

Haematozoa in forest birds from southern Chile: Latitudinal gradients in prevalence and parasite lineage richness

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Abstract The existence of latitudinal gradients in species richness and their abundance is known for many free living organisms but few cases have been reported for parasitic diseases. In addition, asymmetries between the Northern and Southern Hemispheres in several characteristics may affect the distribution and diversity of species at all ecological levels. In this respect, we study the distribution of several genera of blood parasites infecting birds along a latitudinal gradient that includes the world's southernmost forests ecosystems. Birds were mist-netted and sampled for blood in localities across Chile ranging from 33°S to 55°S during the years 2003–06. Overall, 26 bird species were sampled and 27 parasite lineages were identified. The latter belonged to three genera: *Plasmodium* (8), *Haemoproteus* (8) and *Leucocytozoon* (11). We found a positive significant relationship between prevalence and latitude for *Leucocytozoon* lineages and a negative relationship for *Haemoproteus*, *Plasmodium* and mixed infections. However, we did not find a significant relationship between parasite diversity and latitude. We found 18 lineages infecting only one species of host, and 19 lineages appear in only one of the localities of sampling. This pattern implies that some parasite lineages may evolve in isolation in some species/localities. In addition, specificity at the host-family level was only found for *Haemoproteus* lineages infecting birds in the family Emberizidae. Individuals of the long distance migrant bird white-crested elaenia (*Elaenia albiceps*), were found infected by the same parasite lineages in localities separated by 20° of latitude. Infections by these lineages were detected in other sedentary birds including juveniles and nestlings of different bird species. Therefore, long distance migrants are able to distort the presence of latitudinal gradients of diseases due to the potential role of migrants in spreading infections. Geographical gradients in prevalence of avian haematozoa differ between parasite genera and hemispheres, probably in relation to the existence of appropriate vector–parasite–host interactions.

Key words: blood parasites, DNA analysis, host specificity, migratory birds, vectors.

INTRODUCTION

Although many studies have shown the existence of latitudinal gradients in species richness of free living organisms (Pianka 1966; Rohde 1992; Stevens 1992; Huston 1999; Chown & Gaston 2000), knowledge about these potential gradients in pathogenic organisms is scarce (see Hillebrand *et al.* 2001; Curtis *et al.*

2002; Nee 2003; Guernier *et al.* 2004; Guégan *et al.* 2005). The scarcity of studies addressing latitudinal variation in pathogenic microorganisms is especially marked for the Southern Hemisphere. For example, Guernier *et al.* (2004) showed a decline in the number of parasitic and infectious diseases affecting humans at higher latitudes in both hemispheres, but far more data and range of latitude were provided for populations in the north. In this respect, the different distribution of landmasses between both hemispheres may cause differences in biodiversity distribution as pointed out by

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several authors (See Chown *et al.* 2004). One of the main asymmetries between the Northern and Southern Hemispheres is their proportion of oceanic to land surface. Thus, most of the world's emerged land is situated between latitudes 30 and 60° north. This produces a ratio of area covered by water to area covered by land of 1:1 in comparison with a ratio of approximately 16:1 for the same range of latitudes in the southern hemisphere (Chown *et al.* 2004). The result is a marked oceanicity of southern land-masses that has a considerable effect on climate conditions and consequently to the distribution and diversity of species at all ecological levels, where microorganisms are not an exception (Merino & Potti 1996; Bonan 2002; Harvell *et al.* 2002).

South America is the only continental landmass reaching latitudes further south than 40 degrees where coexisting forests, forest birds, and their blood parasites can be encountered. Moreover, the Chilean forest ecosystems are especially interesting due to their isolation from other forest regions. Great orographic and climatic barriers separate them from the nearest tropical forests by at least 1500 km. To the north lies the Atacama Desert, the driest in the world, to the west and south the Pacific Ocean, and to the east rise the high Andean Mountains. In this way, the Chilean temperate forests become a biogeographical island (Medel & Vásquez 1994; Armesto *et al.* 1998; Rozzi *et al.* 2000). Toward the extreme south, the Chilean forest region becomes an archipelago composed of numerous islands of different size very close to each other and to the continent. Moreover, with the exception of *Elaenia albiceps*, there are no migratory forest birds that could spread diseases from the tropical areas to the extreme south.

Recently, avian diseases have gained attention due to their potential implication in the spread of zoonoses and diseases of economic importance among different countries (Rappole *et al.* 2000; Chen *et al.* 2006). Avian haematozoa commonly infect birds across the world (Bennett *et al.* 1982b; Bishop & Bennett 1992; Valkiūnas 2005) and represent good candidates to study latitudinal distribution of diseases in wild animals. Moreover, some phylogeographical studies have shown that parasite diversification is the result of frequent host-switching, rapid parasite dispersal and local specificity in new hosts (Fallon *et al.* 2005). Thus, there exists a potential effect of migrant birds working as a disruptive factor of latitudinal gradients, as they may spread parasite lineages across their migratory routes, which later specialise and diversify in other host species.

In this study, we present data on the latitudinal incidence and lineage richness of three blood protozoa genera (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) infecting forest birds in Chile between latitudes 33 and 55°S. We specifically study (i) the existence of latitudinal

gradients in prevalence and richness of three different genera of blood parasites; (ii) the presence of asymmetries between hemispheres in latitudinal gradients for these blood parasites in birds; (iii) the potential effect of host family specificity in reducing possibilities of parasite lineage spreading; and (iv) the potential effect of migrants as disruptors of these gradients through spreading parasite lineages.

