4.340	
	-Sub-antarctic			
Leucocytozoon sp.	(Intercept)	−5.9303 ± 1.26	−4.672	***
	COLDTEMP	0.1689 ± 0.07	2.260	*
	MAXPREC	0.0158 ± 0.00	3.369	***
	HABITAT	−0.1113 ± 0.76	−0.145	***
	-Rainy	0.5298 ± 0.90	0.588	
	-Relict-Semiarid	6.4467 ± 1.44	4.450	
	-Sub-antarctic			

Year and locality were introduced as random effects. *** < 0.0001; * < 0.05.

3.4. Phylogenetic analysis

The Bayesian phylogenetic analysis of the haemosporidian parasites sequences found in this study showed these grouped in four mainly clades (Fig. 2). *Leucocytozoon* was grouped into two main clades, *Plasmodium* into one main clade, and *Haemoproteus* into one main clade (Fig. 2). The most frequent lineages for *Leucocytozoon* (AspChL1, AspChL4) were grouped in the same clade and were linked mainly to sub-Antarctic habitat (higher latitude), but AspChL1 was present in all types of habitat (Fig. 2; Table 2), the other clade of *Leucocytozoon* was associated mainly with rainy habitat and less frequent to Mediterranean habitat (Fig. 2; Table 2). While lineages of *Plasmodium* and *Haemoproteus* were frequent in Mediterranean and rainy habitats in Central Chile (Fig. 2; Table 2).

Genetic distances between lineages of *Leucocytozoon* found in this study and lineages previously recorded in Andean region have indicated firstly, that most of the lineages of *A. spinicauda* were closely linked between them, lineage AspChL1 was related (gd = 0.002) to AspChL3, lineage AspChL2 was related (gd = 0.035) to AspChL12, lineage AspChL4 was related (gd = 0.035) to AspChL11, lineage AspChL5 was related (gd = 0.002) to AspChL6. Secondly, three lineages (AspChL7, AspChL8, AspChL9) were closely related (gd = 0.012–0.009) to a previously lineage recorded in Colombia (Genbank No. KF699313). Finally, lineage AspChL10 was related (gd = 0.012) to AspChL4 and with a previously lineage recorded in Chile (Genbank No. EF153661), and the only one lineage in our study previously documented in Perú (Genbank No. KF767431) was related (gd = 0.002) with a previously lineage recorded in Chile (Genbank No. EF153657) and with a lineage of Colombia (Genbank No. KF717054) (Table 5).

4. Discussion

In this study, we evaluated the prevalence, diversity, and the factors that influence the probability of infection by haemosporidian parasites (*Leucocytozoon*, *Plasmodium* and *Haemoproteus*) in the Thorn-tailed Rayadito along a latitudinal gradient that encompass all the distribution of the above-mentioned species. We observed an overall prevalence of 28.3% for haemosporidian parasites in the Thorn-tailed Rayadito along its latitudinal distribution of ~3000 km (30°–56° S). *Leucocytozoon* was the most common haemosporidian parasite genus, with high prevalence (25.8%), belonging to 91% of all infections. We only detected 3.5% of prevalence for *Plasmodium* and *Haemoproteus*. High prevalence of *Leucocytozoon* (25.8%) in the Thorn-tailed Rayadito was similar to the 24% and 27% of *Leucocytozoon* prevalence in the blue and great tit, respectively in Europe (Jenkins and Owens, 2011). However, our results differ from the 15.4% of prevalence observed at community level in Chile (Merino et al., 2008) and 1.2% in Andean mountains of Colombia (Lotta et al., 2019). This may be explained by multiple biotic and abiotic factors involving in the transmission, distribution and diversity of haemosporidian parasites (Valkiūnas, 2005; Sehgal, 2015). For example, in our study, most birds were sampled during reproductive season which is related to the relapse stage of chronic haemosporidian parasite infection (Valkiūnas, 2005; Asghar et al., 2011). Additionally and according to other studies (McCurdy et al., 1998; Fecchio et al., 2015), no sex-biased haemosporidian prevalence was observed in the Thorn-tailed Rayadito, this could be in concordance with the similar reproductive costs and exposure to vectors for monogamous species in both mates (McCurdy et al., 1998; Fecchio et al., 2015).

