Short Communication

The prevalence and molecular characterisation of blood parasites infecting the vulnerable Tamarugo Conebill (*Conirostrum tamarugense*) and other birds in the Pampa del Tamarugal, Chile

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Abstract. Blood parasites exert significant selective pressure, which can alter population dynamics, and the introduction of new parasite lineages in a region could lead to the extinction of endemic avian species that have not coevolved with them. The avifauna of the Pampa del Tamarugal in the desert of Atacama, Chile, is highly depauperate, with two species accounting for the greatest numbers: the widespread Rufous-collared Sparrow (*Zonotrichia capensis*) and the endemic Tamarugo Conebill (*Conirostrum tamarugense*). We used molecular and microscopic methods to estimate the prevalence of haemoparasites in birds inhabiting the Pampa del Tamarugal. The molecular screening of the samples from the most common species indicated that the Tamarugo Conebill was only infected by parasites of the genus *Leucocytozoon* (prevalence 3.5%) and the Rufous-collared Sparrow was uninfected. Only two other bird species (of a total of seven) were infected, the House Sparrow (*Passer domesticus*), by *Plasmodium relictum* (prevalence 30%), and the Eared Dove (*Zenaida auriculata*), by *Haemoproteus multipigmentatus* (prevalence 100%). The occurrence of House Sparrows infected with the *Plasmodium* haplotype GRW4 (involved in Hawaiian bird extinctions) may entail the risk of potential host-switching, something particularly relevant for the conservation of the endemic and vulnerable Tamarugo Conebill.

Introduction

The strong negative effects of parasitism have been reported in both managed and wild populations of organisms (Watson 2013). In birds, parasitism can have adverse effects on clutch-size, hatching and fledging success, and adult survival. These detrimental effects have been related to the presence of both ectoparasites and endoparasites (Merino and Potti 1995; Merino *et al.* 2000). Among avian parasites, blood parasites like haemosporidians (e.g. *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) have been widely studied in birds since species belonging to this protozoan group are quite prevalent worldwide (Clark *et al.* 2014) and some of them are highly pathogenic depending on the particular host–parasite system (Valkiūnas 2005).

Haemosporidian parasites have heteroxenous life-cycles and are transmitted by dipteran vectors whose larvae are aquatic.

Thus, environmental constraints related to rainfall determine the incidence of these horizontally transmitted parasitic diseases (Sehgal 2015). Horizontal disease transmission has been related to higher virulence, especially if generalist parasites infect novel hosts (Combes 2005). In fact, parasite host-switching events have been reported with dramatic consequences for species without previous contact with these parasites. The role of avian malaria introduced with non-native bird species in the decline and extinction of native Hawaiian birds is a paradigmatic example (van Riper *et al.* 1986). The *Plasmodium* haplotype GRW4 seems to be solely responsible for these extinctions. The possibility of introduced host species functioning as disease reservoirs has been postulated as a serious threat to native birds in New Zealand (Tompkins and Gleeson 2006), on Sao Miguel Island, the Azores (Hellgren *et al.* 2011) and on Robinson Crusoe Island, Chile (Martínez *et al.* 2015).

Surveys on avian haemosporidians are biased towards European and North American passerines (Clark *et al*. 2014). Some studies have been performed in South America (Durrant *et al*. 2006; González *et al*. 2015), including Chile (Merino *et al*. 2008). However, there are no studies of haemoparasites infecting birds in Pampa del Tamarugal, a national reserve located in the Tarapacá region of Chile. This reserve, characterised by Tamarugo (*Prosopis tamarugo*) forest, is in the Atacama Desert, the most arid desert in the world. Owing to extreme climatic conditions (i.e. high thermic amplitude and very low rainfall), biodiversity is very low, and only 17 species of bird have been recorded (Estades 1995). The Rufous-collared Sparrow (*Zonotrichia capensis*), Tamarugo Conebill (*Conirostrum tamarugense*) and Scaly-throated Earthcreeper (*Upucerthia dumetaria*) are the most abundant species (Estades 1995).

