

Hemosporidian blood parasites in seabirds—a comparative genetic study of species from Antarctic to tropical habitats

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Abstract Whereas some bird species are heavily affected by blood parasites in the wild, others reportedly are not. Seabirds, in particular, are often free from blood parasites, even in the presence of potential vectors. By means of polymerase chain reaction, we amplified a DNA fragment from the cytochrome b gene to detect parasites of the genera *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* in 14 seabird species,

ranging from Antarctica to the tropical Indian Ocean. We did not detect parasites in 11 of these species, including one Antarctic, four subantarctic, two temperate, and four tropical species. On the other hand, two subantarctic species, thin-billed prions *Pachyptila belcheri* and dolphin gulls *Larus scoresbii*, were found infected. One of 28 thin-billed prions had a *Plasmodium* infection whose DNA sequence was identical to lineage P22 of *Plasmodium relictum*, and one of 20 dolphin gulls was infected with a *Haemoproteus* lineage which appears phylogenetically clustered with parasites species isolated from passeriform birds such as *Haemoproteus lanii*, *Haemoproteus magnus*, *Haemoproteus fringillae*, *Haemoproteus sylvae*, *Haemoproteus payevskiyi*, and *Haemoproteus belopolskyi*. In addition, we found a high parasite prevalence in a single tropical species, the Christmas Island frigatebird *Fregata andrewsi*, where 56% of sampled adults were infected with *Haemoproteus*. The latter formed a monophyletic group that includes a *Haemoproteus* line from Eastern Asian black-tailed gulls *Larus crassirostris*. Our results are in agreement with those showing that (a) seabirds are poor in hemosporidians and (b) latitude could be a determining factor to predict the presence of hemosporidians in birds. However, further studies should explore the relative importance of extrinsic and intrinsic factors on parasite prevalence, in particular using phylogenetically controlled comparative analyses, systematic sampling and screening of vectors, and within-species comparisons.

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Introduction

Parasites exert important ecological and evolutionary pressures on their hosts. Blood parasites (hematozoa) were

considered to be organisms of low pathogenicity in wild populations, but it has been demonstrated that hematozoa can have important implications for bird community structure and conservation (e.g., van Riper et al. 1986), as well as life history traits of avian hosts (e.g., Merino et al. 2000; Hōrak et al. 2001; Sanz et al. 2001a, b; Sol et al. 2003; Marzal et al. 2005). Knowledge about the prevalence of hematozoa is thus essential for understanding the ecological and evolutionary implications of these parasites on their hosts and, ultimately, on ecosystems (see Sol et al. 2003).

Birds are infected by a number of intracellular blood parasites, including Hemosporidia of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, and Haemogregarinidae of the genus *Hepatozoon* and piroplasmids of the genus *Babesia*. It has been established that once birds become infected with hemosporidian parasites, they remain infected either for life or many years (Garnham 1966; Valkiūnas 2005).

The intracellular hematozoa occurring in vertebrates belong to the phylum Apicomplexa, and their life cycle generally consists of three major phases (Bush et al. 2001). Schizogony is an asexual phase in which multiple mitoses take place, resulting in daughter cells. Schizogony occurs within the vertebrate host and causes disease by destroying host erythrocytes (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*) or leucocytes (*Leucocytozoon*, *Hepatozoon*). The remaining phases of the life cycle, gametogony and sporogony, occur within an arthropod vector and result in the production of infective sporozoites. Transmission can occur through different hematophagous arthropods. *Plasmodium* is transmitted by mosquitoes (Culicidae) and less commonly by sandflies (Psychodidae). *Haemoproteus* is transmitted by louse flies (Hippoboscidae) and biting midges (Ceratopogonidae) and *Leucocytozoon* by blackflies (Simuliidae). *Hepatozoon* is most commonly transmitted by ticks (Ixodidae, Smith 1996).