In South America, *Leucocytozoon* has been recorded previously in resident birds from highlands of Andes Mountains in Colombia, Peru, Chile, and Ecuador (Merino et al., 2008; Galen and Witt, 2014; Harrigan et al., 2014; Matta et al., 2014; Lotta et al., 2015, 2016; 2019; Martínez et al., 2016; this study), and lowlands from Chile (Merino et al., 2008; Rodrigues et al., 2019; this study), and recently in lowlands from Amazonas (Fecchio et al., 2018). In this study, we support the existence of a latitudinal associated distribution of Leucocytozoids in South America (Merino et al., 2008; Matta et al., 2014). As we expected, and similar to the observed by Merino et al. (2008) in Chile and Oakgrove et al. (2014) in Alaska, the higher *Leucocytozoon* prevalence was increased toward higher latitudes (Table 2). Additionally, genetic lineage richness was increased toward central and higher latitudes (Table 3), similar to the reported in higher latitudes from Alaska (Oakgrove et al., 2014). Contrary to the review of Clark (2018) at global scale who showed no effect of latitude on *Leucocytozoon* and other haemosporidian parasites diversity. The existence of specific local environmental and host conditions that drive diversity and distribution of haemosporidian parasites (Ellis et al., 2017; Fecchio et al., 2019b), and parasite life cycle and transmission (Bordes et al., 2010; Santiago-Alarcón et al., 2012) can explain these results.

Distribution of *Leucocytozoon* in the latitudinal gradient may be attributable to several factors including competent vector-parasite-host
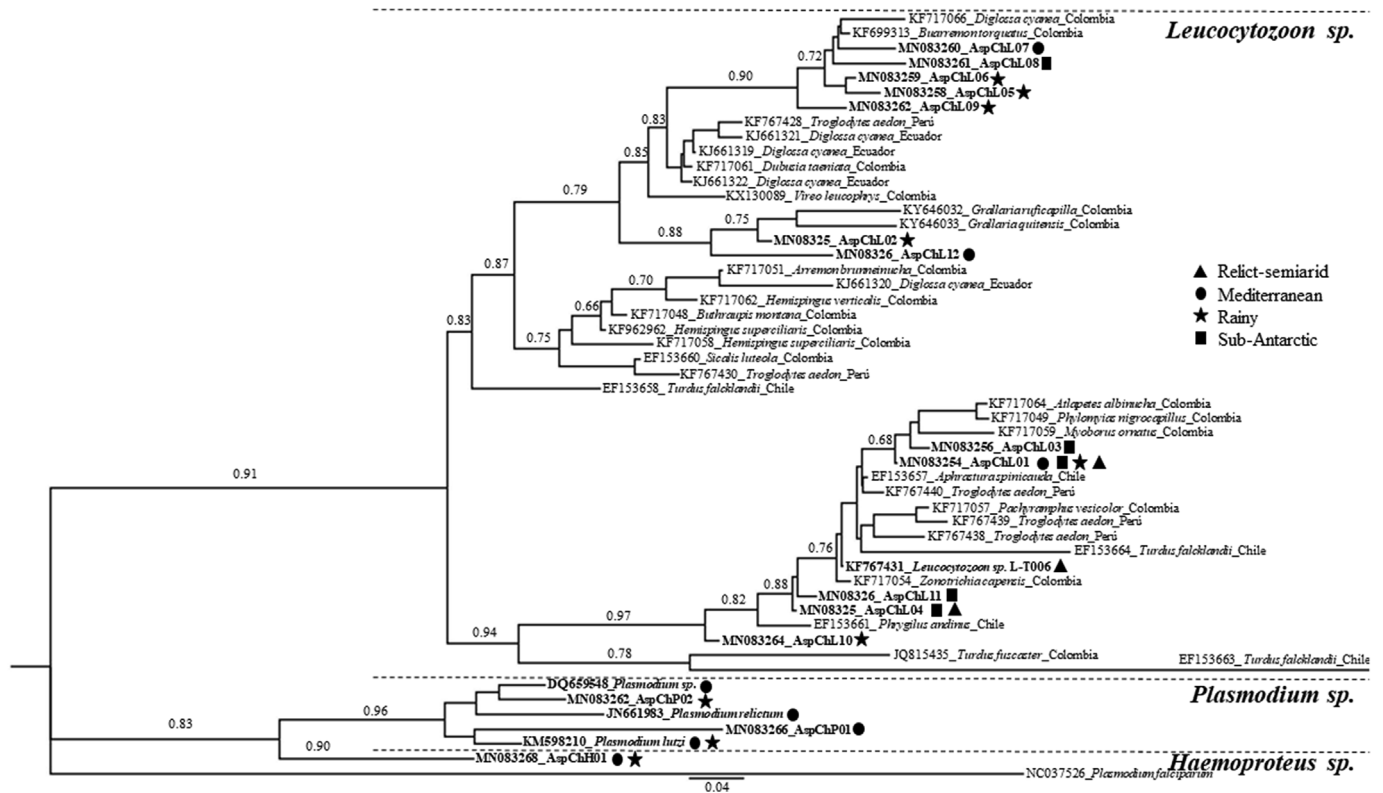
Fig. 2. Bayesian phylogenetic reconstruction of 409 bp haemosporidian cytochrome *b* sequences from positive Thorn-tailed Rayadito samples and lineages found in passerine species for Andean region. *Plasmodium falciparum* was used as outgroup. Lineage names of sequences from GenBank accession numbers are given followed by the passerine bird species and the country in which were recorded. Lineages found in this study are highlighted in bold with figures that represent the type of habitat in which lineages were found. Posterior support values are shown for each node greater than 0.5.