METHODS

Birds were trapped with mist-nets situated at the edge of forest areas in several localities across Chile during 2003, 2004 and 2005 (Table 1). Each bird was individually marked with numbered or coloured bands to avoid re-sampling. For each bird, a drop of blood was obtained from the brachial vein and preserved in a buffer composed of 100 mM TRIS pH = 8.0, 100 mM of ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl sulphate (SDS) (see Jarvi *et al.* 2002) for DNA analyses. We used the same protocol in the spring of 2005/2006 except that samples were stored on FTA classic cards (Whatman International Ltd. UK; see Gutiérrez-Corchero *et al.* 2002). For a subsample of birds another drop of blood was smeared on a slide, air-dried and fixed in 96% ethanol. Blood smears were stained with Giemsa stain (1/10 v/v) for 40 min and scanned in search of blood parasites following the methods described in Merino *et al.* (1997).

Five areas of study were selected for the capture and sampling of birds (Fig. 1, Table 1): (i) Temperate areas: (1) In *Rinconada de Maipú* (nearby Santiago), birds were captured during May–August 2005. This area is covered by dry sclerophyllous shrubland dominated by *Acacia caven* and the climate is Mediterranean (Tapia 2005). (2) *Pantaniillos* is comprised of a mosaic of deciduous *Nothofagus glauca* forests and pine (*Pinus radiata*) plantations. The climate is humid with a short dry summer (Márquez 1992). Birds in this area were captured in November 2004. (3) *Ancud*, Senda Darwin Biological Station and Fundo los Cisnes, Chiloé Island. The area is composed of second growth forests with a mixture of several evergreen species (Gajardo 1994). The climate is mild and wet. Bird captures were carried out during November and December of 2003 and 2004. (ii) Extreme South areas: (4) *Outskirts of Punta Arenas* on an ecotonal zone between *Nothofagus* deciduous forests and the Patagonian steppe (Gajardo 1994). Climate is cold with low rainfall and snow in the winter. Birds were mist netted nearby Punta Arenas during December 2005. (5) Omora ethnobotanical Park on *Navarino Island* on a mosaic of evergreen broadleaf forests dominated by *Nothofagus betuloides* and *Drymis winteri*, deciduous *Nothofagus* forests of *Nothofagus pumilio* and *Nothofagus antarctica*, and mixed patches of evergreen

Table 1. Localities of sampling, indicating latitude, longitude, number of birds infected and sampled individuals (%), number of birds infected by each parasite genus and mixed infections (%) and number of lineages identified for each parasite genus; (ChH: *Haemoproteus*, ChP: *Plasmodium*, ChL: *Leucocytozoon*)

Locality	Latitude and Longitude	Birds infected/ sampled	H	P	L	Mixed	Number of host species	Parasite lineages
1. Rinconada de Maipú	33°32'S 70°6'W	13/61 (21.3%)	12 (19.7%)	6 (9.8%)	3 (4.9%)	7 (11.5%)	12	ChH: 5, 6, 7, 8 ChP: 6 ChL: 7, 8, 11
2. Pantanillos	35°25'S 72°8'W	20/79 (25.3%)	14 (17.7%)	8 (10.1%)	6 (7.6%)	7 (8.9%)	10	ChH: 1, 6 ChP: 1, 4, 6, 7 ChL: 3, 6, 9
3. Ancud, Chiloé Island	41°51'S 3°49'W	22/165 (13.3%) [†]	9 (5.5%)	15 (9.1%)	10 (6.1%)	9 (5.5%)	15	ChH: 1, 6 ChP: 1, 2, 3, 4, 8 ChL: 1, 4, 5, 9, 10
4. Punta Arenas	53°08'S 0°55'W	3/42 (7.1%)	2 (4.8%)	1 (2.4%)	1 (2.4%) [‡]	1 (2.4%)	7	ChH: 2, 4 ChP: 5 ChL: ?
5. Navarino Island	54°56'S 7°36'W	37/270 (13.7%)	3 (1.1%)	1 (0.4%)	34 (12.6%)	1 (0.4%)	13	ChH: 1, 3, 6 ChP: 1 ChL: 1, 2, 3
Total		95/617 (15.4%)	40 (6.5%)	31 (5.0%)	55 (8.9%)	25 (11.5%)	26	8ChH, 8ChP, 11ChL

[†]1/143 (0.7%) for nestlings sampled from nest-boxes (see *Methods*). [‡]Detected only in blood smears.

(*N. betuloides*) and deciduous *Nothofagus* (Pisano 1977; Rozzi *et al.* 2006). Rainfall is distributed more or less uniformly throughout the year and part of the precipitation is in the form of snow. Birds were captured on Navarino Island during December 2005 and January 2006. Bird nomenclature follows Jaramillo *et al.* (2003). In the area of study (3) Chiloé Island, 54 southern house wrens (*Troglodytes musculus*) nestlings and 89 thorn-tailed rayaditos (*Aphrastura spinicauda*) nestlings from a population breeding in nest boxes (Moreno *et al.* 2005) were sampled. Data from nestlings were treated separately in analyses as they do not share the same probability of contact and/or time to develop infections as compared to adults.

Although our areas of study include several localities on islands, the proximity to mainland and size and habitat complexity on them preclude the potential island effect on the diversity of parasites.

DNA analyses

Genomic DNA from samples conserved in lysis buffer was obtained using the UltraClean DNA BloodSpin kit (MO BIO Laboratories, Inc., Solana Beach, CA, USA). Genomic DNA on FTA cards was extracted to a soluble solution before polymerase chain reaction (PCR) using the following protocol: cored samples were transferred to collection vials with 250 µL of SET buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH = 8) at 4°C for 6 h. Afterwards, SDS 20%

(7 µL) and proteinase K (50 µg) were added to the vials and incubated at 55°C overnight. After incubation, ammonium acetate 4 M (250 µL) was added to the vials at room temperature for 30 min. Subsequently, vials were centrifuged at 13 000 *g* for 10 min. After removing the pellet, DNA was precipitated with ethanol and re-suspended in sterile water.