The prevalence of infection varies greatly among different bird orders (e.g., Bennett et al. 1993a, b; Valkiūnas 2005), but the reasons for interspecific variation in parasite prevalence or diversity are still poorly understood (Scheuerlein and Ricklefs 2004). Absence or scarcity of blood parasites has been reported from avian groups such as seabirds (e.g., Peirce and Brooke 1993; Merino et al. 1997a; Merino and Minguez 1998; Engström et al. 2000), swifts (Tella et al. 1995), waders (Figuerola et al. 1996), raptors (e.g., Tella et al. 1996), and parrots (Masello et al. 2006). There is also some evidence that blood parasites are less common in certain habitats such as the Arctic tundra (e.g., Bennett et al. 1992), arid environments (e.g., Little and Earlé 1995; Valera et al. 2003), island environments (e.g., Little and Earlé 1994), or marine environments (e.g., Piersma 1997; Figuerola 1999; Jovani et al. 2001).

Most previous studies used blood smears to detect blood parasites. However, it has been suggested that blood smears

may not detect parasites if the intensity is low (e.g., *Plasmodium* and *Haemoproteus* spp. during early spring; Valkiūnas 2005). Genetic methods, using sensitive polymerase chain reaction (PCR), 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; Parker et al. 2006; Merino et al. 2008). However, false negatives are not completely excluded by molecular methods (Gomez-Diaz et al. 2010). Furthermore, molecular methods are still inaccurate for the identification of the parasite species and should be combined with light microscopy (Valkiūnas et al. 2008).

In the present study, we used genetic methods to detect parasites in a suite of 14 seabird species, ranging from Antarctica to the tropical Indian Ocean. Specifically, we aimed to validate the hypothesis that hemosporidian blood parasite prevalences are low among seabirds and especially in cold climates.

Methods

Study species

Wilson's storm-petrels *Oceanites oceanicus* were sampled in their Antarctic breeding grounds at King George Island (62°14' S, 58°40' W), during fieldwork described in detail previously (summarized in Quillfeldt 2004; Büßer et al. 2008; Gladbach et al. 2009). Out of previously collected blood samples (breeding season 1999–2000), we randomly selected samples of six males and six females for the present analysis. We observed no biting insects at the breeding grounds of Wilson's storm-petrels, the only ectoparasites found being the Mallophaga *Philoceanus robertsi* (Phthiraptera: Ischnocera; Quillfeldt et al. 2004a).

Subantarctic species were sampled at New Island, Falkland Islands (51°43' S, 61°17' W). We included samples of two seasons (2007–2008 and 2008–2009) into the analysis for thin-billed prions *Pachyptila belcheri*, imperial shags *Phalacrocorax atriceps*, and southern rockhopper penguins *Eudyptes chrysocome*. General fieldwork techniques have been described in detail previously for thin-billed prions (e.g., Quillfeldt et al. 2003, 2007a, b, 2008) and southern rockhopper penguins (e.g., Poisbleau et al. 2008). Additionally, in 2008–2009, we collected samples from dolphin gulls *Larus scoresbii*, Magellanic penguins *Spheniscus magellanicus*, and gentoo penguins *Pygoscelis papua*. All adult birds in New Island were sampled at their nest when attending young chicks. Adults were captured by hand, blood-sampled and measured (for sample sizes, see Table 1). Potential arthropod vectors in the New Island seabird community include fleas *Parapsyllus* sp. that are common in nest burrows of thin-billed

Table 1 Data on the bird species sampled in the present study

Species	Scientific name	Breeding site	Number	Parasites found	Prevalence ^a
Antarctic					
Wilson's storm-petrel	<i>Oceanites oceanicus</i>	King George Island (62°14' S, 58°40' W)	12 (6f, 6m)	–	–
Subantarctic					
Rockhopper penguin	<i>Eudyptes chrysocome</i>	New Island (51°43' S, 61°17' W)	28 (14f, 14m)	–	–
Magellanic penguin	<i>Spheniscus magellanicus</i>	New Island (51°43' S, 61°17' W)	18 (9f, 6m, 2u)	–	–
Gentoo penguin	<i>Pygoscelis papua</i>	New Island (51°43' S, 61°17' W)	15 (9f, 6m)	–	–
Thin-billed prion	<i>Pachyptila belcheri</i>	New Island (51°43' S, 61°17' W)	28 (12m, 16f)	<i>Plasmodium</i> (N=1)	3.6±6.9%
Imperial shag	<i>Phalacrocorax atriceps</i>	New Island (51°43' S, 61°17' W)	25 (12m, 13f)	–	–
Dolphin gull	<i>Larus scoresbii</i>	New Island (51°43' S, 61°17' W)	20 (10m, 10f)	<i>Haemoproteus</i> (N=1)	5±9.5%
Temperate					
Cory's shearwater	<i>Calonectris diomedea</i>	Berlenga Island (39°24' N, 9°30' W)	15 (7f, 8m) ^b	–	–
Manx shearwater	<i>Puffinus puffinus</i>	Skomer Island (51°44' N, 5°17' W)	12 (6f, 6m)	–	–
Tropical					
Abbott's booby	<i>Papasula abbotti</i>	Christmas Island (10°25' S, 105°40' E)	12 (6f, 6m)	–	–
Red-footed booby	<i>Sula sula rubripes</i>	Christmas Island (10°25' S, 105°40' E)	12 (6f, 6m)	–	–
Brown booby	<i>Sula leucogaster plotus</i>	Christmas Island (10°25' S, 105°40' E)	12 (6f, 6m)	–	–
Red-tailed tropicbird	<i>Phaethon rubricauda</i>	Christmas Island (10°25' S, 105°40' E)	12 (6f, 6m)	–	–
Christmas Island frigatebird	<i>Fregata andrewsi</i>	Christmas Island (10°25' S, 105°40' E)	9 (6f, 3m)	<i>Haemoproteus</i> (N=5)	55.6±32.46%

