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Molecular characterisation of haemoparasites in forest birds from Robinson Crusoe Island: Is the Austral Thrush a potential threat to endemic birds?

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Summary

The Juan Fernández Firecrown *Sephanoides fernandensis* and Juan Fernández Tit-Tyrant *Anairetes fernandezianus* are two endemic forest birds inhabiting Robinson Crusoe Island and are classified as 'Critically Endangered' and 'Near Threatened' respectively by IUCN. Previous research concluded that the two main factors involved in the decline of these birds were habitat degradation and the introduction of predator / competitor species. However, the potential role of parasitic diseases has not yet been explored. In order to explore hypothetical host-switching phenomena, we genetically identified the haemoparasites present in four bird species, the two endemic species mentioned above and two recent colonisers, Green-Backed Firecrown *Sephanoides sephaniodes* and Austral Thrush *Turdus falcklandii*. We failed to find infections by different blood parasites (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Trypanosoma*, *Babesia* and *Isospora*) in the endangered Juan Fernández Firecrown. However, the Juan Fernández Tit-Tyrant was infected with some parasites shared with the Austral Thrush. The latter species may function as a key-host species on the island as it showed both the higher hemoparaasitic diversity and prevalence. The role of Green-Backed Firecrowns is apparently of lower importance because only one individual was found parasitized. The Austral Thrush could be responsible of the introduction of some parasites also isolated from the Juan Fernández Tit-Tyrant and represent a potential threat to the endemic firecrown due to its role as a reservoir. The spread of Austral Thrushes could increase the contact between species, increasing the probability of a switching event.

Introduction

Insular ecosystems are very susceptible to environmental changes as indicated by the high extinction rates present in oceanic islands (Ricklefs and Schluter 1993, Terborgh *et al.* 2001). In fact, 93% of extinct bird species and 81% of extinct mammal species over the last five centuries were inhabitants of islands (see King 1985 and Ceballos and Brown 1995 respectively). Moreover, 70% of the endemic flora of a number of islands is listed as threatened, rare, or extinct (Davis *et al.* 1986). Islands show low species richness, limited available habitat, small population sizes and high susceptibility to species introduction. These factors do not facilitate the buffering of disturbances to these ecosystems (MacArthur and Wilson 1967, Abbott 1978, Elton 2000). Nowadays disturbances caused by direct or indirect anthropogenic factors are usually more common and deleterious than those with a natural origin (Diamond 1989).

The Pacific Juan Fernández Archipelago (situated about 670 km off the coast of Chile) was declared a Biosphere Reserve in 1977 and represents a clear example of insular ecosystem degraded by human activity, being considered a mini-hotspot along with Galapagos Islands (Mittermeier *et al.* 1999). The Juan Fernández Archipelago presents the highest rate of both endemic plant species per area (Stuessy 1992, Stuessy *et al.* 1992) and plant species richness per area (Arroyo 1999) on oceanic islands. However, much of the native forest has been destroyed and attempts at regeneration have been hampered by prior introductions of animals (e.g. goat *Capra aegagrus hircus* and rabbit *Oryctolagus cuniculus*) and plants (e.g. bramble *Rubus ulmifolius* and maqui *Aristotelia chilensis*) (Wester 1991, Cuevas and Van Leersum 2001).

In contrast, the Juan Fernández Islands have a very limited native fauna, with no land mammals, reptiles, or amphibians. There are seventeen breeding land and sea bird species including five endemic species and three endemic subspecies, two of them inhabiting Robinson Crusoe Island's forests (Juan Fernández Firecrown *Sephanoides fernandensis* and the Juan Fernández Tit-Tyrant *Anairetes fernandezianus*). Several non-endemic bird species have long-established populations in the archipelago (King 1839, Reed 1874): Green-Backed Firecrown *Sephanoides sephaniodes*, Austral Thrush *Turdus falcklandii*, and three birds of prey (*Falco sparverius fernandensis*, *Buteo polyosoma exsul* and *Asio flammeus*). Another two landbird species were directly introduced by humans (House Sparrow *Passer domesticus* and Rock Dove *Columba livia*) (Hahn *et al.* 2009, 2011a).

The Juan Fernández Firecrown has a small range and fragmented habitat. It is catalogued as 'Critically Endangered' (IUCN 2012) although historical records (684 individuals in 1988–1989) indicate smaller populations than new ones (1,100 individuals, Hahn *et al.* 2006, Hahn *et al.* 2009; even 2,500–3,000 individuals were estimated by Hodum *in litt.* 2007; see BirdLife International 2013 and Oikonos 2012). The Juan Fernandez Tit-Tyrant also has a small range and is classified as 'Near Threatened'. Its population is considered stable or slightly declining since 1994 (Hahn *et al.* 2006). Brooke (1987) estimated around 5,000 individuals but more recent estimations show that the population size is about 2,500–3,000 individuals (Hahn *et al.* 2006).

