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SHORT COMMUNICATION

Absence of haemosporidian parasite infections in the long-lived Cory's shearwater: evidence from molecular analyses and review of the literature

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Abstract The apparent scarcity or absence of blood parasites in some avian groups, such as seabirds, has been related to intrinsic and extrinsic factors including host immunological capacity, host-parasite assemblage, and ecological parameters, but also to reduced sensitivity of some methods to detect low parasite prevalence/intensities of infection. Here, we examined the haemosporidian parasite prevalence in a breeding population of Cory's shearwater *Calonectris diomedea borealis*, a long-distance migrant seabird, nesting in the Macaronesian region, in the Eastern Atlantic. Previous studies on *Calonectris diomedea* complex were based on small sample sizes providing weak evidence for a lack of infections by haemoparasites. Here, we investigated the presence of both parasite infections in *C. d. borealis* and larvae of potential mosquito vectors on the area. By employing a PCR-based assay, we extensively examined the prevalence of blood parasites belonging to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* in 286 individuals from different life stages (i.e., chicks, immatures, sabbatical, and breeding adults), facing their specific energetic trade-offs (immunological functions vs. life history activities). We sampled immatures and adult shearwaters, of different sexes, ages, and migratory origins, from two sub-colonies. None of the sampled individuals were infected by these parasites, supporting the hypothesis that there was no in situ or ex situ transmission of vector-borne parasites in marine habitats irrespective of host's life stage and in spite of the presence of the potential *Plasmodium* vector *Culiseta longiareolata* breeding in the area. These results suggest that the lack of transmission of haemosporidian parasites on Selvagem Grande may be related to the lack of suitable dipteran vectors at the study sites, which may result from the geographic isolation of this area.

Keywords Avian malaria parasites · Insect vectors · Procellariiformes · Remote island · Seabirds

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Introduction

Birds are commonly infected by several species of blood parasites causing detrimental effects in terms of body condition and survival (Merino et al. 2000; Martínez-de la Puente et al. 2010). Prevalence and intensity of these parasites are highly variable in space, with individuals of the same species showing clear geographical variation, probably linked to differences in the susceptibilities of host populations and environmental characteristics determining the distribution of suitable vectors (Sol et al. 2000; Martínez-Abraín et al. 2004). However, factors determining this geographical variation may be diverse, potentially differing between species. Birds living in marine, saline, arid, open, or alpine/high-latitude habitats have often been reported to be free from blood parasites (Piersma 1997; Figuerola 1999), even in the presence of

potential insect vectors (Ruiz et al. 1995; González-Solís and Abella 1997). In seabirds, the most commonly observed blood parasites belong to the genus *Plasmodium* that is transmitted by mosquitoes (Fam. Culicidae). *Haemoproteus* is transmitted by biting midges (Fam. Ceratopogonidae) and louse flies (Fam. Hippoboscidae) while black flies (Fam. Simuliidae) are the main vectors of *Leucocytozoon* (Valkiūnas 2005). However, seabirds usually show a low prevalence or total absence of haemosporidian parasites, and this apparent scarcity of blood parasites may be linked to ecological or evolutionary factors (Quillfeldt et al. 2010, 2011). The most plausible hypotheses (Martínez-Abraín et al. 2004) relate those findings to (i) low host density or insufficient time for the coevolution between birds and parasites to occur, (ii) short exposure time to parasite infections, (iii) lack of a suitable host-parasite assemblages (i.e., referring mainly to physiological compatibility to allow parasite development), and (iv) high immunological capacity of the avian hosts to fight off infections. Furthermore, failure to detect parasite may also result from methodological limitations. For example, it has been suggested that the traditional microscopic inspections of blood smears (Peirce 1981a, b) are generally less sensitive to detect *Plasmodium* and *Haemoproteus* parasites if the intensity is low (e.g., winter when transmission does not occur; Valkiūnas 2005). Molecular-based approaches using sensitive polymerase chain reactions (PCRs) can detect some parasitemia missed by blood smears (e.g., Feldman and Freed 1995; Bensch et al. 2000; Perkins and Schall 2002; Ricklefs et al. 2005; Merino et al. 2008). This may result in the underestimation of parasite prevalence, especially when infections are of low intensity (Jarvi et al. 2002; Ricklefs et al. 2005). However, false negatives are not completely excluded by molecular methods (Gomez-Diaz et al. 2010) and established PCRs are still inaccurate for the identification of some parasite species (Valkiūnas et al. 2016) and should be combined with light microscopy (Bernotienė et al. 2016), especially for the case of birds suffering coinfections by different lineages (Martínez et al. 2009; Gutierrez-Lopez et al. 2016). Underestimation of parasite prevalence is an issue of great relevance in parasitological studies because the real prevalence in a population (i.e., population prevalence) is generally unknown and its uncertainty is higher at low sample sizes (Jovani and Tella 2006). Although there is a significant number of studies on parasite infections in seabirds (Quillfeldt et al. 2011), these were performed on relatively modest sample sizes (Jovani and Tella 2006).

