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Supplementary material

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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Section S1. Characteristics of the three sites selected for sampling

La Huayca, a site where there are large *Prosopis* trees, located around 4-6 km south of the village with the same name (see Figure S1; 20°25' S, 69°35' W, 985 m.a.s.l.). This area is extremely arid, with no record of rain.

Canchones, a field site of the Arturo Prat University (see Fig. S1; 20°26' S, 69°33' W, 980 m.a.s.l.), where there are a few plantations due to irrigation from water wells as well as *Prosopis* trees, located within an extremely arid area.

Lastly, a site located at the border of the town of Pica (see Fig. S1; 20°30′ S, 69°20′ O, 1325 m.a.s.l.), where there was also irrigation and plantations such as citrus fruits. In Pica there are a few green areas and crops are grown via irrigation. In this areas it is possible to observe some bird species not commonly reported in the desert such as *Passer domesticus*, *Zenaida meloda* and *Z. auriculata* (see Jaramillo 2003).

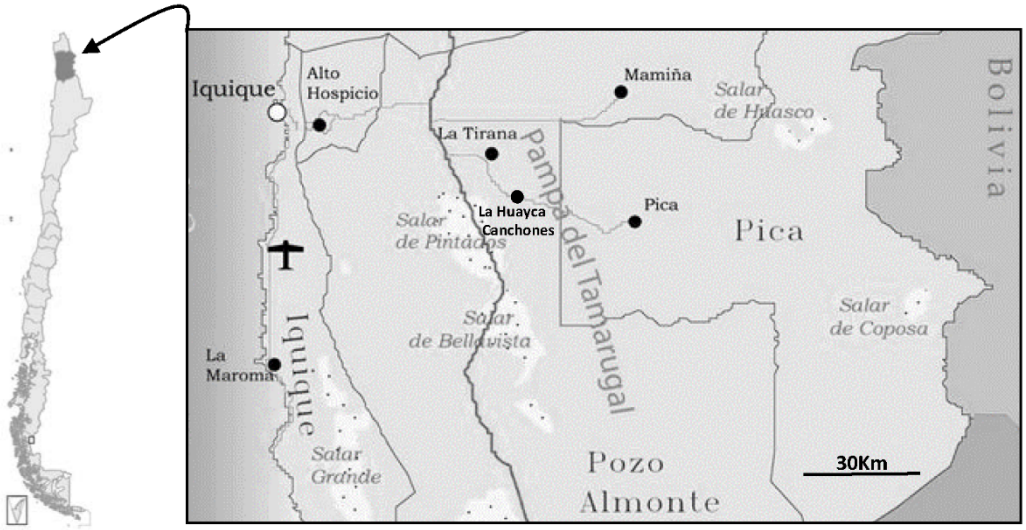

Fig. S1. Map of the study area showing the three sampling sites (i.e., La Huayca, Canchones and Pica) in Pampa del Tamarugal, Tarapacá region, Chile.

Reference

Jaramillo, A. (2003) Birds of Chile. Princeton University Press, Princeton, NJ, USA.

Section S2. Parasite screening

Parasite screening: microscopy

Blood smears were fixed with absolute methanol for 3 min and later stained with Giemsa stain (1/10 v/v) for 45 min in the lab. Blood smears were scanned for hemoparasites at the microscope as follows. In order to facilitate the screening, a half of the symmetric smear was scanned at 20X in search of extracellular parasites (*Trypanosoma* and microfilariae) and large intracellular parasites (*Leucocytozoon*). In the other half of the smear, 100 fields were scanned at 100X in search of small intracellular parasites (*Haemoproteus* and *Plasmodium*). All samples were screened by the same person (A.D-F).

Parasite screening: molecular analyses

PCR reactions consisted of 10 µL reaction volumes containing between 20 and 100 ng of template DNA, 0.25 µM of each primer and SYBR® Select Master Mix (Applied Biosystems, Foster City, CA, USA). The reactions were cycled using 7500 Fast Real-Time PCR System (Applied Biosystems). The diagnosis was performed by visualizing the melting curve of the amplicons. Sequences of the primers, size of the amplicons, and PCR conditions are showed Table S1. After screening, positive samples were amplified again to obtain larger amplicons that facilitate the identification of haplotypes. PCR reaction volume (20 µL) contained between 20 and 100 ng of template DNA, 0.25 M of each primer, and Supreme NZYTaq 2x Green Master Mix (NZYTech, Lda. - Genes and Enzymes, Portugal). Data on primers and PCR conditions are detailed in Table S2. All amplicons 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 detected amplicons in negative controls added in each PCR batch. A positive control for each pair of primers was routinely used.

Identification of haplotypes was performed using the Basic Local Alignment Search Tool (BLAST) implemented by the National Center for Biotechnology Information (NCBI). Megablast option was routinely used. This tool finds regions of local similarity between sequences. In addition, we also used MalAvi database to identify haplotypes. Lastly, prevalence was estimated by using the on line WinEpi tool (http://www.winepi.net/).

Table S1. Primers used to perform the parasitic screening in the present study

All primers were designed for this study except PALU-R (Martinez *et al.* 2009)

primer	sequence 5' → 3'	bp	annealing	extension	parasite (gen)
PALU-Fq	caaggtagctctaatcctttagg	201	54°C-30s	60°C-30s	*Plasmodium*/*Haemoproteus* (cyt b)
PALU-R	dggaacaatatgtaraggagt				
L180	gagaactatggagtggatgg	221	60°C-30s	60°C-30s	*Leucocytozoon* (cyt b)
Leunew1R	cccagaaactcatttgwcc				
TryR	atgcactaggcaccgtcg	121	60°C-30s	60°C-30s	*Trypanosoma* (18S rRNA)
TryF	ggagagggagcctgagaaata				
NF110	gctaatacatgcaccaaagctcc	119	60°C-30s	60°C-30s	microfilariae (18S rRNA)
NR228	caagaccatgcgatcagc				

Table S2. Primers used to amplify positive samples

primer	sequence 5' → 3'	bp	annealing	extension	parasite (gen)
PALU-F	gggtcaaatgagtttctgg	391	56°C-30s	72°C-30s	*Plasmodium*/*Haemoproteus* (cyt b)
PALU-R	dggaacaatatgtaraggagt				
PALU-Fq	caaggtagctctaatcctttagg	422	54°C-30s	72°C-50s	*Plasmodium*/*Haemoproteus* (cyt b)
H15725	catccaatccataataaagcat				
L14902	ttattagccacttgttatactcc	470	54°C-30s	72°C-50s	*Plasmodium*/*Haemoproteus* (cyt b)
PALU-Rq	cctaaaggattagagctaccttg				
L180	gagaactatggagtggatgg	787	58°C-30s	72°C-60s	*Leucocytozoon* (cyt b)
L970	gcatagaatgtgcaaataaacc				

Reference

Martínez, J., Martínez-de la Puente, J., Herrero, J., del Cerro, S., Lobato, E., Rivero-de Aguilar, J., Vásquez, R. A., Merino, S. 2009. A restriction site to differentiate *Plasmodium* and *Haemoproteus* infections in birds: on the inefficiency of general primers for detection of mixed infections. *Parasitology* **136**, 713–722. doi:10.1017/S0031182009006118