Several ecological studies have predominantly focused on the Juan Fernandez Firecrown. The currently accepted drivers of the Juan Fernandez Firecrown's decline are (i) the decline of certain endemic plants used by the Juan Fernández Firecrown as food source, (ii) the introduction of predators such as rats *Rattus* spp. and cats *Felis catus* and (iii) the unequal sex ratio (Cuevas and Van Leersum 2001, Bourne *et al.* 1992, Jaksic 1998, Roy *et al.* 1999, Hahn and Römer 2002, Ricci 2006). In addition, Austral Thrushes could have a potential dual detrimental effect on Juan Fernández Firecrowns. On the one hand they could act as nestling predators and on the other hand contribute to seed dispersal of introduced plants that compete with the endemic species used as a food source by firecrowns (Hahn *et al.* 2011a, Hahn and Römer 2002).

Previous work on the proximate causes behind the decline in endemic forest bird species in the Juan Fernández Archipelago have not accounted for the possible impact of avian diseases. It is well known that introduction of vector-borne diseases by alien species has had deleterious consequences for endemic fauna in insular ecosystems, especially in birds (Lapointe *et al.* 2012, Atkinson and Samuel 2010). Island species evolve in environments virtually free of diseases, making them especially susceptible to pathogens (Murray 2001). The role of introduced avian malaria in the decline and extinction of native Hawaiian birds is a paradigmatic example (van Riper III *et al.* 1986, Jarvi *et al.* 2001). The endemic bird's susceptibility and the occurrence of suitable vectors and reservoirs determine the transmission of avian malaria caused by *Plasmodium relictum* in Hawaii (Lapointe *et al.* 2012). The virulence of this haemosporidian on Hawaiian avifauna is high, showing 50% mortality in hatch-year birds and 25% in adults (Atkinson and Samuel 2010). However, the Hawaiian thrushes appear to have a high tolerance to malaria, developing chronic disease with low parasitemia (Atkinson *et al.* 2001), thus probably playing a role as reservoir of avian malaria for susceptible species. This role was also suggested for other thrush species in New Zealand (Tompkins and Gleeson 2006)

and Sao Miguel Island, Azores (Hellgren *et al.* 2011). In this sense, the identification of reservoir species (key-host species) to assess its potential impact on endemic ones is essential to determine if it is necessary to act by reducing or even removing their populations (Hellgren *et al.* 2011).

Disease caused by a novel parasite could be highly pernicious to the host, with susceptibility increased in the presence of other concurrent factors such as predation pressure (Kilpatrick 2006). As commented above, the Juan Fernandez Firecrown is actually exposed to different stressors (habitat degradation and predators) which may reduce its resistance to parasites. In this sense, an additional threat to the Juan Fernandez Firecrown lies with the Austral Thrush, a recent coloniser of the Juan Fernandez Archipelago, which shares habitat with this endemic bird (Hahn *et al.* 2011b). Previous studies have shown that Austral Trushes are commonly infected by different haemoparasites on the mainland (Merino *et al.* 2008). If this is also the situation on Juan Fernández Island then the occurrence of host-switching events between thrushes and other bird species is feasible. Another recent coloniser that could also operate as a reservoir is the Green-backed Firecrown, though this is perhaps unlikely due to the low prevalence of blood parasites exhibited by individuals of that species captured on the mainland (Merino *et al.* 2008). Although the Juan Fernandez Firecrown population appears subjected to several stressors and therefore might seem more vulnerable to the introduction of a novel parasite, other endemic bird species, like the Juan Fernandez Tit-Tyrant, may also be threatened by the arrival of pathogens.

The introduction of a novel parasite into a new ecosystem can produce unknown consequences for hosts (Martinez and Merino 2011) and study of the parasitic fauna infecting both endemics and recent colonisers is essential to determine the occurrence of novel parasites and their potential impact. In this sense, we have focused the present study on haemoparasites. Many of them (*Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Trypanosoma*) have previously been detected in the mainland populations of Austral Thrush and Green-backed Firecrown (Merino *et al.* 2008). Parasites within genera *Isospora* and *Babesia* are infrequently recorded in birds (Bennett *et al.* 1982) but the lack of knowledge on the parasites present in the Juan Fernandez Archipelago prompt us to include them in the screening.