Previous studies on Cory's shearwaters, *Calonectris diomedea* complex, including here the two seabird sister species *Calonectris diomedea borealis* and *Calonectris diomedea diomedea* (Sangster et al. 2012), were based on small sample sizes screened almost exclusively by means of traditional microscopic inspection of blood smears. This fact could limit the sensitivity of parasite detection, as this method usually fails to detect low ($\leq 0.001\%$) parasitemia which is commonly found in wild birds (Jarvi et al. 2002; Valkiūnas et al. 2016), especially in seabirds (Quillfeldt et al. 2011, 2014). That provides weak evidence of infections by haemoparasites in this group (Wink et al. 1979; González-Solís and Abella 1997; Quillfeldt et al. 2010; Hervías et al. 2013, Table 1; and the MalAvi—database for avian haemosporidian parasites, Bensch et al. 2009). *C. d. borealis* is a long-lived migratory seabird nesting colonially on small isolated islands throughout the Macaronesian region (North-East Atlantic) and overwintering in different areas of the South Atlantic (Dias et al. 2011).

Here, we carried out a detailed study on this species where we investigated the presence of both haemosporidian parasite infections in a population breeding at Selvagem Grande Island (Madeira archipelago) and larvae of potential mosquito vectors. More specifically, because Cory's shearwater has delayed maturity (age of first reproduction 8.9 ± 1.7 (mean ± SD) years Mougin et al. 2000) and adult breeders can skip reproduction during several years, we screened parasite prevalence: (a) among individuals in different stages of the life cycle (i.e., chick, immature of different ages, sabbatical, and breeding adults) and (b) between breeding sub-colonies of different sizes. We used the broadly used PCR-based approach by Hellgren et al. (2004) to determine the prevalence (and eventually the genetic identity) of the parasites belonging to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* commonly found in infecting birds. Finally, we determined the presence of potential parasite vectors by sampling mosquito larvae in the only quasi-permanent freshwater pool located close to the study colonies.

Methods

Host species

Cory's shearwater, a medium sized, pelagic seabird, including two sister species *C. d. borealis* and *C. d. diomedea*, nests colonially in marine habitats in the North-East Atlantic Ocean (Macaronesian region) and throughout the Mediterranean region, respectively. *C. d. borealis* arrives at its breeding ground in the NE Atlantic (Azores, Berlenga, Madeira, Selvagem, and Canary Island) in March after a trans-equatorial migration and leave the breeding ground in the beginning of November heading to their wintering areas located mainly in the South Atlantic Ocean (González-Solís et al. 2007; Dias et al. 2011). Incubation starts at the end of May (Zino et al. 1987) while hatching begins only at the end of July (Granadeiro 1991). By then, most pre-breeder individuals have already come ashore to visit their natal colonies (authors' pers. obs.). Females lay a single egg per clutch, and chicks fledge in late October to early November (Granadeiro 1991).