Initially, partial amplification of the cytochrome B gene was accomplished by PCR using the non-specific primers 3760F (5'-GAG TGG ATG GTG TTT TAG AT-3') and 4292Rw (5'-TGG AAC AAT ATG TAR AGG AGT-3') as previously used for detection of *Haemoproteus/Plasmodium* (see Beadell *et al.* 2004). Among samples found to be positive for infection by using those primers we tried to detect double infections by using new designed specific primers: PF (5'-GGA TTT GTG GTG GAT ATC TTG-3') and 4292Rw for *Plasmodium* and HML (5'-GCT ACT GGT GCT ACA TTT GT-3') and HMR (5'-CCT AAA GGA TTA GAG CTA CC-3') for *Haemoproteus*. These pairs of primers have been found to be specific for the detection of *Plasmodium* or *Haemoproteus* in several bird species (Martinez *et al.* unpubl.data, 2007). Partial amplification of the cytochrome B gene was accomplished using the newly designed primers LDLd (5'-CAT TCY ACW GGT GCA TCT TT-3') and LDRd (5'-CTG GAT GWG ATA ATG GWG CA-3') for *Leucocytozoon*. The criteria used for selection of these new designed primer pairs were: (i) a short product (<600 nucleotides), which would be more likely to be recovered in samples with mainly

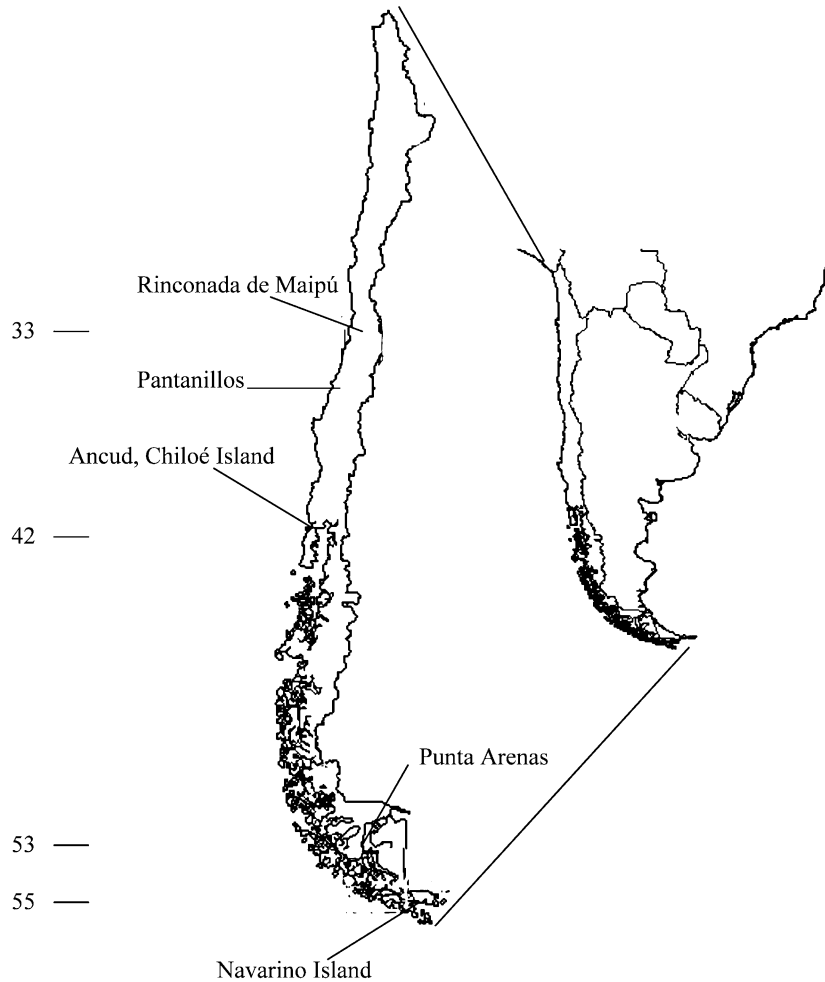


Fig. 1. Map of Chile showing the localities of sampling and latitude.

host DNA (Saiki 1990); and (ii) primer sites conserved across the parasites but different enough from bird DNA to prevent its amplification. PCR reactions consisted of 25- μ L reaction volumes containing 20 ng template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 MgCl₂, 0.2 mM of each dNTP, 1 μ M of each primer, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The reactions were cycled at the following parameters using a thermal cycler (MasterCycler Personal, Eppendorf): 94°C for 10 min (polymerase activation), 40 cycles at 95°C for 40 s, 58°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. All the DNA fragments obtained after PCR assays were recovered from agarose gels and subjected to direct sequencing. DNA fragments obtained were sequenced using an ABI 3130 (Applied Biosystems) automated sequencer. For potential cases of under-amplification (i.e. weak signal obtained) we repeated the PCR procedure under the same conditions. All of these samples were considered as

negative because the second amplification failed to render a positive band. Thus, all samples rendering a clear PCR product were sequenced and identified to genus. A sample previously identified as infected by both methods of blood smear and molecular sequencing, was included at each PCR as a positive control.