The objectives of the present study are: (i) determine the genera and diversity of haemoparasites infecting both endemic birds (Juan Fernández Firecrown and Juan Fernández Tit-Tyrant) and recent colonisers (Green-backed Firecrown and Austral Thrush) and (ii) assess the role of the latter (Green-backed Firecrown and Austral Thrush) as potential reservoirs of parasites.

Materials and methods

Capture and sampling of birds was conducted on the Robinson Crusoe Island for five days during January 2010 (14–18 January). The islands have a temperate oceanic climate. Data from the settlement's meteorological station indicate that average annual precipitation is 1,081 mm varying from 318 mm to 1,698 mm across the year. During late fall and the winter months (May to August) rainfall is higher than 100 mm (24 mm for January). We captured birds near to the CONAF (Corporacion Nacional Forestal) center located in San Juan Bautista village (S33°36.5', W78°50.5'). During the following four days the capture was performed in Plazoleta El Yunque (S33°38.975', W078°50.568'), a recreational area located inside the island's protected area as a National Park.

The birds were captured using mist nets. Once the nets were installed we checked them every five minutes to avoid excessive stress to the birds. All captures were performed between 08h00 and 13h00. The captured birds were weighed and measured (tarsus, wing, tail, and beak length). A slight puncture was performed on the tarsus or wing vein with a needle (0.5 mm). Approximately 20 µL of blood was taken to perform smears (see Merino *et al.* 1997, Merino *et al.* 2008) and molecular analysis (stored on FTA cards, Whatman). Before releasing the birds, their claws were painted with white varnish to avoid resampling recaptured birds.

DNA analyses

Genomic DNA from samples stored in FTA cards was extracted according to Martínez *et al.* (2009). Partial amplification of the cytochrome *b* gene was accomplished by PCR using the non-specific primers PALU-F and PALU-R for detecting *Haemoproteus*/*Plasmodium* species (Martinez *et al.* 2009) and primers Leunew1F (designed for this study, see Table 1) and LDRd for *Leucocytozoon* species (Merino *et al.* 2008). In addition, partial amplification of the 18S ribosomal RNA gene was performed using the primers Try-F and Try-R for detecting *Trypanosoma* species (designed for this study, see Table 1) and the generic primers hep900F and hep1615R for various hematic parasites as piroplasms, *Hepatozoon*, hemococcidians, and hematic stages of intestinal coccidians (Merino *et al.* 2008; the primer hep800F mentioned by these authors is identical to primer hep900F). The sequences of the primers, size of the amplicons, and PCR conditions are shown in Table 1. All amplicons obtained after PCR assays were recovered from agarose gels and subjected to direct sequencing using an ABI 3730 XL automated sequencer (Applied Biosystems). To prevent contamination, we used different sets of pipettes and filter tips for extraction, PCR set up and downstream fragment analyses. DNA extraction and PCR set up were always performed in different laminar flow cabinets. We never amplified DNA from negative controls added in each PCR batch. A positive control for each pair of primers was routinely used.

As the reference sequences deposited in GenBank are larger than the amplicons obtained with the primers used in the screening, we obtained a larger DNA fragment of the 18S ribosomal RNA gene by using the primers hep50F/EimRodR and EimRodF/hep1615R for *Isospora* and NBA1-bab/hep1615R for *Babesia* (Table 2). These longer fragments allow us to perform a more powerful phylogenetic analysis. Identity analysis was performed using BioEdit software (Hall 1999).

Phylogenetic analysis

The sequences used in the phylogenetic analysis were obtained from the MalAvi database (2013) or GenBank. We selected *Plasmodium* and *Leucocytozoon* haplotypes isolated in continental Chile and lineages involved in the decline of some bird populations in other parts of the world (Merino *et al.* 2008; Bensch *et al.* 2000). In addition, we selected *Babesia* haplotypes isolated from birds and mammals, and *Eimeria* and *Isospora* haplotypes isolated from birds.