Table 1 Available studies on the blood parasites of Cory's shearwaters and analytical procedure used

Study area	Study species	Sample size	Method employed to detect blood parasites	Reference
Aegean Isls. (unknown)	*C. d. diomedea*	26	SMEAR	Wink et al. (1979)
Chafarinas Isl. (135°11′ N, 2°26′ E)	C. *d. diomedea*	38	SMEAR	González-Solís and Abella (1997)
Berlenga Isl. (39°24′ N, 9°30′ W)	*C. d. borealis*	15	PCR and SMEAR	Quillfeldt et al. (2010)
Corvo Isl. (39° 40′ N, 31° 7′ W)	*C. d. borealis*	53	SMEAR	Hervías et al. (2013)

Study area

During the breeding season of 2014, we studied a population of *C. d. borealis* breeding on Selvagem Grande in the Macaronesian region. Selvagem Grande Island has a surface area of 270 ha (30°09′ N, 15°52′ W) and is located ca. 300 km south of Madeira island, 160 km north of the Canary Islands and approximately 350 km from the coast of North Africa. The island hosts the largest population of *C. d. borealis* of the world (ca. 30,000 breeding pairs, Granadeiro et al. 2006).

Data collection

This study was performed from July to August 2014 (early-chick rearing). Chicks and adult breeders were blood-sampled by puncturing the inter-digital membrane, and a blood sample of approximately 0.2 mL was collected with a capillary tube and maintained in absolute ethanol. In Selvagem Grande, we collected blood samples from immature birds of different ages and sabbatical individuals (see Campioni et al. 2016). Breeders and nonbreeders from a different sub-colony (i.e., Baía das Cagarras) of the island (< 500 m away) were also sampled to address potential differences between sub-colonies. Due to the long-term monitoring of the Selvagem Grande colony carried out since 2004, the age, sex, and reproductive status of most individuals were known (Granadeiro et al. 2006). Furthermore, 14 individuals were equipped with geolocators in previous years, which provided information on their wintering areas, and these individuals were sampled before and after their migration in 2014/2015. Finally, all individuals were sexed employing a molecular-based method following Griffiths et al. (1998). In March 2017, we also collected and identified 113 mosquito larvae found in the only quasi-permanent freshwater pool located 400 m from the nearest study nests.

Parasite analysis: blood sampling and DNA extraction

Genomic DNA was isolated from blood samples using a standard chloroform/isoamyl alcohol procedure (Gemmell and Akiyama 1996; Ferraguti et al. 2013), with minor modifications. Each sample was introduced into individual tubes containing 300 μl of lysis buffer (100 mM NaCl, 50 mM Tris-HCl pH 8, 50 mM EDTA pH 8, 1% SDS), 5 μl of proteinase K (20 mg/ml), and 10 μl of DDT (1 M) and then kept on a shaker incubating at 55 ° C overnight. The following day, an equal volume (320 μl) of 5 M LiCl was added to each tube and then each sample was mixed by inversion for 1 min after adding 630 μl chloroform/isoamyl alcohol (24:1). After shaking the tubes, the samples were centrifuged for 15 min at 16,200×*g* and the supernatant (500 μl) was carefully removed and transferred into a new tube, where 1 ml of absolute ethanol was added to precipitate the DNA overnight at − 18 ° C. The next day, the DNA was recovered by centrifugation at 16,200×*g* for 15 min. The pellet was dried and washed with 70% ethanol and resuspended in 20 μl of milliQ water. For some samples, with low concentration of red blood cells, a Maxwell kit (Maxwell®16 LEV system Research (Promega, Madison, WI) was used to extract DNA following the manufacturer instructions. We analyzed the prevalence of haemosporidian parasites belonging to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* using the protocol detailed by Hellgren et al. (2004) based on the amplification of a fragment of the mitochondrial cytochrome b gene. In brief, this method consists in a first PCR to amplify a DNA fragment of the three parasite genera, followed by a nested PCR to amplify each *Plasmodium*, *Haemoproteus*, or *Leucocytozoon* DNA. Positive amplifications are assigned to parasite genus/lineages by blast comparisons with available sequences deposited in GenBank database (National Center for Biotechnology Information Blast). Positive and negative controls were included in each plate. Negative samples in a first screening were repeated twice to minimize the possibility of false negatives.