m males, f females, u unknown sex

^a Prevalence ±95% confidence interval

^b Chick samples (age 18 to 36 days) of Cory's shearwater, all other samples were from breeding adults

prions and Magellanic penguins, as well as in gentoo penguin colonies (De Meillon 1952). *Ixodes* sp. ticks are abundant in nest burrows of Magellanic penguins and in imperial shag nesting areas. Although rockhopper penguins breed close to the imperial shags, ticks are not usually observed on this species (PQ & JFM, pers. obs.).

Two temperate species were sampled. Cory's shearwater *Calonectris diomedea* chicks of 18 to 36 days of age were blood-sampled at Berlenga Island, Portugal (39°24' N, 9°30' W, see Quillfeldt and Masello 2004; Träger et al. 2006; Quillfeldt et al. 2007c) in August 2003. Manx shearwater *Puffinus puffinus* adults were blood-sampled at Skomer Island, Southwest Wales, UK (51°44' N, 5° 17' W) (e.g., Quillfeldt et al. 2004b; Hamer et al. 2006; Riou and Hamer 2010; Riou et al. 2010). Possible vectors include fleas (*Ornithopsylla laetitiae* in the Manx shearwater and *Xenopsylla gratioiosa* in Cory's shearwater); the trombiculid mite *Neotrumbicula autumnalis*, which is commonly found on Skomer; and *Ixodes* ticks (Brooke 1990; Gomez-Diaz et al. 2008).

Tropical species were sampled on Christmas Island (CI), Indian Ocean, Australia (10°25' S, 105°40' E). Samples were taken from July to September 2007 and additional samples of CI frigatebirds were collected in May 2008.

Adults of the ground-breeding brown booby and red-tailed tropicbird were caught on their nests using a 1.2-m-long noose pole. Nests of the tree breeding CI frigatebird, Abbott's booby, and red-footed booby were accessed by tree climbing, and birds were captured on their nests by hand. After sampling, adult brown boobies, Abbott's boobies, and red-tailed tropicbirds were put back onto their nests, whereas CI frigatebirds and red-footed boobies were released from the ground and returned onto their nests within 2–7 min. Vectors here include mosquitoes Culicidae which are abundant all over the island and at the colonies of all study species. Louse flies Hippoboscidae were found on individuals of all study species but ticks Ixodidae were mainly found on the ground-breeding brown booby and red-tailed tropicbird.

Sample collection

All adults were sampled during the breeding season, on their nest or in their nest burrow, when incubating eggs or attending young. Each bird was individually marked with numbered rings or electronic tags in penguins, to avoid re-sampling. For each bird, a drop of blood was obtained from the brachial vein and preserved in 80% ethanol for

DNA analyses. We used the same protocol in 2008–2009 except that samples were stored on FTA classic cards (Whatman International Ltd. UK).