The alignments were performed using the MUSCLE algorithm implemented on the website of the European Bioinformatics Institute (European Bioinformatics Institute 2012). The Gblocks program (Castresana 2000, Talavera and Castresana 2007) was used to eliminate poorly aligned positions and divergent regions of the 18S RNA alignments or to establish the tails of the cytochrome *b* alignment. Using the cytochrome *b* sequence AY099045 from *Haemoproteus majoris* (1123 bp) as a reference, primers Palu F/R amplify from position 403 to 793 and primers LeunewF1/LDRd from 404 to 743. Primers hep50F/hep1615R amplify from position 81 to 1634 (using as a reference 18S rRNA gene from *Isospora gryphoni*; AF080613; 1797 bp) and primers NBA1bab/hep1615R amplify from position 137 to 1559 (using as a reference 18S rRNA gene from *Babesia* sp. EU1; AY046575; 1727 bp). *Plasmodium*/*Leucocytozoon*, *Babesia* and *Isospora*/*Eimeria* alignments contained 421, 1396, and 1460 sites, respectively. The alignments were analysed using Bayesian inference implemented in the program MrBayes v3.2 (Ronquist and Huelsenbeck 2003). The analysis consisted of two runs of four chains each, with 2,000,000 generations per run and a burn-in of 500,000 generations (30,000 trees for consensus tree) for *Plasmodium-Leucocytozoon* species, 3,000,000 generations per run and a burn-in of 750,000 generations (45,000 trees for consensus tree) for *Babesia* species, and 200,000 generations per run and a burn-in of 50,000 generations (3,000 trees for consensus tree) for *Isospora-Eimeria* species. The substitution model (nst=2 and rates=invgamma) was selected using MEGA5 software (Tamura *et al.* 2011). The final standard deviation of the split frequencies was < 0.01. Convergence was checked using the Tracer v1.5 software (Rambaut and Drummond 2007). All of the model parameters were higher than 100.

Table 1. Pairs of primers used in the screening in the present study (PaluF/PaluR, Leunew1F/LDRd, TryF/TryR, and Hep900F/Hep1615R).

Primers	Sequence 5´→ 3´	Size bp	Annealing	Extension	Parasites (gene)
Palu-F	GGGTCAAATGAGTTTCTGG	390	54°C / 30s	72°C / 40s	*Plasmodium*/*Haemoproteus* (cytochrome B)
Palu-R	DGGAACAATATGTARAGGAGT				
Leunew1F	GGWCAAATGAGTTTCTGGG	340	56°C / 30s	72°C / 30s	*Leucocytozoon* (cytochrome B)
LDRd	CTGGATGWGATAATGGWGCA				
Try-F	GGAGAGGGAGCCTGAGAAATA	120	56°C / 30s	72°C / 30s	*Trypanosoma* (18S ribosomal RNA)
Try-R	ATGCACTAGGCACCGTCG				
Hep900F	GTCAGAGGTGAAATTCTTAGATTTG	732	58°C / 30s	72°C / 60s	*Hepatozoon*/Piroplasmid/Coccidians (18S ribosomal RNA)
Hep1615R	AAAGGGCAGGGACGTAATC				

Table 2. Pairs of primers used to obtain a larger 18S ribosomal RNA gene fragment for *Isospora* and *Babesia* (Hep50F/EimRodR, EimRodF/Hep1615R, and NBA1bab/Hep1615R).

Primers	Sequence 5´→ 3´	Size bp	Annealing	Extension	Parasites
Hep50F	GAAACTGCGAATGGCTCATT	978	58°C / 30s	72°C / 70s	*Isospora*
EimRodR	GCATTTCCCTATCTCTAGTCGG				
EimRodF	CCGACTAGAGATAGGGAAATGC	596	58°C / 30s	72°C / 50s	*Isospora*
Hep1615R	AAAGGGCAGGGACGTAATC				
NBA1-bab	GGATAACCGTGCTAATTGT	1424	58°C / 30s	72°C / 90s	*Babesia*
Hep1615R	AAAGGGCAGGGACGTAATC				

Table 3. Bird species captured in the present study.

Common name	Scientific name	Individuals	% vs total captures
Green-Backed Firecrown	*Sephanoides sephaniodes*	44	57.9
Juan Fernández Firecrown	*Sephanoides fernandensis*	17	22.4
Austral Thrush	*Turdus falcklandii*	8	10.5
Juan Fernández Tit-Tyrant	*Anairetes fernandezianus*	7	9.2

Results

Data on bird species captured in the present study are shown in Table 3. No individual was recaptured. Only six birds were found infected by microscopic examination of blood smears. Two tit-tyrant individuals were infected by trypanosomes and another two by both trypanosomes and *Leucocytozoon*, while one Austral Thrush appeared infected by *Plasmodium* and other by *Leucocytozoon* parasites. In all cases intensity of infection was low (less than 1 parasite per 10,000 erythrocytes). All infections detected by microscopy were also detected by molecular analyses.