Results

None of the 286 *C. d. borealis* sampled at Selvagem Grande Island were infected by any of the three analyzed genera tested (Table 2). All the mosquito larvae ($n = 113$) collected corresponded to the species *Culiseta longiareolata*.

Table 2 Number of *C. d. borealis* analyzed in this study with respect to bird sex, colony, and the parasite prevalence. Analyzed blood samples (Total) are shown according to life cycle stage, sex, and sub-colony of origin (Plateau, Baía das Cagarras, B.C.)

Life cycle	Sex[a]		Colony		Blood parasite	
Stage	Male	Female	Plateau	B.C.	Total	Prevalence
Chick	71	60	140	–	140	0
Immature	44	6	54	–	54	0
Breeder	31	17	28	25	53	0
Sabbatical	10	9	19	–	19	0
Unknown	11	8	3	17	20	0
Total	167	100	244	42	286	0

[a] Sample size includes only birds successfully sexed

Discussion

The results of this study showed that despite extensive sampling of a large variety of individuals, birds from two subcolonies from Selvagem Grande Island were free from haemosporidian parasites during all stages of their life cycle, from the early stage as nestlings to that of experienced breeder (range 8–26 years). The absence of infections in *C. d. borealis* supports observations from previous studies on Cory's shearwaters corroborating the general trend of parasite occurrence recorded in seabirds. Only four studies have been conducted on the haemosporidian parasites infecting Cory's shearwater until now (Table 1). Total absence of these parasites has been reported in previous studies using inspection of blood smears (only one used PCR combined with smears; see Table 1) on both *C. d. borealis* and *C. d. diomedea* from the Macaronesian and Mediterranean regions, respectively. Also, no reports of infections appear in the MalAvi database for bird species belonging to the *Calonectris* genus. Seabirds comprise species for which the prevalence of blood parasite infections is generally low (mean haematozoa prevalence 8.7%, range 0–27.7%, across 113 seabird species; Quillfeldt et al. 2011) compared, for example, to 26% in a sample of 14,812 European passerines (Scheuerlein and Ricklefs 2004). However, most of the studies on seabirds, especially those on Cory's shearwaters, relied on small sample sizes (Table 1), which could have biased parasite prevalence estimations (Jovani and Tella 2006). With one exception, previous studies only used microscopic smear screening which could fail to identify the presence of parasite infections at very low parasite load (e.g., those $\leq 0.001\%$; Jarvi et al. 2002, see Table 1). In addition, the absence of *Plasmodium*, *Haemoproteus*, or *Leucocytozoon* parasites was recorded when both screenings of blood smears and molecular methods were used to identify haemosporidian infections in this species (Quillfeldt et al. 2011). Thus, although the molecular approach may fail to amplify some particular parasite lineages potentially biasing our estimation of parasite prevalence (Valkiūnas et al. 2016; Bernotienė et al. 2016; Gutierrez-Lopez et al. 2016), this possibility is of low probability. Furthermore, the studies on shearwaters were limited to analyzing birds from a single life cycle stage potentially differing in their susceptibility to parasite infections (Forero et al. 2006).

Beyond the analytical issues, within-population variation in susceptibility to parasite infections may arise in relation to intrinsic and extrinsic ecological factors. For this reason and because sexual differences in immunocompetence may lead to different susceptibilities to parasite infections (Forero et al. 2006; Lobato et al. 2008), we extensively sampled birds from both sexes. Similarly, because birds with different wintering origins may differ in their exposure to parasite infections (Gutierrez-Lopez et al. 2015), we sampled ($n = 9$) adult and ($n = 5$) immature shearwater overwintering in distinct areas (7% off the coast of Brazil, 72% South Africa, or 21% Canary Islands). However, we did not find any birds infected by haemosporidians.

