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**SUPPORTING INFORMATION**

# Unforeseen biogeographical patterns in a multiple parasite system in Macaronesia

Juan Carlos Illera, Ángela Fernández-Álvarez, Carmen N. Hernández-Flores and Pilar Foronda

**Appendix S1** Details of the molecular procedures used and the number of potential hosts for haemosporidians in Macaronesia.

## Haemosporidian

Haemosporidian pathogens were screened using a nested PCR, using primers described by Hellgren *et al.* (2004) that amplify a 507 base pair (bp) fragment of the mitochondrial cytochrome *b* gene. For the first PCR, reactions were set up in a final volume of 10 µL including 5 µL of GoTaq® Green Master Mix 2× (Promega Corporation, Madison, WI, USA), 0.4 µL (10 mM) of primers HaemNFI and HaemNR3 and 1 µL of DNA. Reactions were performed on a G-Storm GS2 thermal cycler (Somerton Biotechnology Centre, Somerset, UK) under the following conditions: initial denaturation at 96 ºC for 3 min followed by 20 cycles of denaturation at 94 ºC for 20 s, annealing at 50 ºC for 30 s, and extension at 72 ºC for 45 s, and a final extension at 72 ºC for 10 min. The second reaction was set up in a final volume of 10 µL including 5 µL of GoTaq® Green Master Mix 2× (Promega), 0.4 µL (10 mM) of primers HaemF and HaemR2 (to amplify *Plasmodium*/*Haemoproteus*) or HaemFL and HaemR2L (for *Leucocytozoon*), and 1 µL of amplicon from the first reaction. Amplifying conditions were: initial denaturation at 96 ºC for 3 min followed by 40 cycles of denaturation at 94 ºC for 30 s, annealing at 51 ºC (for *Plasmodium*/*Haemoproteus*) or 57 ºC (for *Leucocytozoon*) for 45 s, and extension at 72 ºC for 45 s, followed by a final extension at 72 ºC for 10 min. PCR products were checked by electrophoresis in 1.5% agarose gels stained with GelRed™ nucleic acid gel stain (Biotium, Inc., Hayward, CA, USA). Two positives of *Plasmodium/Haemoproteus* and *Leucocytozoon*, and two negatives (distilled water) were used in each PCR reaction, and all samples were screened twice to ensure the accuracy of the results. Sequencing reactions were performed using the Perkin Elmer BigDye v. 3.1 (Applied Biosystems, Carlsbad, CA, USA) terminator reaction mix in a volume of 10 µL using 1 µL of PCR product and the primers HaemF,

HaemR2, HaemFL and HaemR2L. PCR conditions were: initial denaturation at 94 ºC for 2 min followed by 25 cycles of denaturation at 94 ºC for 30 s, annealing at 50 ºC for 30 s, and extension at 60 ºC for 2 min, followed by a final extension at 60 ºC for 1 min. The final product was purified and sequenced on an ABI PRISM® 3130xl Genetic Analyzer.

**Gastrointestinal parasites**

PCR reactions were set up in a final volume of 50 μL containing 200 μM of each dNTP (Bioline, London, UK), 0.2 μM of each primer, 2.2 mM $MgCl_2$ (Bioline, London, UK), 1 unit of Taq DNA polymerase (Bioline, London, UK), 1× PCR buffer (Bioline, London, UK), and 2 μL of DNA template. Reactions were performed on a Labnet thermal cycler (Labnet International, Edison, NJ, USA), with the following conditions: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. As with the haemosporidian pathogens, samples were sequenced, and either identified as known species if there was 100% of coincidence with homologous sequences published in the NCBI, or new species if there was at least one bp difference.

**Number of potential hosts for haemosporidians in Macaronesia**

The number of potential dipteran vector species transmitting avian haemosporidian parasites per archipelago was obtained from Arechavaleta *et al.* (2005, 2010) and Borges *et al.* (2008). The number of potential vertebrate hosts (i.e. number of extant bird species) from Illera *et al.* (2012). The blood-sucking dipteran families considered as potential invertebrate hosts for haemosporidians were: Culicidae, Hippoboscidae, Simuliidae and Ceratopogonidae (Santiago-Alarcon *et al*., 2012).

| **Number of extant species** | **Madeira** | **Canary Islands** | **Cape Verde** |
|---|---|---|---|
| **Birds** | 37 | 77 | 37 |
| **blood-sucking dipteran** | 21 | 62 | 12 |

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**Appendix S2** Expected prevalence (%) of haemosporidian haplotypes from all spectacled warblers (*Sylvia conspicillata*) sampled. Values obtained after 10,000 bootstrap replicates on each group (sample size in brackets). Expected prevalence is shown as the mean (± standard error) and confidence intervals (95%) in brackets. NSP = uninfected individuals. * $P << 0.0001$.