The molecular analysis showed that all sampled Austral Thrushes were parasitized by at least one haemoparasite species (see Table 4). *Leucocytozoon* was found in all individuals (100%), *Plasmodium* in seven (87.5%), *Babesia* in five (62.5%), *Isospora* in three (37.5%), and *Trypanosoma* in two (25%). The Juan Fernández Tit-Tyrant was the second most frequently parasitized species. Four of the seven captured individuals were infected by at least one haemoparasite species (see Table 5). *Trypanosoma* was detected in four individuals (57%), *Leucocytozoon* in two (28%), and *Plasmodium* in just one of them (14%). In contrast, only two Green-Backed Firecrown individuals were parasitized (4.5%; in all cases by *Trypanosoma*) and the Juan Fernández Firecrowns were not found infected by haemoparasites. We did not find any bird parasitized by *Haemoproteus*.

Only one haplotype was detected for *Plasmodium* (haplotype ChP2 with 331 bp), *Trypanosoma* (haplotype JFT1 with 100bp) and *Babesia* (haplotype JFB1 with 1350 bp). The *Plasmodium* haplotype was previously isolated from Austral Thrushes captured on the continent (Merino *et al.* 2008) but also in other thrush species such as *T. migratorius* and *Hylocichla mustelina* from the USA (Ricklefs and Fallon 2002, Martinsen *et al.* 2007, 2008) and *T. rufiventris* from Uruguay (Durrant *et al.* 2006). The *Trypanosoma* haplotype isolated in the present study had 100% identity with *T. avium*. However, the *Babesia* haplotype was detected for the first time, being *Babesia kiwiensis* the closest species (98% identity). On the other hand, we detected three and five haplotypes belonging to *Isospora* and *Leucocytozoon* genera, respectively. The *Isospora* haplotypes JFI1 and JFI2 (1522 and 1486 bp, respectively) differed in only one base but the haplotype JFI3 (725bp) showed a 98.8% identity with each JFI1 and JFI2. Three *Leucocytozoon* haplotypes (JFL1, JFL2 and JFL3) were detected for first time. The haplotypes JFL1 and JFL2 (301 bp) differed in just one nucleotide but their genetic identities with the haplotype JFL3 (283 bp) were close to 87%. However, the other two *Leucocytozoon* haplotypes (ChL9 and ChL2) were previously isolated from birds captured on the continent, the first from Austral Thrushes and the second from several bird species which do not inhabit the archipelago (Merino *et al.* 2008). The haplotypes isolated

Table 4. Haplotypes of the parasites found in each Austral Thrush individual.

Individual	*Plasmodium*	*Leucocytozoon*	*Trypanosoma*	*Babesia*	*Isospora*
1	ChP2	ChL9, JFL1	JFT1	JFB1	-
2	ChP2	JFL2	-	JFB1	-
3	ChP2	ChL9, JFL3	JFT1	-	JFI1
4	ChP2	JFL1	-	-	JFI2
5	ChP2	ChL9	-	JFB1	-
6	-	JFL3	-	-	JFI3
7	ChP2	JFL1, JFL2	-	JFB1	-
8	ChP2	ChL9, JFL1	-	JFB1	-

Table 5. Haplotypes of the parasites found in each Juan Fernández Tit-Tyrant individual.

Individual	*Plasmodium*	*Leucocytozoon*	*Trypanosoma*	*Babesia*	*Isospora*
1	-	-	-	-	-
2	ChP2	ChL2	JFT1	-	-
3	-	-	-	-	-
4	-	-	JFT1	-	-
5	-	-	JFT1	-	-
6	-	ChL2	JFT1	-	-
7	-	-	-	-	-

from each individual are shown in Tables 4 and 5. The geographical distribution of the haemosporidian haplotypes detected in the Juan Fernández birds is indicated in Figure 1.

Phylogenetic analysis

The *Plasmodium* haplotype ChP2 isolated from Austral Thrushes was clustered together with the European haplotype Padom16, which corresponds to the morphospecies *Plasmodium rouxi*, and the American haplotype ChP4 (Figure 2).

The novel *Leucocytozoon* haplotypes JF1 and JF2 were grouped together with haplotype ChL4 which was previously isolated from Austral Thrushes captured in continental Chile (Figure 2). In addition, the novel haplotype JFL3 was closely related to the haplotype ChL9 isolated from Austral Thrush and forming a sister group with six American haplotypes included in the tree (Figure 2). The haplotype ChL2 exclusively detected in Juan Fernández Tit-Tyrants was clustered together with other American *Leucocytozoon* haplotypes but it was not closely related to the haplotypes isolated from other birds in the archipelago (Figure 2).