Selvagem Grande is an isolated oceanic island characterized by low precipitation, high salinity, and exposure to winds and as a consequence, relatively unsuitable for insect vectors (e.g., Ceratopogonidae, Simuliidae, and many species of mosquitoes) requiring an aquatic larval stage to complete their life cycles (see Super and van Riper III 1995). These meteorological characteristics especially windy conditions have been suggested limiting the presence of insect vectors (Fredeen and Mason 1991; Martin et al. 1994; Martínez-de la Puente et al. 2009) in marine habitats (Jovani et al. 2001; Valkiūnas 2005) and as a consequence of parasites, in comparison with inland areas (i.e., mainland and/or large islands) thus providing support to Piersma's hypothesis (1997). A differential distribution of the vector *Culex pipiens* between islands of the Madeiran archipelago (being absent in Selvagem Grande and Deserta Grande) has been argued by Spurgin et al. (2011) as the main reason explaining the variable prevalence of blood parasites in Berthelot's pipits from this area. While infected Berthelot's pipits were trapped in islands of the Macaronesia where the vector is present, none of the birds captured in Selvagem Grande (2006, $n = 34$; 2009, $n = 42$) were infected by blood parasites (screened by PCR-based assay, Illera et al. 2008; Spurgin et al. 2011). Despite that, the surveillance of mosquito larvae conducted in the study area allowed us to confirm the presence of *Culiseta longiareolata*, an ornithophilic species of the family Culicidae, competent for the transmission of two avian *Plasmodium* parasites, the widespread *Plasmodium relictum* and *P. polare* (Santiago-Alarcon et al. 2012). Nevertheless, the role of *Culiseta* spp. as vectors of avian malaria remains insufficiently investigated and no clear results are reported in the literature. In addition, Spurgin et al. (2011) observed significant effects of both island size and isolation on pathogen species richness across islands, with Selvagem Grande being the most isolated one, free from the

avian malaria parasites and poxvirus. All this evidence suggests that the absence of a parasite able to infect *Calonectris diomedea* group and/or the lack of a suitable vector may explain the absence of haemosporidian parasites in shearwaters breeding in small and highly isolated islands, such as Selvagem Grande.

Conclusion and future directions

Our study strongly suggests that shearwaters are free of blood parasites during their long lifetime. The absence of in situ or ex situ transmission of vector-borne parasites in *C. d. borealis* is likely due to the harsh environmental conditions characterizing a marine location and geographic isolation of Selvagem Grande and the pelagic life style of shearwaters during the nonbreeding period. However, the reason(s) behind the absence of haemosporidian parasites among Cory's shearwater populations across its broad breeding range is difficult to ascertain based on the information currently available (Table 1). The lack of knowledge about the concurrent presence of the host species and potential vectors (but see González-Solís and Abella 1997) on different islands prevents us from drawing conclusions for this species. Further studies should focus on the occurrence of haemosporidian parasites in the whole bird community breeding on Selvagem Grande in order to understand the absence of parasites in *C. d. borealis* and in particular to test the resistance of this and other phylogenetically related species to infection by haemosporidians.

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References

Bensch S, Hellgren O, Pérez-Tris J (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol Ecol Resour 9:1353–1358. https://doi.org/10.1111/j.1755-0998.2009.02692.x

Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, Pinheiro RT (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proc R Soc London Ser Biol Sci 267:1583–1589. https://doi.org/10.1098/rspb.2000.1181

Bernotienė R, Palinauskas V, Iezhova T, Murauskaitė D, Valkiūnas G (2016) Avian haemosporidian parasites (Haemosporida): a comparative analysis of different polymerase chain reaction assays in detection of mixed infections. Exp Parasitol 163:31–37. https://doi.org/10.1016/j.exppara.2016.01.009

Campioni L, Granadeiro JP, Catry P (2016) Niche segregation between immature and adult seabirds: does progressive maturation play a role? Behav Ecol 27:426–433. https://doi.org/10.1093/beheco/arv167