DNA sequences were aligned using the CLUSTALW program (Thompson *et al.* 1994). The BIOEDIT program (Hall 1999) was used to edit the sequences. The MEGA3.1 (Kumar *et al.* 2004) software package was used in phylogram construction/drawing. Computer programs were set at their default parameters in all analyses. Phylogenetic analyses were carried out using the parsimony method and the Kimura model. Tree was estimated by bootstrap analysis with 100 replications. As both methods render similar results, we present only the results for the Kimura model. Phylogenetic analysis was carried out using sequences with a length of 405 bp after removing columns containing gaps or missing data. The

length of sequences from lineages ChP7 and ChP8 were 315 bp and 259 bp, respectively. Thus, both lineages were excluded in phylogenetic analysis. Several GenBank sequences from parasites recovered from birds of the same families were included in the phylogenetic trees to compare affinities between our samples and those from other localities.

Statistics

We looked for relationships between prevalence and parasite richness as dependent variables, and latitude and the two measures of sampling effort (number of samples at each locality and number of host species sampled at each locality; see Gregory 1990) as independent variables by means of GLM (general linear model). Variables were eliminated step-by-step from the model when $P > 0.05$ and models retained all significant variables. Prevalences were arcsine square root transformed. As some of the variability in prevalence between localities may be due to the host species composition at each locality, we conducted similar analyses but using only the three more frequently sampled bird species (*Aphrastura spinicauda*, *Elaenia albiceps*, *Troglodytes musculus*) from each of four localities (all except locality 1: Rinconada where these species were scant; see Appendix 1 at <http://www.ecolsoc.org.au/What%20we%20do/Publications/Austral%20Ecology/AE.html>). In these analyses, variables to control for sampling effort only included number of samples as the same number of hosts species (three) were used for each locality. Latitude was introduced as its absolute value that ranged from 33.32°S to 54.56°S (Table 1). We also made comparisons of prevalence of infections between temperate areas (localities 1, 2 and 3) versus extreme south areas (localities 4 and 5) by means of χ^2 tests.

RESULTS

Using DNA analyses 95 samples from 617 adult birds and one sample from 143 nestlings were found to be infected (Table 1). We detected 40 birds (6.5%) belonging to several species, infected by *Haemoproteus* (excluding nestlings), 31 birds (5.0%) infected by *Plasmodium* and 55 birds (8.9%) infected by *Leucocytozoon*. Mixed infections (several lineages of blood parasites; Table 1) were carried by 25 birds. Eight lineages of *Plasmodium*, eight of *Haemoproteus* and 11 of *Leucocytozoon* were identified as different lineages when their sequences differed in at least one bp (see Appendix 2 at <http://www.ecolsoc.org.au/What%20we%20do/Publications/Austral%20Ecology/AE.html>). Minimum and maximum lineage divergence was 0.3–7.5% (1–31 bp) for *Haemoproteus*, 1.5–

7.7% (6–32 bp) for *Plasmodium* and 0.3–13.9% (1–58 bp) for *Leucocytozoon*. Five lineages of *Plasmodium*, five of *Haemoproteus* and eight of *Leucocytozoon* appeared as host-specific, as they were found in only one bird species (see Appendix 2 at <http://www.ecolsoc.org.au/What%20we%20do/Publications/Austral%20Ecology/AE.html>). In addition, 19 lineages were detected in only one of the localities (five of *Plasmodium*, six of *Haemoproteus*, and eight of *Leucocytozoon*) (see Appendix 2 at <http://www.ecolsoc.org.au/What%20we%20do/Publications/Austral%20Ecology/AE.html>).

For the subsample of blood smears, we detected 17 infections from 198 birds (8.6%), four corresponding to *Haemoproteus* (2.0%), five to *Plasmodium* (2.5%) and eight to *Leucocytozoon* (4.0%). All infections detected in blood smears were also detected by amplification of parasite DNA except one infection by *Leucocytozoon* in a patagonian sierra finch (*Phrygilus patagonicus*) from Punta Arenas. However, by molecular methods we detected 20 other infections by the different genera of parasites that were not detected in blood smears.

Overall prevalence of infection (by the three genera of parasites together) was negatively related to latitude but not significantly, once the number of hosts sampled and the number of samples examined were eliminated from the model (Table 2). When we analysed the prevalence by each parasite genus separately, we found a significant negative relationship between *Haemoproteus* prevalence and latitude (Fig. 2a; Table 2) once we controlled for the number of hosts sampled. The number of samples was not retained in the model for *Haemoproteus* prevalence. A similar result was obtained for the relationship between latitude and *Plasmodium* prevalence (Fig. 2b; Table 2), but the number of samples and the number of hosts sampled were not retained in the model. In addition, we found a significant positive relationship between *Leucocytozoon* prevalence and latitude (Fig. 2c; Table 2) once we controlled for the positive effect of number of hosts sampled and number of samples examined. A significant negative relationship between mixed infections and latitude was also detected (Fig. 2d; Table 2) once the number of hosts sampled and number of samples examined were eliminated from the model. Parasite richness was not significantly related to latitude and was the second variable eliminated from the model, which did not retain significant variables (Table 2). Parasite richness for each parasite genus was not significantly related to any of the variables in the model (Table 2).

A comparison of prevalences between temperate (localities 1, 2 and 3) versus extreme south areas (localities 4 and 5) also rendered latitudinal differences, with *Haemoproteus*, *Plasmodium* and mixed infections being more common in temperate than

Table 2. Results of GLMs (general linear models) relating prevalence and diversity for each parasite genera and latitude, number of samples and number of host sampled. Variables were eliminated step-by-step from the model when $P > 0.05$

	Latitude		Number of samples		Number of hosts	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Prevalence						
H	144.78	0.01	0.07	0.83	19.82	0.05
P	22.96	0.02	0.36	0.61	0.67	0.56
L	386.29	0.03	854.45	0.02	242.18	0.04
Mixed	57.06	0.005	1.74	0.32	0.002	0.97
Total	6.57	0.08	83.11	0.07	57.28	0.08
Diversity						
H	0.23	0.66	0.11	0.78	0.0006	0.98
P	0.68	0.47	0.005	0.96	0.34	0.62
L	3.25	0.21	0.81	0.53	18.60	0.02
Total	2.93	0.23	0.02	0.92	5.50	0.10

Statistical values before to be eliminated from each model are presented for non-significant variables. H, *Haemoproteus*; L, *Leucocytozoon*; Mixed, Mixed infections; P, *Plasmodium*; Total, sum of infections by each parasite genera.