The Tamarugo Conebill is considered endemic to the deserts of northern Chile and southern Peru (López-Calleja and Estades 1996). It is a vulnerable insectivorous bird (BirdLife International 2012) whose conservation seems to be tightly linked to management of Tamarugo forests, which support the Conebill's critical food resource, *Leptotes trigemmatus* caterpillars (López-Calleja and Estades 1996), especially during spring. Populations of Tamarugo Conebills winter at high altitudes in southern Peru (i.e. Arequipa, Tacna and Moquegua) where arid plants of the genera *Gynoxys* and *Polylepis* are common at 3400–4050 m above sea level (asl).

The Tamarugo Conebill can be considered a restricted-range species because its breeding range is small, estimated at only 108 km^2 (Estades 1996). Its small breeding range combined with the low avian diversity and extreme climatic conditions in that range may have allowed it to avoid some parasite lineages in the past. In addition, the high-altitude climatic characteristics of its winter range in southern Peru may also have limited encounters with some parasites transmitted by mosquitoes, such as *Plasmodium*, since optimal conditions for the reproduction of these vectors are lacking (LaPointe *et al*. 2010). Thus, in the present study, we have estimated the occurrence of haemoparasites (i.e. *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Trypanosoma* and microfilariae) in the avian community of the Pampa del Tamarugal arid region to identify potential parasitic threats to the vulnerable Tamarugo Conebill.

Materials and methods

Capture and sampling of birds was conducted within or near the Pampa del Tamarugal National Reserve for 7 days during January 2012 (12–20 January) at three sites: La Huayca (3 days) and Canchones (2 days) within the National Reserve, and Pica, 20 km east of Pampa del Tamarugal (2 days) (see section S1 in supplementary material available online). During January mean maximum and minimum temperatures are slightly above 30°C and 10°C, respectively, and rainfall is very low, if any (Arenas 2013; Hajek and Di Castri 1975).

The Pampa del Tamarugal is a vast plain within the Atacama Desert, located 70–100 km east of the city of Iquique, at ~1000 m asl. Some areas are vegetated with *Prosopis tamarugo*, a flowering tree species belonging to the family Fabaceae. This tree species grows without rainfall and obtains water via a root-system that reaches deep ground-water supplies and possibly also obtains

water from dew. The three sampling sites were selected based on previously observed bird activity (see section S1).

The birds were captured in mist-nets, which were checked at 5-min intervals to avoid undue stress or overheating of birds. All mist-netting was done between 1000 and 1215 hours. Approximately 20 µL of blood was extracted by puncture of the tarsal or wing vein with a 0.5-mm gauge needle for blood smears and molecular analyses. Blood smears were done in the field at the time of blood extraction (see Merino *et al*. 1997) and blood for molecular analyses were stored on Whatman FTA cards (GE Healthcare Life Sciences, Little Chalfont, UK) to preserve biological samples for downstream DNA analysis. Genomic DNA was extracted from samples stored on FTA cards according to Martínez *et al*. (2009). Details of the screening of parasites by microscopy and PCR (Tables S1 and S2) are available in section S2 of the supplementary material.

Results

The numbers and species of birds captured and examined at the three sampling sites, and the number and proportion of infected birds are shown in Table 1. The most commonly captured bird species were the Tamarugo Conebill and Rufous-collared Sparrow, which together comprised >90% of the individuals captured. Overall, the prevalence of haemoparasites was low in the present survey (10 infected of 197 individuals examined = 5% (95% confidence level 2–8.1%)). Infections were found in three Tamarugo Conebills, with *Leucocytozoon* (3 of 89 examined = 3.4% (0–7.1%)); three House Sparrows, with *Plasmodium* (3 of 10 examined = 30% ((1.6–58.4%)); and four Eared Doves, with *Haemoproteus* (4 of 4 examined= 100%). No mixed infections or infections by other blood parasites were detected (Table 1).

The haplotypes isolated from the Tamarugo Conebills (GenBank accession KT363871, 719 bp), House Sparrows (GenBank accession KT363870, 824 bp) and Eared Doves (GenBank accession KT363869, 326 bp) correspond to *Leucocytozoon* sp., *Plasmodium relictum* and *Haemoproteus multipigmentatus*, respectively, as indicated by the BLAST analyses (Table 2). Lineage names, genetic identities, query cover, E value and other hosts from which these haplotypes were previously isolated are detailed in Table 2.

Microscopic examinations of blood smears, corresponding to PCR positive samples, were negative for *Plasmodium* and *Leucocytozoon* but positive for *Haemoproteus*.