interactions (Valkiūnas, 2005) and abiotic environmental factors such as, temperature and precipitation. The natural geographical isolation of the South American temperate forests from another forests regions provides contrasting environments (relict-semiarid, mediterranean, rainy, and sub-antarctic, see methods for details). In consequence, *Leucocytozoon* showed contrasting patterns of prevalence in those types of habitat (Table 3), because forest type and variation in forest structure influence the probability to be infected (Renner et al., 2016). Our GLMMs results indicated that *Leucocytozoon* prevalence was higher in the sub-antarctic HABITAT (higher latitude), with cooler temperatures (COLDTEMP) and higher precipitations (MAXPREC) (Table 4). It has been observed that habitat is an important predictor to *Leucocytozoon* (Oakgrove et al., 2014; Lutz et al., 2015; Sehgal, 2015; Lotta et al., 2016; Illera et al., 2017; Padilla et al., 2017). Also, temperature and precipitation have been described to be essential environmental drivers of *Leucocytozoon* prevalence (Oakgrove et al., 2014; Harrigan et al., 2014; Illera et al., 2017; Padilla et al., 2017). In this sense, our findings are not unexpected, since *Leucocytozoon* species are adapted to develop and transmit below 15 °C (Valkiūnas, 2005). In addition, *Leucocytozoon* has been described as to complete their life cycle at higher latitudes and elevations in mountain regions (Haas et al., 2012; Van Rooyen et al., 2013a; Harrigan et al., 2014; Matta et al., 2014; Illera et al., 2017). The mountains regions provide favorable habitat for blackflies, the vectors for *Leucocytozoon* (Haas et al., 2012; Lotta et al., 2016). Vectors are habitat dependent (Santiago-Alarcón et al., 2012) and blackflies are recorded in all environments from different altitudes and latitudes (Coscarón and Arias, 2007). In the Andes Mountains of Colombia, it has proposed that transmission occurs at low temperatures (0–14 °C) (Matta et al., 2014; Lotta et al., 2015, 2016), which temperatures conditions are similar in higher latitudes from Chile. Localities in higher latitudes such as, Navarino island (sub-antarctic HABITAT) it is composed by several mountain streams that provides suitable conditions for parasite

development and transmission (Merino et al., 2008), reflecting in our prevalence records in the Thorn-tailed Rayadito. In fact, the high overall prevalence in our study was underlain by Navarino island locality, the higher latitude (55°40′S) at which haemosporidian parasites have been recorded in the world, given the absence of positive samples after this latitude. Hence, prevalence and diversity may be driven by the presence of competent vectors, which needs to be taken into account in future studies.

High genetic lineage richness was recorded for *Leucocytozoon*, we found 13 *Leucocytozoon* lineages and only one was recorded previously in the Peruvian Andes (Galen and Witt, 2014). Surprisingly, those lineages were different to previously lineages recorded in Chile (Merino et al., 2008; Martínez et al., 2016; Rodrigues et al., 2019), even they were different to three lineages previously found in the Thorn-tailed Rayadito (Merino et al., 2008). Those findings might indicate that genetic diversity of *Leucocytozoon* is higher for this species in Chile, despite we include localities covering all the latitudinal distribution. Phylogenic relationships using sequences previously reported in South America (Andean region), showed two mainly clades. Lineages recorded in this study were present in both clades, most of them with a close relationship among the other lineages found in the Thorn-tailed Rayadito (Fig. 2; Table 5). However, some of the lineages were closely linked to lineages recorded in other passerine species from Andean regions of Colombia, Perú and Chile (Fig. 2; Table 5). Our observations suggest that some lineages tend to be generalists, because are distributed across a wide range of host and locations along the Andean regions (Merino et al., 2008; Galen and Witt, 2014; Lotta et al., 2015, 2016). Nonetheless, some lineages might be exclusively to Furnariidae family (Fig. 2), as was observed with some lineages in Turdidae family (Lotta et al., 2016). However, we cannot asseverate the existence of host-specificity, since limited evidence was described for Leucocytozoids below the host-order level (Valkiūnas, 2005; Forrester and