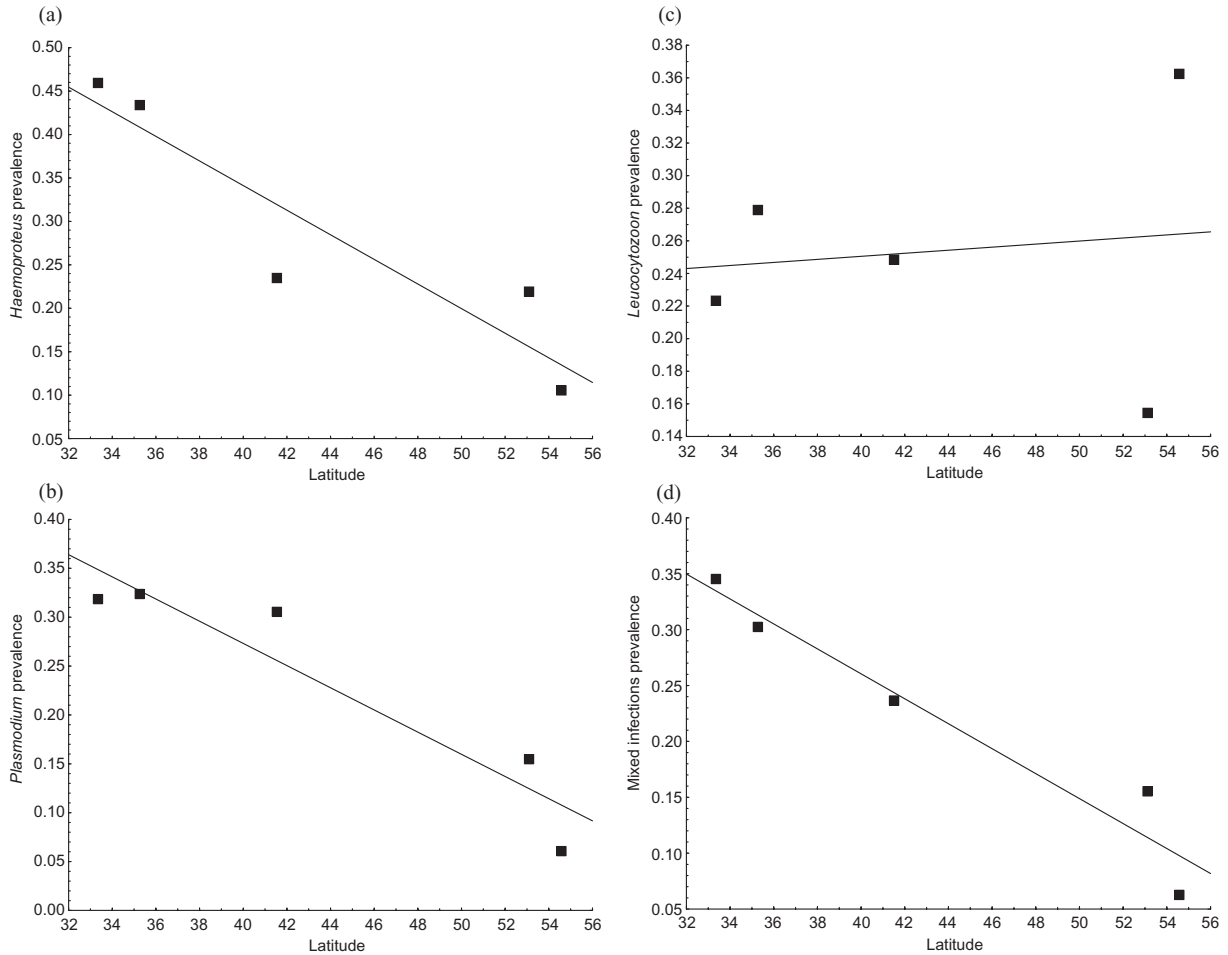


Fig. 2. Relationship between prevalence (arcsine square root transformed) for each parasite genus and latitude for the localities included in the study. (a) *Haemoproteus*, (b) *Plasmodium*, (c) *Leucocytozoon*, (d) Mixed infections. Lines indicate the pattern of the relationship. Raw data are shown.

extreme south areas (Yates $\chi^2_1 = 23.19$, $P < 0.0001$, Yates $\chi^2_1 = 23.58$, $P < 0.0001$ and Yates $\chi^2_1 = 17.15$, $P = 0.0001$, respectively). However, *Leucocytozoon* infections were more common in extreme south areas than in temperate ones (Yates $\chi^2_1 = 4.20$, $P = 0.04$). Parasite richness was higher in temperate (28 lineages) than in extreme south localities (11 lineages) but not significantly so (Mann–Whitney U -test, $Z = 1.73$, $P = 0.08$).

For the subsample of three host species being sampled at each of four localities we obtained a significant and negative relationship between prevalence of *Plasmodium* and latitude ($F_{1,3} = 27.75$, $P = 0.034$). In addition, a similar pattern was found for relationships between latitude and prevalence of mixed infections but did not reach significance ($F_{1,3} = 16.77$, $P = 0.08$). Other relationships were not significant (data not shown). The comparison between temperate and extreme south areas for the pool of these three host species also rendered some differences in prevalences: higher prevalence of *Haemoproteus* and *Plasmodium* were found in temperate areas (Yates $\chi^2_1 = 8.66$, $P = 0.003$ and Yates $\chi^2_1 = 10.67$, $P = 0.001$, respectively). In addition, higher prevalence of *Leucocytozoon* was found in extreme south localities (Yates $\chi^2_1 = 7.57$, $P = 0.006$).