Discussion

In this study, we present preliminary data on the prevalence of haemoparasites in the avian community inhabiting the Pampa del Tamarugal. Tamarugo Conebills and Rufous-collared Sparrows together comprised >90% of the individual birds captured, with other bird species scarce in the community. Overall, the prevalence of haemoparasites was low (5.08%). However, a more exhaustive sampling effort should be conducted to obtain adequate sample sizes for all species to enable an accurate estimation of overall prevalence. Nevertheless, we can provide a reasonable estimate of haemoparasite prevalence for the two most abundant bird species in the region: Rufous-collared Sparrows were uninfected, and only three of 89 Tamarugo Conebills were infected (prevalence 3.37%), with parasites of the genus *Leucocytozoon*.

Table 1. Numbers of each species captured and examined at the three sampling sites, and number and proportion (%) of infected birds

Under Number examined, values in parentheses are the proportion of individuals of a species as a percentage of the total of all birds captured ($n = 197$). Under Number infected, values in parentheses are the number infected as a proportion of the number of that species captured

Common name	Scientific name	Number captured and examined (proportion of total captures)	Number of infected birds (proportion of captures of each species infected)	Number captured at each sampling site Huayca	Canchones	Pica
House Sparrow	*Passer domesticus*	10 (5.08%)	3 (30.0%)[A]	0	1	9
Eared Dove	*Zenaida auriculata*	4 (2.03%)	4 (100%)[B]	0	0	4
Tamarugo Conebill	*Conirostrum tamarugense*	89 (45.2%)	3 (3.37%)[C]	33	19	37
Cinereous Conebill	*Conirostrum cinereum*	5 (2.54%)	0	2	0	3
Rufus-collared Sparrow	*Zonotrichia capensis*	87 (44.1%)	0	20	56	11
Vermilion Flycatcher	*Pyrocephalus rubinus*	1 (0.51%)	0	0	1	0
Scaly-throated Earthcreeper	*Upucerthia dumetaria*	1 (0.51%)	0	0	1	0
Total		197	10 (5.08%)	55	78	64

[A]All infected with *Plasmodium* and captured in Pica.
[B]All infected with *Haemoproteus* and captured in Pica.
[C]All infected with *Leucocytozoon*, two captured in Pica, the other in Canchones.

Table 2. Identification of amplicons detected in the present study using BLAST analysis

Amplicons in this study	Genus	Lineage	Identity	Query cover	E value	Hosts
Conirostrum tamarugense (GenBank KT363871; 719 bp)	*Leucocytozoon*	L-T087 or TROAED06[D]	100%	66%	0.0	Cinereous Conebill (*Conirostrum cinereum*), Junin, Peru, (GenBank JQ988442); Buff-browed Foliage-gleaner (*Syndactyla rufosuperciliata*), Cuzco, Peru (GenBank JQ988495); Rufous-breasted Chat-tyrant (*Ochthoeca rufipectoralis*), Junin, Peru (GenBank JQ988532); House Wren (*Troglodytes aedon*), Peru (GenBank KF767441 and KF767437)
Passer domesticus (GenBank KT363870; 824 bp)	*Plasmodium*	GRW04[A] or P15	100%	100%	0.0	Hawai'i 'amakihi (*Hemignathus virens*), Hawaii (GenBank AY733090); Great Reed-Warbler (*Acrocephalus arundinaceus*), Sweden (GenBank AF254975); House Sparrow (*Passer domesticus*), USA (GenBank AY172850); *Culex pipiens quinquefasciatus*, Japan (GenBank AB308050)
Zenaida auriculata (GenBank KT363869; 326 bp)	*Haemoproteus*	SocH6	100%	99%	1e-167	Mourning Dove (*Zenaida macroura*), Socorro Island, Mexico (GenBank JN788937)

[A]Lineage TROAED06 and GRW04 from MalAvi database.

The three Tamarugo Conebills were PCR positive for the parasite but smears were negative, indicating a very low parasitaemia. The obtained haplotype seems to be generalist since it was previously detected in other species of birds, such as the Cinereous Conebill (*Conirostrum cinereum*), Buff-browed Foliage-gleaner (*Syndactyla rufosuperciliata*), Rufous-breasted Chat-Tyrant (*Ochthoeca rufipectoralis*) and House Wren (*Troglodytes aedon*) (Table 2). It is noteworthy that these bird species were mainly captured in high-altitude areas in Peru (i.e. Cuzco and Junin) where simulids (vectors of *Leucocytozoon* species) can find optimal conditions, especially mountain streams, to complete their life-cycle (Greiner *et al.* 1975; Merino *et al.* 2008). As the Tamarugo Conebill migrates to high altitudes in southern Peru, it is likely that the three parasitised individuals were infected in these areas before returning to their breeding regions in Pampa del Tamarugal where simulids have not yet been reported.