The only haplotype belonging to *Babesia* genus formed a well-supported cluster together with *Babesia kiwiensis* (Figure 3). However, it was not related to other *Babesia* species isolated from seabirds (*B. bennetti* and *B. poelea*). The *Isospora* haplotypes JFI1 and JFI2 were clustered together with *I. robini* which was previously isolated from American Robin *Turdus migratorius*. However, the haplotype JFI3 was located as sister group of the latter clade with a low support (Figure 4). As we could only obtain a short DNA fragment for *Trypanosoma* (100 bp of the 18S ribosomal RNA gene) we did not build a phylogenetic tree. Nevertheless, as mentioned above this fragment showed 100% identity with *T. avium*.

Discussion

Our results indicate that the Austral Thrush is a key-host species for the bird blood parasite community due to its high parasite prevalence and diversity. In fact, this species seems to be the potential source for two host-switching events involving *Plasmodium* and *Trypanosoma* parasites. Apparently, the most threatened endemic, Juan Fernandez Firecrown, is free of haemoparasites. However, we cannot rule out the possibility that infections were lethal for these birds, as we only captured healthy individuals. The endemic Juan Fernandez Tit-Tyrant appears affected by the hypothetical parasite switches. The *Plasmodium* haplotype ChP2 and *Trypanosoma* were detected in both host species, Austral Thrush and Juan Fernandez Tit-Tyrant. The haplotype (ChP2) was previously detected with a high prevalence (75%) in continental thrushes in the Chilean locality of Ancud (Merino *et al.* 2008). In addition, it seems to be host-specific because it was not detected in 14 other bird species sampled in the same locality, including tit-tyrants closely related to the endemic species on Robinson Crusoe Island (Merino *et al.* 2008). Thus, a recent host-switching event would be the most plausible explanation for the presence of this parasite in Juan Fernandez Tit-Tyrants. On the other hand, parasites belonging to the genus *Trypanosoma* were found in Juan Fernández Tit-Tyrants (prevalence 57%), Austral Thrushes (prevalence 25%), and Green-backed Firecrowns (prevalence 4.5%)

Figure 1. Geographical distribution of the haemosporidian haplotypes detected in Juan Fernández forest birds from both Robinson Crusoe Island and continental Chile. The dotted lines indicate the hypothetical origin of the haplotypes. The haplotypes JF were exclusively detected on the island. The haplotypes marked in bold were detected on both the island and continent. The rest of haplotypes were exclusively detected on the continent. The size of Robinson Crusoe Island is arbitrarily enlarged with respect to the American continent.

inhabiting Robinson Crusoe Island. Trypanosomes also infect Austral Thrushes on the mainland but apparently no Green-backed Firecrowns or other species of tit-tyrants (authors' unpubl. data, based on microscopic examination of blood smears). This fact points to an introduction of this parasite to the island by Austral Thrushes. At the moment, the impact of *Plasmodium* and *Trypanosoma* parasites on the stability of the Juan Fernández Tit-Tyrant population remains unknown. However, this bird species is presently suffering from a severe population decline (Hahn *et al.* 2006). As *Trypanosoma* infection is related with poor development and immune responses in nestlings of some passerine species (Merino *et al.* 1996, Martínez-de la Puente *et al.* 2013), it will be necessary to assess the virulence of these parasites by measuring the mortality caused on hatch-year and adult birds (see Atkinson and Samuel 2010).

Four *Leucocytozoon* haplotypes were detected in the Austral Thrushes. One of them (haplotype ChL9) was previously found in continental individuals (Pantanillos and Ancud localities; Fig. 1; see Merino *et al.* 2008) and three of them were detected for the first time in the archipelago