Table 5
Genetic distance table between cytochrome b lineages of *Leucocytozoon* shown in Fig. 2. Calculations were made using Kimura two-parameter model of substitutions.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	**MN083259**																									
2	KIF767431	0.080																								
3	KF717054COL	0.083	0.002																							
4	**MN083254**	0.080	0.005	0.007																						
5	**MN083257**	0.080	0.005	0.007	0.010																					
6	**MN083264**	0.082	0.007	0.005	0.012	0.002																				
7	**MN083265**	0.064	0.094	0.091	0.099	0.088	0.085																			
8	EF153661CHILE	0.074	0.010	0.012	0.015	0.005	0.007	0.088																		
9	EF153664CHILE	0.094	0.038	0.040	0.038	0.038	0.040	0.091	0.040																	
10	**MN083263**	0.066	0.017	0.020	0.017	0.012	0.015	0.091	0.012	0.045																
11	**MN083261**	0.022	0.088	0.091	0.088	0.088	0.091	0.072	0.082	0.091	0.080															
12	**MN083258**	0.002	0.083	0.085	0.077	0.083	0.085	0.066	0.077	0.096	0.069	0.025														
13	KF699313	0.012	0.077	0.080	0.077	0.077	0.080	0.061	0.072	0.085	0.069	0.010	0.015													
14	**MN083260**	0.022	0.088	0.091	0.088	0.088	0.091	0.066	0.083	0.091	0.080	0.015	0.025	0.010												
15	**MN083262**	0.020	0.085	0.088	0.085	0.083	0.085	0.072	0.077	0.096	0.074	0.022	0.022	0.012	0.022											
16	EF153658CHILE	0.064	0.085	0.083	0.085	0.080	0.077	0.058	0.080	0.083	0.072	0.066	0.066	0.061	0.067	0.067										
17	KF717049COL	0.088	0.012	0.015	0.007	0.012	0.015	0.102	0.017	0.040	0.020	0.094	0.085	0.085	0.096	0.091	0.088									
18	KJ661322ECU	0.046	0.091	0.094	0.091	0.085	0.088	0.069	0.085	0.094	0.088	0.043	0.043	0.038	0.038	0.043	0.072	0.094								
19	KF767440PERU	0.091	0.010	0.012	0.010	0.010	0.012	0.099	0.015	0.043	0.022	0.099	0.088	0.088	0.099	0.088	0.091	0.012	0.091							
20	EF153657CHILE	0.083	0.002	0.005	0.002	0.007	0.010	0.096	0.012	0.040	0.020	0.091	0.080	0.080	0.091	0.088	0.088	0.010	0.088	0.007						
21	EF153660CHILE	0.058	0.080	0.083	0.085	0.075	0.077	0.056	0.075	0.091	0.072	0.066	0.061	0.061	0.061	0.067	0.048	0.088	0.059	0.085	0.083					
22	KJ661319ECU	0.035	0.085	0.088	0.091	0.080	0.083	0.064	0.080	0.088	0.083	0.033	0.038	0.028	0.028	0.033	0.067	0.094	0.010	0.091	0.088	0.053				
23	**MN083255**	0.051	0.083	0.080	0.083	0.077	0.074	0.035	0.077	0.080	0.080	0.053	0.053	0.043	0.053	0.048	0.043	0.085	0.056	0.088	0.085	0.053	0.051			
24	**MN083256**	0.083	0.007	0.010	0.002	0.012	0.015	0.102	0.017	0.040	0.020	0.091	0.080	0.080	0.091	0.088	0.088	0.010	0.094	0.012	0.005	0.088	0.094	0.085		
25	*NC037526*	0.240	0.264	0.267	0.257	0.260	0.264	0.233	0.264	0.239	0.257	0.247	0.236	0.246	0.253	0.257	0.229	0.260	0.240	0.264	0.260	0.243	0.240	0.216	0.260	

Lineages of *Leucocytozoon* obtained in this study are indicated in bold. Lineage of *Plasmodium falciparum* was used as outgroup, and it is indicated in italics.