Two migratory species were present in our sample: the Chilean swallow *Tachycineta meyeni* with migratory populations in southern Chile and resident populations in central Chile, and the white-crested elaenia (*Elaenia albiceps*) which is a long distance migrant with wintering grounds in northern South America. Swallows were not captured in localities 1 and 2 where the species is resident, and 26 individuals were found uninfected by the three parasite genera in the other three localities. In contrast white-crested elaenias were captured in all localities except in the northernmost one, Rinconada de Maipú. Eight different lineages were detected in this species (three *Haemoproteus*, four *Plasmodium* and one *Leucocytozoon*) and only two were species-specific (ChH2 and ChP7). Of the other six lineages infecting this species, ChH1 was the most frequently found and it infected elaenias in localities 2, 3 and 5. This parasite lineage also infected one adult and one nestling thorn-tailed rayadito at localities 2 and 3, respectively; two rufous-crowned sparrows and one green-backed firecrown at locality 2, and one southern house wren at locality 3 were also infected (see Appendix 2 at <http://www.ecolsoc.org.au/What%20we%20do/Publications/Austral%20Ecology/AE.html>).

Phylogenetic trees showed certain specificity at the family level for *Haemoproteus* infections within the avian family Emberizidae, as most of the lineages from birds of this family were grouped with a bootstrap value of 88 (see Fig. 3). No clear host family pattern appears for *Plasmodium* and *Leucocytozoon* lineages.

DISCUSSION

The latitudinal range covered in this study included almost the entire distribution of the native forests of Chile, bounded to the north by the Atacama Desert and to the south by Pacific and Atlantic oceans (i.e. The Drake pass). In fact, Navarino Island, one of the largest islands in the Cape Horn Biosphere Reserve, includes the southernmost forests of the world (Rozzi *et al.* 2006).

With respect to our first objective, the existence of latitudinal gradients in prevalence and richness of three different genera of blood parasites, prevalences of *Haemoproteus*, *Plasmodium* and mixed infections increased at lower latitudes (Fig. 2a,b,d). Similar results were obtained for prevalence of *Plasmodium* when only three host species captured at each of four localities of sampling were analysed. In addition, there were clear differences between temperate (localities 1–3) and extreme south areas (localities 4 and 5), even when the analyses were restricted to the three common host species at four localities. Higher prevalence of *Plasmodium* and *Haemoproteus* parasites occurred in temperate areas as compared to extreme south areas. These results were in agreement with previous studies for other diseases in humans and wild animals. For example, Nunn *et al.* (2005) concluded either that the greater abundance and diversity of biting arthropods in the tropics or that the climatic effects on vector behaviour and parasite development were responsible for the relationship between latitude and parasite richness increasing towards the equator. Guernier *et al.* (2004) showed a similar latitudinal pattern for several human diseases across the world. The greater abundance and/or diversity of vectors toward the equator may also explain our results for the relationship of prevalences of *Plasmodium*, *Haemoproteus* and mixed infections and latitude. Contrary to those previous studies, we failed to detect a significant negative relationship between latitude and parasite richness. This may partly be due to the isolation of Chile from the rest of the continent due to geographical and ecological barriers (the Andes Mountains, Atacama Desert, Pacific Ocean), thus preventing the arrival of a higher diversity of parasites. Also diversity gradients may be more intense at lower latitudes. Although the use of smaller screening fragments could allow detection of more parasite lineages, we expect that the probability of missed lineages was similar at the different areas of study and therefore should not affect our results.

The relationship found here between the prevalence of *Haemoproteus* and latitude agrees with data from other localities in South America. Durrant *et al.* (2006) found a higher prevalence of *Haemoproteus* and *Plasmodium* in 53 bird species from Guyana when compared to 111 bird species from Uruguay. Overall prevalence and parasite richness in the study by