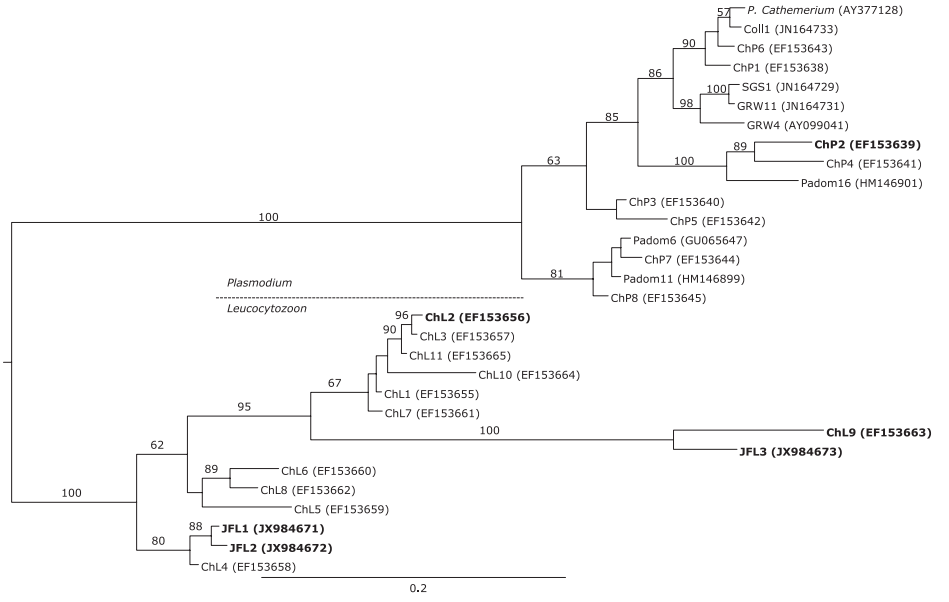

Figure 2. Phylogenetic analysis of the *Plasmodium* and *Leucocytozoon* haplotypes detected on the Juan Fernández Archipelago and continental Chile. Bayesian inference implemented in Mr Bayes program was used to build the tree. There were a total of 421 positions in the final dataset. The haplotypes detected in the present survey are in bold.

(JFL1, JFL2 and JFL3). However, the haplotype ChL2 was only detected in Juan Fernández Tit-Tyrant. This haplotype was previously detected in some bird species from the mainland (Navarino locality; Merino *et al.* 2008) but not in Austral Thrushes or in the close relative Tufted Tit-Tyrant *Anairetes parulus*. Thus, its origin on the island is uncertain.

In a recent study performed on Sao Miguel Island (Azores) on the haemosporidians present in a bird community, the authors described some parasitological aspects similar to those found in the present survey (Hellgren *et al.* 2011). First, the Eurasian Blackbird *Turdus merula* was the main bird species harbouring haemoparasites, exhibiting the highest prevalence and parasite diversity. Second, most of the other sampled bird species were uninfected and only three of the 11 sampled bird species presented only one infected individual. However, these species showed higher prevalence when they were sampled in continental Europe. In our case, Green-Backed Firecrown and Austral Thrush are the only two species sampled that inhabit the archipelago and the continent, and their parasite diversity is higher on the mainland (see Merino *et al.* 2008 and Pérez-Rodríguez *et al.* 2013 for a similar result on other islands). Third, *Haemoproteus* has not been found on the islands, possibly indicating a lack of suitable vectors.

The occurrence of the Austral Thrush as a parasite reservoir appears to be a threat to the two endemic Juan Fernández species, an actual threat to the Juan Fernández Tit-Tyrant and a potential threat in the case of the Juan Fernández Firecrown. Additionally, the well-known effects of habitat degradation and the pressure exerted by alien predators could play a role as immunosuppressive agents for the endemic birds (Kilpatrick 2006). In fact, in a stress context some *Plasmodium* haplotypes could become pathogenic as previously reported in New Zealand for the system *P. elongatum* / Saddleback *Philesturnus carunculatus* (Alley *et al.* 2010). Paradoxically, the same Austral Thrushes could play a role in the system as a stressor agent, promoting the displacement of native plants by dispersing foreign seeds and acting as predators at least to the Juan Fernández Firecrown (Smith-Ramírez *et al.* 2013). Habitat loss and degradation on oceanic islands are not only leading to population decline of endemic birds but also facilitating the spread of invasive bird species