| Haemosporidian | Mainland (84) | Macaronesia (371) | Madeira (45) | Canary Islands (218) | Cape Verde (108) |
|---|---|---|---|---|---|
| **P-LK06*** | 21 ± 4 (13–31) | 31 ± 2 (27–36) | 35 ± 7 (22 ± 49) | 40 ± 3 (33–46) | 13 ± 3 (7–19) |
| **P-SC-CV1** | 0 | 0.3 ± 0.3 (0–1) | 0 | 0 | 1 ± 1 (0–3) |
| **P-GRW06** | 0 | 0.3 ± 0.3 (0–1) | 0 | 0 | 1 ± 1 (0–3) |
| **P-SC-IP1** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **P-GRW11** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **H-CWT3** | 0 | 3 ± 1 (1–4) | 0 | 0 | 9 ± 3 (4–15) |
| **H-SC-IP1** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **L-SC-GC1*** | 1 ± 1 (0–3) | 3 ± 1 (1–5) | 20 ± 6 (9–31) | 1 ± 1 (0–2) | 0 |
| **L-SC-GC2*** | 0 | 3 ± 1 (1–4) | 9 ± 4 (2–18) | 3 ± 1 (1–5) | 0 |
| **L-SC-MO1** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **L-H027** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **L-SC-IP2** | 1 ± 1 (0–3) | 0 | 0 | 0 | 0 |
| **SYAT22** | 0 | 0.5 ± 0.4 (0–1) | 4 ± 3 (0–11) | 0 | 0 |
| **L-SC-PO1** | 0 | 0.3 ± 0.3 (0–1) | 2 ± 2 (0–7) | 0 | 0 |
| **L-SC-TF1** | 0 | 0.3 ± 0.3 (0–1) | 0 | 0.5 ± 0.5 (0–1) | 0 |
| **L-SC-PO2** | 0 | 0.3 ± 0.3 (0–1) | 2 ± 2 (0–7) | 0 | 0 |
| **NSP*** | 71 ± 5 (62–81) | 63 ± 2 (58–68) | 53 ± 7 (38–67) | 58 ± 3 (52–65) | 76 ± 4 (67–83) |

**Appendix S3** Haemosporidian and coccidian (*Isospora* spp.) haplotypes found in the spectacled warbler (*Sylvia conspicillata*) and others bird species in this study. The avian host recorded hitherto for each haemosporidian haplotype is also shown. SC = *Sylvia conspicillata*. AB = *Anthus berthelotii*. FN = *Falco naumanni*. SA = *Sylvia atricapilla*. SCOM = *Sylvia communis*. SECA = *Serinus canarius*. PLOCU = *Ploceus cucullatus*. EUPOR = *Euplectes orix*. GEN = generalist (infecting more than three species). ? = unavailable species yet.

| | **Avian host** | **GenBank** | **Reference** |
|---|---|---|---|
| **Haemosporidian** | | | |
| *Plasmodium* | | | |
| P-LK06 | SC / AB / FN | EU883534 | Illera *et al*. (2008) |
| P-SC-CV1 | SC | KP688295 | This study |
| P-GRW06 | GEN | JX029877 | MalAvi |
| P-SC-IP1 | SC | KP688296 | This study |
| P-GRW11 | GEN | AY831748 | Pérez-Tris & Bensch (2005) |
| *Haemoproteus* | | | |
| H-CWT3 | SC / SCOM | DQ368343 | Pérez-Tris *et al*. (2007) |
| H-SC-IP1 | SC | KP688297 | This study |
| H-SYAT-CV1 | SA | KP688298 | This study |
| *Leucocytozoon* | | | |
| L-SC-GC1 | SC / ? | KP688299 | This study / MalAvi |
| L-SC-GC2 | SC / SECA / PLOCU / EUPOR | KP688300 | This study / MalAvi |
| L-SC-MO1 | SC | KP688301 | This study |
| L-H027 | GEN | KJ488581 | Drovetski, *et al*. (2014) |
| L-SC-IP1 | SC / ? | KP688302 | This study / MalAvi |
| L-SYAT22 | SC / SA /AB | DQ847236 | MalAvi |
| L-SC-PO1 | SC | KP688303 | This study |
| L-SC-TF1 | SC | KP688304 | This study |
| L-SC-PO2 | SC | KP688305 | This study |
| **Coccidian (*Isospora* spp.)** | | | |
| I-SC-1 | SC | KP688306 | This study |
| I-SC-2 | SC | KP688315 | This study |
| I-SC-3 | SC | KP688307 | This study |
| I-SC-4 | SC | KP688313 | This study |
| I-SC-5 | SC | KP688314 | This study |
| I-SC-6 | SC | KP688308 | This study |
| I-SC-7 | SC | KP688309 | This study |
| I-SC-8 | SC | KP688310 | This study |
| I-SC-9 | SC | KP688311 | This study |
| I-SC-10 | SC | KP688312 | This study |
| I-SC-11 | SC | KP688316 | This study |
| I-SC-12 | SC | KP688317 | This study |

| | | | |
|---|---|---|---|
| I-SC-13 | SC | KP688318 | This study |
| I-SC-14 | SC | KP688320 | This study |
| I-SC-15 | SC | KP688319 | This study |

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