DNA analyses

Blood samples preserved in ethanol were centrifuged at 13,000×g for 15 min. Afterwards, the supernatants were discarded and the pellets resuspended in distilled water, frozen, and lyophilized to remove ethanol. Genomic DNA from lyophilized samples was extracted using the following protocol: dried samples were resuspended in 250 μL of SET buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH=8), and SDS 20% (7 μL) and proteinase K (50 mg) were immediately added to the vials, maintaining the mix in an incubating shaker at 55°C overnight. The following day, ammonium acetate 4 M (250 μL) was added to the vials at room temperature for 30 min. Subsequently, vials were centrifuged at 13,000×g for 10 min. After removing the pellet, DNA was precipitated with ethanol and resuspended in sterile water.

Partial amplification of the cytochrome b gene was accomplished by PCR using the non-specific primers PALU-F (5'-GGGTCAAATGAGTTTCTGG-3') and PALU-R (5'-DGGAACAATATGTARAGGAGT-3') for detection of *Haemoproteus/Plasmodium* species (see Martínez et al. 2009) and primers LDLd (5'-CATTCYACWGGTGCATCTTT-3') and LDRd (5'-CTGGATGWGATAATGGWGCA-3') for detection of *Leucocytozoon* (Merino et al. 2008).

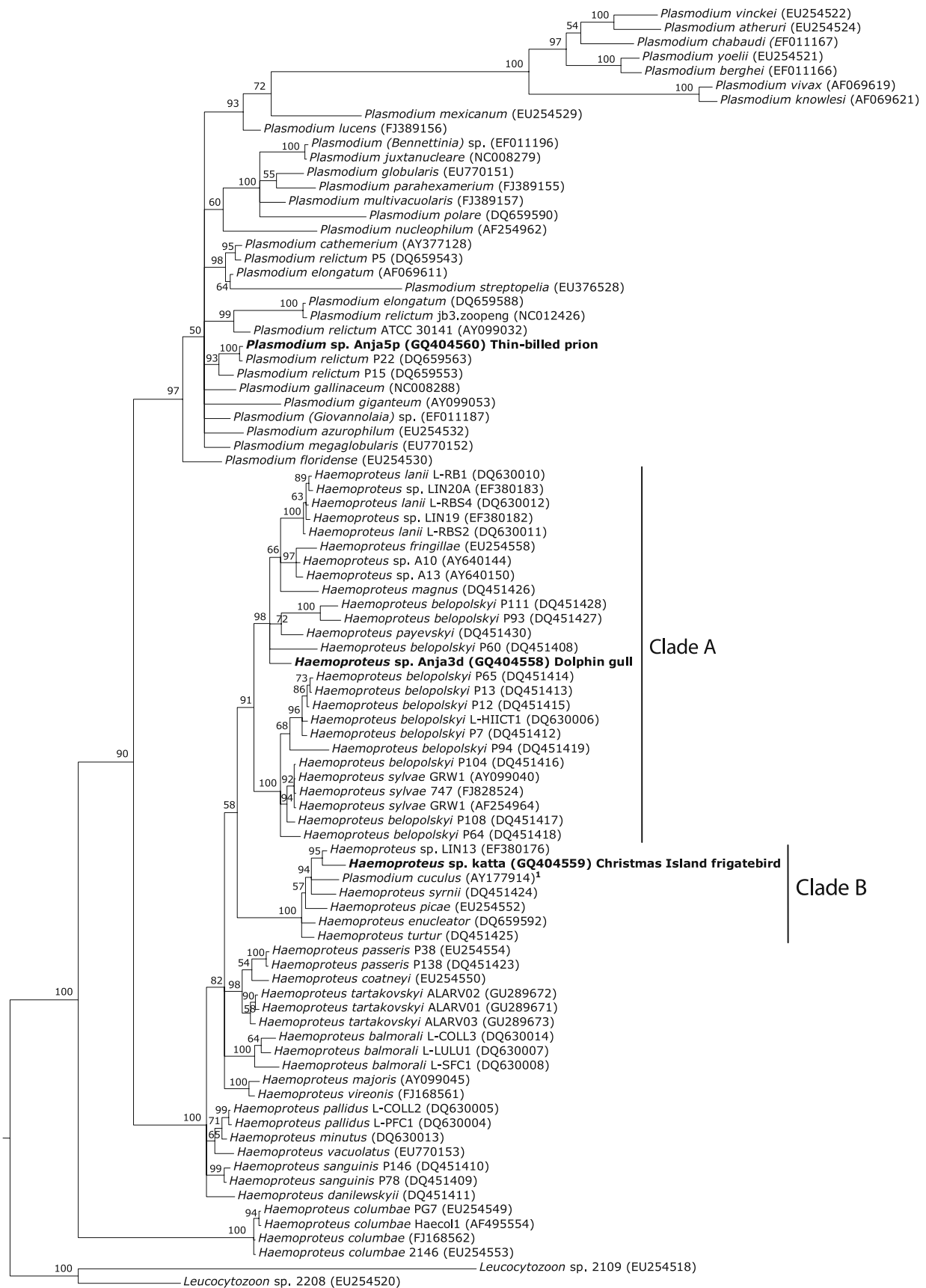
PCR reactions consisted of 25-μL reaction volumes containing 20 ng template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 MgCl₂, 0.2 mM of each dNTP, 0.5 μM of each primer and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The reactions were cycled at the following parameters using a thermal cycler (MasterCycler Personal, Eppendorf): 94°C for 10 min (polymerase activation), 40 cycles at 95°C for 40 s, annealing temperature for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. The annealing temperature was selected at 54°C and 58°C for primers PALU-F/PALU-R and LDLd/LDRd, respectively. All the DNA fragments obtained after PCR assays were recovered from agarose gels and subjected to direct sequencing. DNA fragments obtained were sequenced using an ABI 3130 (Applied Biosystems) automated sequencer. For potential cases of under-amplification (i.e., weak signal obtained), we repeated the PCR procedure under the same conditions. All of these samples were considered as negative because the second amplification failed to render a positive band. Thus, all samples rendering a clear PCR product were sequenced and identified to genus. A sample previously identified as infected by both methods of blood smear and molecular sequencing was included at each PCR as a positive control.