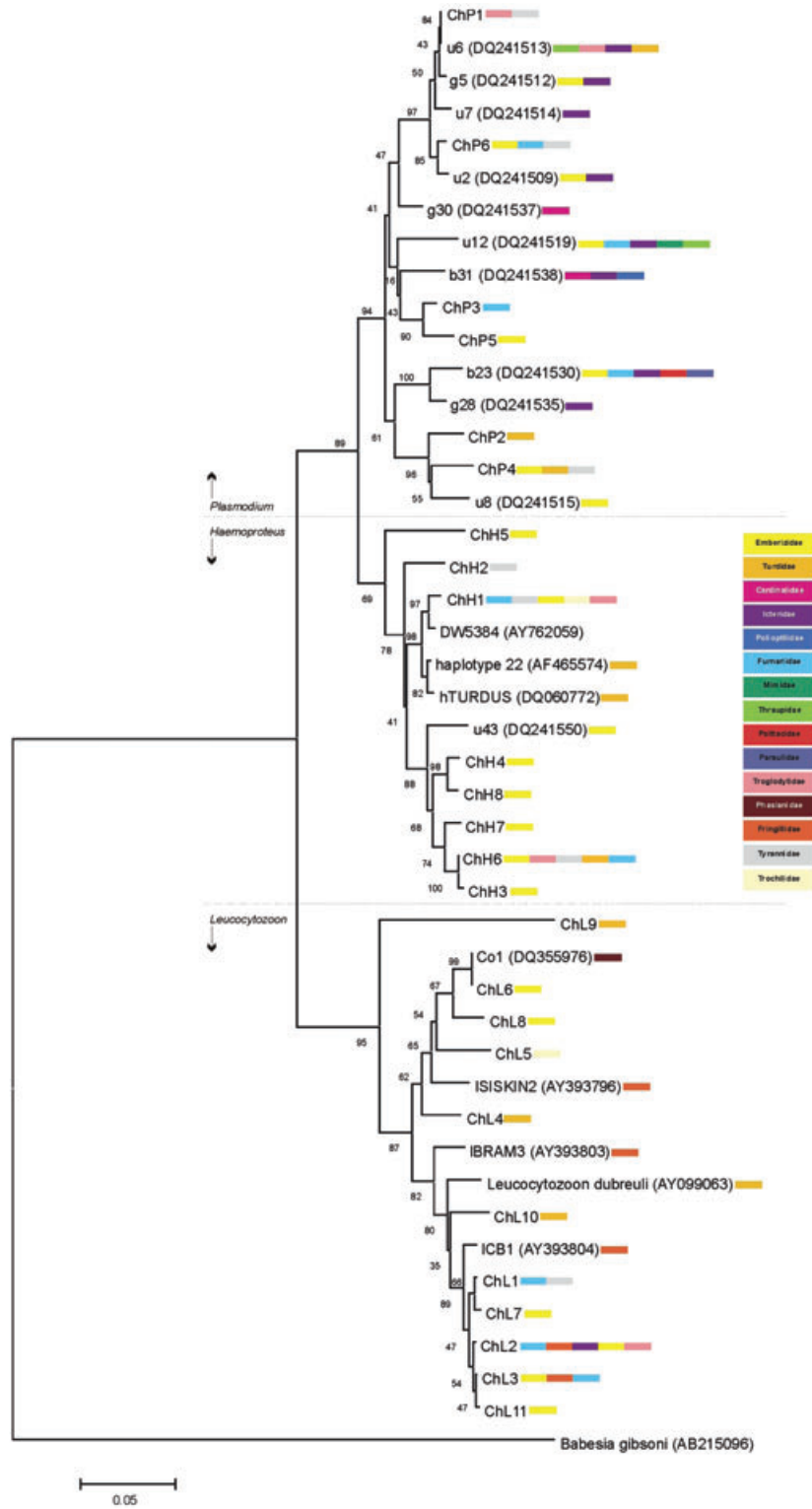


Fig. 3. Phylogenetic tree obtained with the MEGA3 software package using the model of Kimura. Bootstrap values (with 100 replications) are shown at the corresponding nodes. Numbers in brackets after the species name indicate the GenBank accession number of the lineage number for samples from this study.

Durrant *et al.* (2006) was higher than in our study and, contrary to us, they reported a higher prevalence of infection by *Plasmodium* than by *Haemoproteus*. In Uruguay, which is situated at the latitude of our northernmost study site (sampled localities in Uruguay varied from 30.19 to 34.39°S) the prevalence of *Haemoproteus* was clearly lower (3.4%) than in our study area in Rinconada del Maipú (19.7%; Table 1) but the prevalence of *Plasmodium* was higher (14.9% in Uruguay versus 9.8% in Rinconada). These facts may imply a different vector abundance or activity in Chile and Uruguay for blood-sucking mosquitoes (Diptera: Culicidae) that are vectors of *Plasmodium*, and biting midges (Diptera: Ceratopogonidae) and hippoboscids (Diptera: Hippoboscidae) that are vectors of *Haemoproteus* (Valkiūnas 2005). These differences are probably due to differences in habitat and climate conditions between the Atlantic and the Pacific sides of the continent even at the same latitude.

We failed to find similar studies based on DNA analyses for *Leucocytozoon* species in South America, so we can only compare our results with studies based on the analyses of blood smears. White *et al.* (1978) reviewed the literature based on studies of blood smears of the haematozoa from Neotropical birds and reported an overall prevalence of 10.5% for 35 555 birds. They found that *Haemoproteus* was the most prevalent parasite genus (7.4%), followed by *Plasmodium* (1.9%), with *Leucocytozoon* appearing in only 0.2% of the birds. These numbers are not very different from those reported by Valkiūnas (2005; see their table 2, p. 117). White *et al.* (1978) also reported that the Neotropics showed much lower parasite prevalence and a near absence of *Leucocytozoon* in comparison with a similar review of Nearctic avian haematozoan distribution (Greiner *et al.* 1975). In another study conducted in the semiarid northernmost area of Chile, Forrester *et al.* (1977) reported data that also fit well with the patterns of higher *Haemoproteus* prevalence and lower *Leucocytozoon* prevalence at lower latitudes. Our results for the subsample of blood smears showed lower prevalences for *Haemoproteus* and higher prevalences for *Plasmodium* and especially for *Leucocytozoon* than those reported by these authors.

In relation to our second objective, the presence of asymmetries between hemispheres in latitudinal gradients, we found that our data also differed from those reported from North America by Greiner *et al.* (1975) who found 19.5% prevalence for *Haemoproteus*, 17.7% for *Leucocytozoon* and 3.8% for *Plasmodium*. These authors also failed to find a clear pattern of distribution of prevalence among different regions of North America other than a higher prevalence for the north-western Montane region and a lower one for the Arctic barrens region. They found a more or less homogeneous distribution of *Haemoproteus* across most

regions (except the Arctic region) and concluded that vector potential, and thus transmission of this parasite, is rather uniform throughout the continent. Greiner *et al.* (1975) also concluded that transmission of *Leucocytozoon* was restricted by the presence of suitable breeding streams for the vectors (mainly simuliids), and therefore depended on the topography of the region. That is, *Leucocytozoon* prevalence is expected to be higher in montane regions. The picture for *Plasmodium* was not so clear and the relatively short duration of a patent infection was stated as the most important factor for the lower number of infections diagnosed by blood smears (Greiner *et al.* 1975).