Dias M, Granadeiro JP, Phillips RA, Alonso H, Catry P (2011) Breaking the routine: individual Cory's shearwaters shift winter destinations between hemispheres and across ocean basins. Proc R Soc London Ser B Biol Sci 278:1786–1793. https://doi.org/10.1098/rspb.2010.2114

Feldman RA, Freed LA (1995) A PCR test for avian malaria in Hawaiian birds. Mol Ecol 4:663–673

Ferraguti M, Martínez-de la Puente J, Muñoz J, Roiz D, Ruiz S, Soriguer R, Figuerola J (2013) Avian *Plasmodium* in *Culex* and *Ochlerotatus* mosquitoes from southern Spain: effects of season and host-feeding source on parasite dynamics. PLoS One 8(6):e66237. https://doi.org/10.1371/journal.pone.0066237

Forero M, González-Solis J, Igual JM, Hobson K, Ruíz X, Viscor G (2006) Ecological and physiological variance in T-cell mediated immune response in Cory's shearwaters. Condor 108:865–876. https://doi.org/10.1650/0010-5422(2006)108%5B865:EAPVIT%5D2.0.CO;2

Fredeen FJ, Mason PG (1991) Meteorological factors influencing host-seeking activity of female *Simulium luggeri* (Diptera: Simuliidae). J Med Entomol 28:831–840

Figuerola J (1999) Effects of salinity on rates of infestation of waterbirds by haematozoa. Ecography 22:681–685. https://doi.org/10.1111/j.1600-0587.1999.tb00517.x

Gemmell NJ, Akiyama S (1996) An efficient method for the extraction of DNA from vertebrate tissues. Trends Genet 12(9):338. https://doi.org/10.1016/S0168-9525(96)80005-9

Gomez-Diaz E, Doherty PF Jr, Duneau D, McCoy KD (2010) Cryptic vector divergence masks vector-specific patterns of infection: an example from the marine cycle of *Lyme borreliosis*. Evol Appl 3:391–401. https://doi.org/10.1111/j.1752-4571.2010.00127.x

González-Solís J, Abella JC (1997) Negative record of haematozoan parasites on Cory's shearwater *Calonectris diomedea*. Orn Fenn 74:153–155

González-Solís J, Croxall J, Oro D, Ruiz X (2007) Trans-equatorial migration and mixing in the wintering areas of a pelagic seabird. Front Ecol Environ 5:297–301. https://doi.org/10.1890/1540-9295(2007)5%5B297:TMAMIT%5D2.0.CO;2

Granadeiro JP (1991) The breeding biology of Cory's shearwater *Calonectris diomedea borealis* on Berlenga Island, Portugal. Seabird 13:30–39

Granadeiro JP, Dias MP, Rebelo R, Santos CD, Catry P (2006) Numbers and population trends of Cory's shearwaters *Calonectris diomedea* at Selvagem Grande, Northeast Atlantic. Waterbirds 29:56–60. https://doi.org/10.1675/1524-4695(2006)29%5B56:NAPTOC%5D2.0.CO;2

Griffiths R, Double M, Orr K, Dawson R (1998) A simple DNA test to sex most birds. Mol Ecol 7:1071–1076. https://doi.org/10.1046/j.1365-294x.1998.00389.x

Gutiérrez-López R, Gangoso L, Martínez-de la Puente J, Fric J, López-López P, Mailleux M, Muñoz J, Touati L, Samraoui B, Figuerola J (2015) Low prevalence of blood parasites in a long-distance migratory raptor: the importance of host habitat. Parasit Vectors 8:189. https://doi.org/10.1186/s13071-015-0802-9

Gutiérrez-López R, Martínez-de la Puente J, Gangoso L, Yan J, Soriguer RC, Figuerola J (2016) Do mosquitoes transmit the avian malaria-like parasite *Haemoproteus*? An experimental test of vector competence using mosquito saliva. Parasit Vectors 28:609. https://doi.org/10.1186/s13071-016-1903-9

Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J Parasitol 90:797–802. https://doi.org/10.1645/GE-184R1

Hervías S, Ramos JA, Nogales M, Ruiz de Ybáñez R (2013) Effect of exotic mammalian predators on parasites of Cory's shearwater: ecological effect on population health and breeding success. Parasitol Res 112:2721–2730. https://doi.org/10.1007/s00436-013-3443-y

Illera JC, Emerson BC, Richardson DS (2008) Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (*Anthus berthelotii*) in Macaronesia. Parasitol Res 103:1435–1443. https://doi.org/10.1007/s00436-008-1153-7

Jarvi SI, Schultz JJ, Atkinson CT (2002) PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally-infected passerines. J Parasitol 88:153–158. https://doi.org/10.1645/0022-3395(2002)088%5B0153:PDUTPO%5D2.0.CO;2

Jovani R, Tella JL (2006) Parasite prevalence and sample size: misconceptions and solutions. Trends Parasitol 22:214–218. https://doi.org/10.1016/j.pt.2006.02.011

Jovani R, Tella JL, Forero MG, Bertellotti M, Blanco G, Ceballos O, Donázar J (2001) Apparent absence of blood parasites in the Patagonian seabird community: is it related to the marine environment? Waterbirds 24:430–433

Lobato E, Merino S, Morales J, Tomás G, Martínez-de la Puente J, Sánchez E, García-Fraile S, Moreno J (2008) Sex differences in circulating antibodies in nestling pied flycatchers *Ficedula hypoleuca*. Ibis 150:799–806. https://doi.org/10.1111/j.1474-919X.2008.00863.x

Martin FR, McCreadie JW, Colbo MH (1994) Effect of trap site, time of day, and meteorological factors on abundance of host-seeking mammalophilic black flies (Diptera: Simuliidae). Can Entomol 126:283–289. https://doi.org/10.4039/Ent126283-2

Martínez J, Martínez-de la Puente J, Herrero J, Del Cerro S, Lobato E, Rivero-de Aguilar J, Vásquez RA, 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. https://doi.org/10.1017/S0031182009006118

Martínez-Abrain A, Esparza B, Oro D (2004) Lack of blood parasites in bird species: does absence of blood parasite vectors explain it all? Ardeola 5:225–232

Martínez-de la Puente J, Merino S, Lobato E, Rivero-de Aguilar J, Del Cerro S, Ruiz-de-Castañeda R, Moreno J (2009) Does weather affect biting fly abundance in avian nests? J Avian Biol 40:653–657. https://doi.org/10.1111/j.1600-048X.2009.04726.x

Martínez-de la Puente J, Merino S, Tomás G, Moreno J, Morales J, Lobato E, García-Fraile S, Belda EJ (2010) The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. Biol Lett 6:663–665. https://doi.org/10.1098/rsbl.2010.0046

Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc Roy Soc London Ser B Biol Sci 267:2507–2510. https://doi.org/10.1098/rspb.2000.1312

Merino S, Moreno J, Vásquez RA, Martínez J, Sánchez-Monsálvez I, Estades CF, Ippi S, Sabat P, Rozzi R, McGehee S (2008) Haematozoa in forest birds from southern Chile: latitudinal gradients in prevalence and parasite lineage richness. Austral Ecol 33:329–340. https://doi.org/10.1111/j.1442-9993.2008.01820.x

Mougin JL, Jouanin C, Roux F (2000) Démographie du puffin cendré *Calonectris diomedea* de Selvagem Grande. Rev Ecol Terre Vie 55:275–290

Peirce MA (1981a) Distribution and host-parasite check-list of the haematozoa of birds in Western Europe. J Nat Hist 15:419–458. https://doi.org/10.1080/00222938100770321

Peirce MA (1981b) Haematozoa of British birds. VI Redescription of *Haemoproteus larae* Yakunin from the lesser black-backed gull *Larus fuscus*. J Nat Hist 15:459–462. https://doi.org/10.1080/00222938100770331