Fig. 1 Phylogenetic inference of the *Plasmodium* and *Haemoproteus* lineages found in thin-billed prions *Pachyptila belcheri* (Anja5p), Christmas Island frigatebirds *Fregata andrewsi* (katta), and dolphin gulls *Larus scoresbii* (Anja3d). Phylogenetic tree was obtained with the program MrBayes v3.2 using the substitution model GTR+I+G that was previously selected by means of jModelTest (see “Methods”). Accession numbers are indicated between parentheses and the three lineages isolated in the present study are marked in bold. *Superscripted number 1—Plasmodium cuculus* is not a valid species and still needs taxonomic description. Based on the present phylogeny, it is probably a *Haemoproteus* species

The three DNA sequences corresponding to *Haemoproteus* or *Plasmodium* lineages obtained from marine birds were aligned together with the other 84 sequences belonging to identified *Haemoproteus* or *Plasmodium* species previously listed in GenBank. The alignments were performed using the CLUSTALW algorithm implemented in BIOEDIT program (Hall 1999). The final alignment contained 765 positions and 89 taxa including two lineages of *Leucocytozoon* as outgroup. The alignments were analyzed using Bayesian inference implemented in the program MrBayes v3.2 (Ronquist and Huelsenbeck 2003) setting the substitution model GTR+I+G. The model was previously selected using corrected AIC implemented in jModelTest 0.0.1 (Posada 2008). Instead of setting the parameters I (proportion of invariant sites) and G (α parameter of the gamma distribution) obtained from jModelTest, they were estimated by the MrBayes program selecting the “invgamma” option from the command “rates” as suggested by the authors of the program. Metropolis-coupled Markov chain Monte Carlo analyses were run for 20,000,000 generations and sampled every 1,000 generations. At the end of the analysis, we set the burn-in period to 50% where the chains reached stationary phase. The convergence of the parameters and topology was tested using the TRACER (Rambaut and Drummond 2007) and AWTY (Nylander et al. 2008; Wilgenbusch et al. 2004) applications, respectively. In both cases, the statistical data and graphs did not show lack of convergence. The consensus tree was estimated using 20,000 trees. Estimates of evolutionary divergence were conducted using the uncorrected p-distance test using the pair-wise deletion option implemented in MEGA4 (Tamura et al. 2007).

Microscopic analyses

A drop of blood from each bird was immediately smeared and air-dried, fixed with methanol, and later stained with Giemsa stain (1/10 v/v) for 30 min. Blood smears from birds found positive for blood parasite infections by molecular analyses were scanned using an optic microscope (Olympus BX41). Parasites were searched at 100× under oil amplification following the methods described in Merino et al. (1997b).



