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

Javier Martínez A,D, Rodrigo A. Vásquez B, Alberto Marqués A, Alazne Díez-Fernández C and Santiago Merino B

ADepartamento de Biomedicina y Biotecnología, Área Parasitología, Facultad de Farmacia, Universidad de Alcalá, Alcalá de Henares, E-28871 Madrid, Spain.
BInstituto de Ecología y Biodiversidad, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile.
CDepartamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales – Centro Superior de Investigaciones Científicas, J. Gutiérrez Abascal 2, E-28006 Madrid, Spain.

DCorresponding author. Email: francisco.martinez@uah.es

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.

Keywords: avian malaria, haplotype GRW4, host-switching, House Sparrow, *Plasmodium, Leucocytozoon*.
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 (Valkiunas 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 trigemnatus* 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 km² (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 mL 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 2.0–8.1%)). Infections were found in three Tamarugo Conebills, with *Leucocytozoon* (3 of 89 examined = 3.4% (0.0–7.1%)); 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*.

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.)
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 1948a, 1948b). 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 Hippoboscidae), 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-

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 Cone-bills 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.

**Acknowledgements**

During preparation of this work, J. Martínez and S. Merino were supported by funding from the Spanish Ministry of Science and Technology (project CGL2009–09439), the Spanish Ministry of Economy and Competiveness (projects CGL2012–40026-C02–01 and 02) and the Fundación BBVA (project BIOCON06/109). Assistance with travel and accommodation to Pica was provided by the joint project between Consejo Superior de Investigaciones Científicas (CSIC, Spain) and Comisión Nacional de Investigación Científica y Tecnológica (CONICYT, Chile) (2009CL0025 / CONICYT-CSIC 2009–137). Research has been supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT-Chile 1090794, FONDECYT 1140548) and the Institute of Ecology and Biodiversity (ICM-P05–002-Chile, and PFB-23-CONICYT-Chile), to R. A. Vásquez. We also acknowledge the support of A. Vilaxa, C. González and A. Taucare.

**References**


pathogen *Plasmodium relictum* based on MSP1 allelic diversity. Ecography 38, 842–850. doi:10.1111/ecog.01158


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.

<table>
<thead>
<tr>
<th>Common name captured at each sampling site</th>
<th>Scientific name</th>
<th>Number captured</th>
<th>Number of infected</th>
<th>Number captured at each sampling site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(proportion of total captures)</td>
<td>(proportion of captures of each species infected)</td>
<td>Huayca</td>
</tr>
<tr>
<td>House Sparrow</td>
<td>Passer domesticus</td>
<td>10 (5.08%)</td>
<td>3 (30.0%)(^a)</td>
<td>0</td>
</tr>
<tr>
<td>Eared Dove</td>
<td>Zenaida auriculata</td>
<td>4 (2.03%)</td>
<td>4 (100%)(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Tamarugo Conebill</td>
<td>Conirostrum tamarugense</td>
<td>89 (45.2%)</td>
<td>3 (3.37%)(^c)</td>
<td>33</td>
</tr>
<tr>
<td>Cinereous Conebill</td>
<td>Conirostrum cinereum</td>
<td>5 (2.54%)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Rufus-collared Sparrow</td>
<td>Zonotrichia capensis</td>
<td>87 (44.1%)</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Vermilion Flycatcher</td>
<td>Pyrocephalus rubinus</td>
<td>1 (0.51%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Scaly-throated Earthcreeper</td>
<td>Upucerthia dumetaria</td>
<td>1 (0.51%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>197 (10.08%)</td>
<td>10 (5.08%)</td>
<td>55</td>
</tr>
</tbody>
</table>

\(^a\)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>
<thead>
<tr>
<th>Amplicons in this study</th>
<th>Genus</th>
<th>Lineage</th>
<th>Identity</th>
<th>Query cover</th>
<th>E value</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Conirostrum tamarugense</em> (GenBank KT363871; 719 bp)</td>
<td><em>Leucocytozoon</em></td>
<td>L-T087 or TROAED06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100%</td>
<td>66%</td>
<td>0.0</td>
<td>Cinereous Conebill (<em>Conirostrum cinereum</em>), Junin, Peru (GenBank JQ988442); Buff-browed Foliage-gleaner (<em>Syndactyla rufousuperficialis</em>), Cuzco, Peru (GenBank JQ988495); Rufous-breasted Chat-tyrant (<em>Ochthoeca rufipectoralis</em>), Junin, Peru (GenBank JQ988532); House Wren (<em>Troglodytes aedon</em>), Peru (GenBank KF767441 and KF767437)</td>
</tr>
<tr>
<td><em>Passer domesticus</em> (GenBank KT363870; 824 bp)</td>
<td><em>Plasmodium</em></td>
<td>GRW04&lt;sup&gt;a&lt;/sup&gt; or P15</td>
<td>100%</td>
<td>100%</td>
<td>0.0</td>
<td>Hawai`i ʻamakihi (<em>Hemignathus virens</em>), Hawaii (GenBankAY733090); Great Reed-Warbler (<em>Acrocephalus arundinaceus</em>), Sweden (GenBank AF254975); House Sparrow (<em>Passer domesticus</em>), USA (GenBankAY172850); <em>Culex pipiens quinquefasciatus</em>, Japan (GenBank AB308050)</td>
</tr>
<tr>
<td><em>Zenaida auriculata</em> (GenBank KT363869; 326 bp)</td>
<td><em>Haemoproteus</em></td>
<td>SocH6</td>
<td>100%</td>
<td>99%</td>
<td>1e-167</td>
<td>Mourning Dove (<em>Zenaida macroura</em>), Socorro Island, Mexico (GenBank JN788937)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lineage TROAED06 and GRW04 from MalAvi database.