Perkins SL, Schall JJ (2002) A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. J Parasitol 88:978. https://doi.org/10.1645/0022-3395(2002)088%5B0972:AMPOMP%5D2.0.CO;2

Piersma T (1997) Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? Oikos 80:623–631. https://doi.org/10.2307/3546640

Quillfeldt P, Arriero E, Martínez J, Masello JF, Merino S (2011) Prevalence of blood parasites in seabirds—a review. Front Zool 8:26. https://doi.org/10.1186/1742-9994-8-26

Quillfeldt P, Martínez J, Bugoni L, Mancini PL, Merino S (2014) Blood parasites in noddies and boobies from Brazilian offshore islands—differences between species and influence of nesting habitat. Parasitology 141:399–410. https://doi.org/10.1017/S0031182013001649

Quillfeldt P, Martínez J, Hennicke J, Ludynia K, Gladbach A, Masello JF, Riou S, Merino S (2010) Hemosporidian blood parasites in seabirds—a comparative genetic study of species from Antarctic to tropical habitats. Naturwissenschaften 97:809–817. https://doi.org/10.1007/s00114-010-0698-3

Ricklefs RE, Swanson BL, Fallon SM, Martinez-Abrain A, Scheuerlein A, Gray J, Latta SC (2005) Community relationships of avian malaria parasites in southern Missouri. Ecol Monogr 75:543–559. https://doi.org/10.1890/04-1820

Ruiz X, Oro D, González-Solís J (1995) Incidence of a *Haemoproteus lari* parasitemia in a threatened Mediterranean gull: *Larus audouinii*. Orn Fenn 72:159–164

Sangster G, Collinson JM, Crochet PA, Knox AG, Parkin DT, Votier SC (2012) Taxonomic recommendations for British birds: eighth report. Ibis 154:874–883. https://doi.org/10.1111/j.1474-919X.2012.01273.x

Santiago-Alarcon D, Havelka P, Schaefer HM, Segelbacher G (2012) Bloodmeal analysis reveals avian plasmodium infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors.

PLoS One 7(2):e31098. https://doi.org/10.1371/journal.pone.0031098

Scheuerlein A, Ricklefs RE (2004) Prevalence of blood parasites in European passeriform birds. Proc Roy Soc London Ser B Biol Sci 271:1363–1370. https://doi.org/10.1098/rspb.2004.2726

Sol D, Jovani R, Torres J (2000) Geographical variation in blood parasites in feral pigeons: the role of vectors. Ecography 23:307–314. https://doi.org/10.1111/j.1600-0587.2000.tb00286.x

Spurgin LG, Illera JC, Padilla DP, Richardson DS (2011) Biogeographical patterns and co-occurrence of pathogenic infection across island populations of Berthelot's pipit (*Anthus berthelotii*). Oecologia 168:691–701. https://doi.org/10.1007/s00442-011-2149-z

Super PE, van Riper IIIC (1995) A comparison of avian hematozoan epizootiology in two California coastal scrub communities. J Wildl Dis 31:447–461

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton

Valkiūnas G, Ilgūnas M, Bukauskaitė D, Iezhova TA (2016) Description of *Haemoproteus ciconiae* sp. nov. (Haemoproteidae, Haemosporida) from the white stork *Ciconia ciconia*, with remarks on insensitivity of established polymerase chain reaction assays to detect this infection. Parasitol Res 115:2609–2616. https://doi.org/10.1007/s00436-016-5007-4

Wink M, Wink C, Ristow D (1979) Parasitenbefall juveniler und adulter Gelbschnabelsturmtaucher (*Calonectris diomedea*). Bonn Zool Beitr 30:217–219

Zino PA, Zino F, Maul T, Biscoito JM (1987) The laying, incubation and fledging periods of Cory's shearwater *Calonectris diomedea borealis* on Selvagem Grande in 1984. Ibis 129:393–398. https://doi.org/10.1111/j.1474-919X.1987.tb03185.x