Greiner, 2008), and this needs to be explored in birds communities and using new approaches, beyond the classical molecular methods (Lotta et al., 2019).

On the other hand, lineages of *Leucocytozoon* were mainly associated with rainy and sub-antarctic habitats (Table 3, Fig. 2), as was observed previously in Chile, where most lineages were present in the localities with similar environmental characteristics (Merino et al., 2008). This might be explained by geographic distributions of haemosporidian parasites are generally determined by populations of avian host and their abundance (Ellis et al., 2015, 2017). Additionally, in our study two lineages (AspChL1 and AspChL4) underlain overall prevalence of haemosporidian parasites in the Thorn-tailed Rayadito, which are mostly frequent in Navarino island locality (55° S), but present in almost all types of habitats with less frequency (Fig. 2; Table 2). This is consistent with other studies in which haemosporidian lineages with wider distribution had higher local prevalence (Szöllösi et al., 2011; Swanson et al., 2014). This pattern suggests a host-switching (Ellis et al., 2015) along the latitudinal distribution of the Thorn-tailed Rayadito, probably by migratory birds (Durrant et al., 2006), as was suggested by Merino et al. (2008), who observed shared lineages between the white-crested Elaenia (*Elaenia albiceps*) a long-distance migrant bird and other native birds from Chile.

For *Plasmodium* and *Haemoproteus* parasites, we lacked to find a latitudinal associated pattern, and infections were missing toward lower (30°–32° S) and higher (53°–56° S) latitudes, however low prevalence and high diversity was recorded in central latitudes (mediterranean and rainy habitats; 33°–44° S). This association was observed previously in Rufous-collared sparrows in Central Chile (Doussang et al., 2019). Contrary to the observed by Fecchio et al. (2019a), where higher diversity of *Plasmodium* and *Parahaemoproteus* parasites was observed from Patagonia to Amazonia, following the classical latitudinal gradient in diversity increased toward the Equator. Our findings may be due the fact of Central Chile is considered as hotspot of biodiversity (Myers et al., 2000). In consequence, higher haemosporidian lineage richness was associated to central Chile, because the higher number of hosts species increases haemosporidian parasite diversity through parasite lineage sharing and host shifting (Galen and Witt, 2014; Ricklefs et al., 2014; Clark, 2018). Furthermore, it has been observed that *Plasmodium* and *Haemoproteus* exhibits similar diversity to their avian hosts (Clark et al., 2014), and prevalence is positively related to abundance of hosts (Ellis et al., 2017). However, we lacked to find this pattern of prevalence in these high biodiversity areas from Chile. This might be related to a dilution effect of diseases by high diversity of hosts (Keesing et al., 2006) and the association of some lineages for a particular host species in areas where all the haemosporidian parasites genera co-occurred (Clark et al., 2014; Pulgarín-R et al., 2018). Further, previous records of *Haemoproteus* in Chile suggested an association of this parasite for passerine family Emberizidae (Merino et al., 2008). This may be related to the low prevalence of this parasite genus in the Thorn-tailed Rayadito.

The lineages of *Plasmodium* and *Haemoproteus* recorded in this study are more generalist, because distribution was recorded previously in different hosts and geographical areas (Clark et al., 2014; Bensch et al., 2009). Additionally, we found the widely distributed lineage of *Plasmodium relictum* (GRW04), the parasite that contributed to the declined of avifauna in Hawaii (Beadell et al., 2006). This lineage was recorded in Central Chile (Manquehue hill locality) and was previously reported in House sparrows at the North of Chile (Martínez et al., 2016) and recently in bird communities at Central and North of Chile by Doussang et al., (in preparation).

Finally, differences in prevalence and diversity among haemosporidian genus and type of habitat in the Thorn-tailed Rayadito may be explained by their evolutionary history (Ricklefs et al., 2004, 2014; Valkiūnas, 2005; Lutz et al., 2015). Because prevalence and diversity of haemosporidian parasites may be driven by the presence of competent vectors, more studies are needed to reveal the potential roles of local

species of Culicidae, Ceratopogonidae, and Hippoboscidae families, as vectors. Also, more studies focused on temporal prevalence and diversity patterns in bird communities are necessary to fully understand the distribution and possible host-specificity of *Leucocytozoon* and other haemosporidian in South America.

Declaration of competing interest

The author declared that there is no conflict of interest.

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