The high prevalence of *Leucocytozoon* in our study as compared to that of White *et al.* (1978) may also be related to topography as suggested by Greiner *et al.* (1975) as the highest prevalence for this parasite genus appeared in Navarino, where mountain streams are common in comparison with other study areas. The second locality with high prevalence for this parasite was Pantanillos, which is also a relatively mountainous area. However, data from Forrester *et al.* (2001) do not agree with that explanation as they reported a prevalence of 87% for *Leucocytozoon toddi* in blood smears of 15 chimango caracaras (*Milvago chimango*) a bird of prey sampled on Chiloé Island, one of our areas of study not specially characterized by elevated topography. The significant relationship between *Leucocytozoon* prevalence and latitude found in our study may be influenced by the higher prevalence of this parasite genus in Navarino Island. In this respect, it is clear that latitudinal gradients in protozoan infections may be distorted due to distributions of their vectors. However, the abundance of vectors is also high close to the equator and this may render an absence of a clear latitudinal gradient for some parasite genera (e.g. *Leucocytozoon*) when a higher range of latitudes than those reported in the present study are included. This appears to be the case for studies carried out in Europe, where low prevalences of blood parasites in central Europe in comparison with prevalences in northern and southern populations of birds support the hypothesis that destruction of natural habitats has led to a decline of vector populations in central Europe (Bennett *et al.* 1982a; Merilä *et al.* 1995; but see Scheuerlein & Ricklefs 2004). In any case, it appears that latitudinal gradients for haemoparasites of birds differ among genera of parasites and also differ between North and South America, with less clear gradients in the Northern Hemisphere. In fact, Ricklefs *et al.* (2005) reported a lower incidence of haematozoa in tropical areas than in temperate areas of North America based on data obtained from blood smears, thus also pointing to a difference in latitudinal gradients in haematozoa between hemispheres.

In reference to our third objective on host family specificity, *Haemoproteus* and *Leucocytozoon* parasites

have been considered as specific at the family level in their avian hosts (Valkiūnas 2005). Several classic experiments using cross-infection between birds of the same and different families have supported this theory (Fallis & Bennett 1960; Atkinson 1986; Valkiūnas 2005). This fact may reduce possibilities of parasite lineage spreading if they represent parasite species adapted to infect one host family.

Our data show a relatively tight grouping for *Haemoproteus* lineages infecting birds in the family Emberizidae with the exception of those birds infected by the lineages ChH1 and ChH5 (Fig. 3). However, the bird species infected by lineage ChH5 have been proposed to be transferred from Emberizidae to the family Thraupidae based on genetic data (Bledsoe 1988; Burns *et al.* 2002, 2003) and this may explain why ChH5 did not appear grouped with parasites infecting birds in the Emberizidae. On the other hand, ChH1 appeared to be a generalist parasite lineage infecting several bird species, and it is possible that it did not originate in the Emberizidae. This possibility requires further research. The high number of lineages specific at the level of host species and even at the level of locality may imply that parasites show high local and species adaptation. Specificity appears to be linked to birds within a region or within a well-interconnected region, for example via migratory flows, although genetic similarity among potential hosts could allow successful transmissions (see Engelstädter & Hurst 2006).

Finally, on the potential effect of migrant hosts as disruptors of parasitic gradients (fourth objective), we must emphasize that bird migration has been considered as a potential way of spreading these protozoan diseases (Borg 1992), and data based on molecular detection of blood parasites support this assumption (Waldenström *et al.* 2002). Although we failed to confirm infection by any of the three parasite genera here studied in one of the two migrant species sampled, several parasite lineages were found infecting the white-crested elaenias populations across Southern Chile, and most of these lineages were not specific for this species. Elaenias are long-distance migrants and therefore coexist with many more species than resident birds, or even than short-distance migrants, which increases the chances of becoming infected with a higher diversity of lineages (Waldenström *et al.* 2002). Additionally, this bird species may act as a vehicle to transport some parasite lineages between regions within South America. Although molecular studies of these parasites have shown that at least some parasite lineages are capable of infecting birds from different families (Bensch *et al.* 2000), not all of the bird-parasite interactions detected will be stable through time. The fact that one parasite was found in one host may not reflect long-term coevolution between them, but it might be more indicative of the feeding habits of its invertebrate

vector, as mosquitoes and biting midges are generalist feeders (Beadell *et al.* 2004). In this respect, the lineage ChH1 that was found in several individuals of white-crested elaenias across Chile infected one nestling and one juvenile thorn-tailed rayadito and one juvenile green-backed firecrown, but it was absent in adult populations of these species, thus indicating a failure to establish themselves in hosts from the Furnariidae and Trochilidae families. Potential specificity in a family or at given localities and the spread of parasite lineages carried by migratory species may ultimately depend on the availability of appropriate vectors. This appeared to be the most important factor shaping the latitudinal gradients in prevalence observed for these parasites.

In conclusion, distribution of blood parasites across a latitudinal gradient in Chile varied among parasite genera, which was probably a function of the availability and abundance of appropriate vectors. These gradients seem to be absent in North America where a more homogeneous distribution of parasites appears to be the rule. In addition, although we found high specificity among the lineages of parasites in the three genera, some of these are capable of infecting avian species of very different genera, while some of the other lineages appeared restricted to geographical areas, thus pointing to a coadaptation between lineages and the bird fauna present in each locality. Migratory species may disrupt these patterns of coadaptation, spreading these lineages across southern South America, but successful and stable infections probably depend on the existence of appropriate parasite-vector-host associations.

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