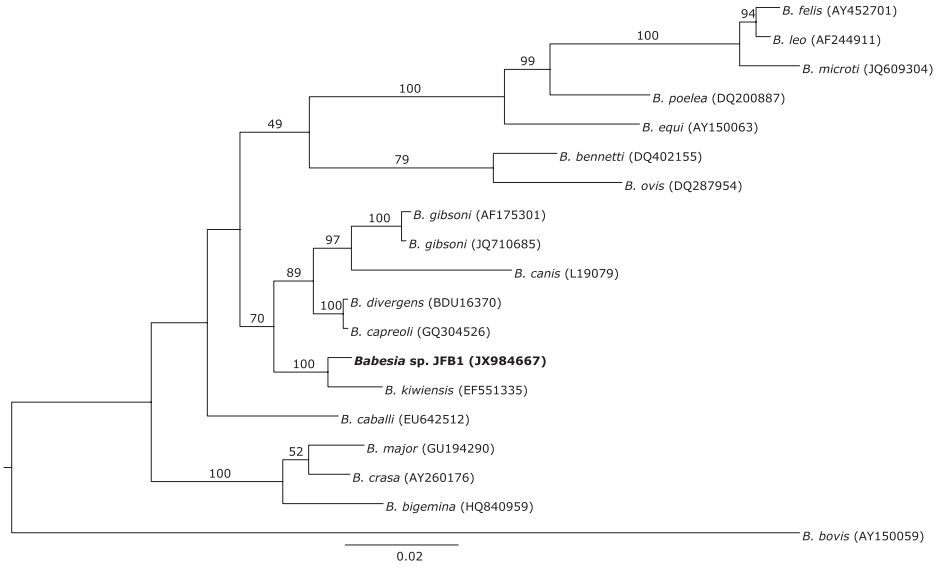

Figure 3. Phylogenetic analysis of the *Babesia* haplotype isolated from Austral Thrush. Bayesian inference implemented in Mr Bayes program was used to build the tree. There were a total of 1,396 positions in the final dataset. Haplotypes detected in the present survey are in bold.

(Catry *et al.* 2000, Byers 2002). Furthermore, an increase in Austral Thrush population density together with the low bird biodiversity present in the island (low dilution effect) would be key factors in enhancing parasite transmission (Martínez and Merino, 2011).

Some examples of introduction of avian blood parasites to islands with severe consequences for endemic avifauna exist. In these cases the bird host is as relevant as the parasitic specificity of the

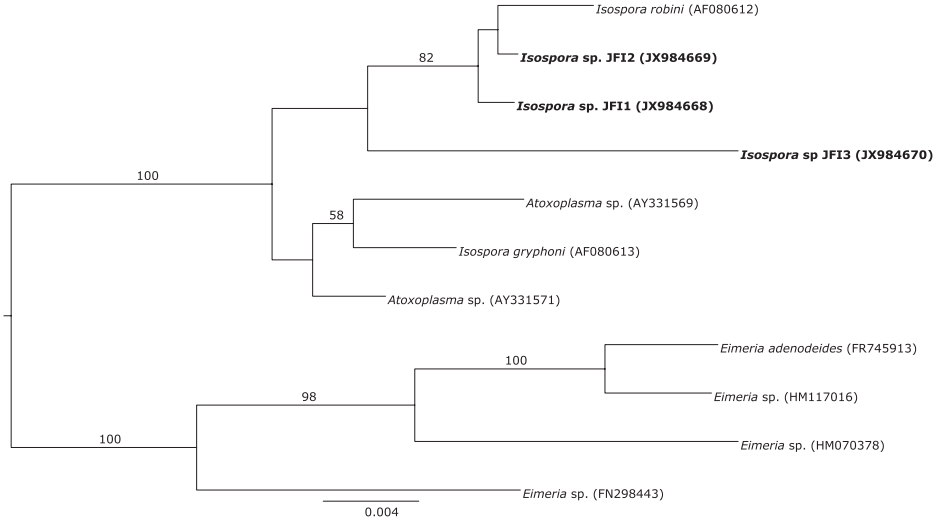

Figure 4. Phylogenetic analysis of the *Isospora* haplotypes isolated from Austral Thrush. Bayesian inference implemented in Mr Bayes program was used to build the tree. There were a total of 1,460 positions in the final dataset. Haplotypes detected in the present survey are in bold.

introduced lineages (Ewen *et al.* 2012) and the vectors present in the colonised region. In this sense, the best example is the introduction of infected sparrows in Hawaii causing a population decline of native birds (van Riper III *et al.* 1986). The low host specificity showed by the *Plasmodium* haplotype introduced by sparrows (GRW4) was an essential factor in facilitating the switch to native species. However, its transmission to native birds was impossible until the main competent vector for alien *Plasmodium* lineages, *Culex quinquefasciatus*, was introduced by humans at the beginning of 19th century (Hardy 1960, Warner 1968). In New Zealand, this vector has spread over the past three decades and recent studies have showed a strong relationship between the avian malaria infections and the geographical distribution of the vector (Tompkins and Gleeson 2006). In this case, one of the main reservoirs of infection to native species is also the non-native Eurasian Blackbird.

In conclusion, our results suggest that the Austral Thrush is a key-host species in the island as it showed both high haemoparasitic diversity and prevalence. The Juan Fernández Tit-Tyrants are infected with parasites possibly introduced by the thrushes. Although the endemic Juan Fernandez Firecrown is free of haemoparasites, the Austral Thrush is a potential threat due to its role as a reservoir. In the near future it would be very interesting (i) to check the virulence of the introduced parasites on the fitness of the Juan Fernández Tit-Tyrants, (ii) to perform a survey of the haemoparasites present in the recently introduced House Sparrow *Passer domesticus*, (iii) to identify potential vectors to assess a hypothetical parasite switch event to the endemic birds, and (iii) to establish a surveillance programme in order to control potential parasite switches and / or increases in parasite prevalence.

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