Only House Sparrows were found to be infected with *Plasmodium* (prevalence 30%). The haplotype detected (GRW4) is thought to have been introduced to Hawaii by the House Sparrow, causing the subsequent decline and extinction of a number of endemic Hawaiian birds (van Riper 1991). This haplotype has been detected in several species of birds. It is a dominant haplotype on some oceanic islands, like Bermuda and French Polynesia (Beadell *et al.* 2006). In North America, it has thus far only been detected in House Sparrows, and has only rarely been detected in continental South America (Marzal *et al.* 2011). In fact, there are only eight reports, all from Argentina and Brazil and all isolated from House Sparrows (Marzal *et al.* 2011).

Therefore, it could be that the House Sparrow is spreading the parasite from north to south through the Americas. In addition, suitable vectors needed either to be present or introduced in parallel to facilitate parasite transmission in the regions invaded by the House Sparrow. In fact, the decline and extinction of endemic Hawaiian birds has been related to the introduction of *Culex pipiens quinquefasciatus* in 1827 (Zimmerman 1948*a*, 1948*b*). This vector has been reported in the village of Pica (González *et al.* 2012) where most of the House Sparrows were captured (9 of 10). It is possible that the life-cycle of *Plasmodium* haplotype GRW4 was predominantly urban but, even so, presents a risk of infection for the endemic Tamarugo Conebill because 37 of 89 (41.6%) captured were caught in the same area of Pica where most House Sparrows were captured. Although encounters between *Plasmodium* and Tamarugo Conebills is possible, the pathogenicity of the lineage GRW4 is not known. It is worth noting that previous studies have demonstrated the genetic diversity of the lineage GRW4 and its possible relationship to pathogenicity. In this sense, several TRAP (thrombospondin-related anonymous protein) and MSP1 (merozoite surface protein 1) alleles have been detected in GRW4 haplotypes isolated worldwide (Farias *et al.* 2012; Hellgren *et al.* 2015). These surface proteins are likely to interact with the immune system and parasite evasion mechanisms of the host. It is possible that parasites bearing different alleles behave as different populations whose transmission and pathogenic characteristics may be specific for a particular host species (Hellgren *et al.* 2015). Thus, it would be interesting to characterise the allelic diversity present in the haplotype GRW4 isolated in the present survey to establish its origin and potential pathogenicity.

The third haemosporidian parasite found in the Pampa del Tamarugal belongs to the subgenus *Haemoproteus*. It was only detected in the Eared Dove, with 100% prevalence (*n* = 4). This parasite is transmitted by louse flies (family Hippobosciidae), which are obligate parasites and many are flightless. Most larval development takes place within the maternal body and pupation occurs almost immediately once in the soil or on the host. As parasites belonging to the subgenus *Haemoproteus* have not yet been isolated from passeriform birds (Santiago-Alarcon *et al.* 2014; Valkiūnas *et al.* 2013; Merino *et al.* 2012), those found in the Eared Dove should not represent a threat to the Tamarugo Conebill.

In conclusion, the overall prevalence of blood parasites in the present survey was low. However, haemoparasites transmitted by hipoboscids, such as *Haemoproteus Multipigmentatus*, could be common in arid regions and overall prevalence may be higher with sufficient samples of scarcer species of birds. Further, although the main threat to Tamarugo Conebills relates to the management of the Tamarugo forests (BirdLife International 2012), the occurrence of House Sparrows infected with *Plasmodium* should be considered a risk. As noted above, a range of ecological factors might have contributed to the lack of past encounters between Tamarugo Conebills and the *Plasmodium* lineage isolated in the present study. As such, Tamarugo Conebills would not have acquired the immunological defence mechanisms to combat this parasite. To establish the actual risk of haemoparasites to the avian community, a more comprehensive survey of parasites and vectors over time, and the study of any negative health effects of these parasites on birds, should be conducted.

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