0.2

Results

Parasites detected

We did not detect parasites in 11 of the 14 species, including one Antarctic, four subantarctic, two temperate, and four tropical species. Blood parasites were found in two species from subantarctic areas: one of 20 dolphin gulls (a female) was infected with *Haemoproteus* (GenBank accession number GQ404558) and one of 28 thin-billed prions (a female) was infected with *Plasmodium* (GenBank accession number GQ404560). The prevalence and the 95% confidence interval of gulls and prions were $5.0 \pm 9.5\%$ and $3.6 \pm 6.9\%$, respectively. However, we did not detect hemoparasites in the blood smears of the infected dolphin gull or the infected thin-billed prion (two smears from each of these birds were scanned in search of blood parasites).

Only one tropical species had higher prevalence. Five of nine Christmas Island frigatebirds ($55.6 \pm 32.46\%$) were infected with *Haemoproteus* (GenBank accession number GQ404559).

Phylogenetic analysis

Bayesian inference indicated that the *Plasmodium* isolated (Anja5p) from thin-billed prion blood forms a monophyletic clade (support of 93%) together with the lineages P22 and P15 identified as *Plasmodium relictum* (Fig. 1). The *Plasmodium* lineages P22 and Anja5p were identical and the genetic distance between *Plasmodium* lineages Anja5p and P15 was 2.2%. The *Haemoproteus* isolated from dolphin gull blood (Anja3d), in contrast, was clustered into a well-supported (91%) monophyletic group (named clade A) containing diverse *Haemoproteus* species (from *Haemoproteus lanii* L-RB1 to *Haemoproteus belopolskyi* P64, see Fig. 1). The genetic distance between Anja3d isolated and the other *Haemoproteus* species included in clade A ranged between 2.7% for A10 *Haemoproteus* isolate and 7.7% for P65 and P13 isolates identified as *H. belopolskyi* (Fig. 1). Finally, the *Haemoproteus* line from Christmas Island frigatebird blood (katta isolate) formed a monophyletic group (named clade B and with support of 100%) with *Haemoproteus* species isolated from non-passeriform birds (*Haemoproteus syrni*, *Haemoproteus picae*, *Haemoproteus enucleator*, and *Haemoproteus turtur*). The LIN13 lineage isolated from Eastern Asian black-tailed gulls *Larus crassirostris* was the closer sequence grouped with the katta lineage and the genetic distance between them was 1.4% (Fig. 1). Finally, the clusters where the *Haemoproteus* isolates from Christmas Island frigatebird (katta) and dolphin gull (Anja3d) are found are sister groups (Fig. 1).

Discussion

Haemoproteus

Haemoproteus are globally distributed in birds with about 100 named species (Peirce 2005) and within seabirds *Haemoproteus* parasites are especially common in frigatebirds (Lowery 1971; Work and Rameyer 1996; Padilla et al. 2006) and gulls (Peirce 1981; Ruiz et al. 1995; Bosch et al. 1997; Padilla et al. 2006). Thus, our present findings of a *Haemoproteus* infection in a frigatebird and a gull are in agreement with this pattern. The *Haemoproteus* isolate from Christmas Island frigatebird (katta) appears to have evolved from an original ancestor specialized in non-passeriform species. The close relation with the *Haemoproteus* lineage found in *L. crassirostris* points to a posterior specialization phenomenon involving marine birds (Fig. 1). However, the only *Haemoproteus* infection detected in *L. scoresbii* was clustered with parasite lineages isolated from birds belonging to Passeriformes. The infection was undetected in blood smears, implying a very low intensity of infection. This fact, along with the result of the phylogenetic analysis which groups this parasite species with those infecting passeriform birds (Fig. 1), suggests that it may be a paratenic or cross infection still not totally, or recently established in this bird (see Beadell et al. 2004; Merino et al. 2008).

In general, *Haemoproteus* parasites are considered relatively benign in birds. Two seabird species affected by *Haemoproteus* have been studied in detail. In magnificent frigatebirds *Fregata magnificens*, 16% of the males were infected with *Haemoproteus iwa*, but all infections were light (<1% of erythrocytes) and were thus classified as chronic (Madsen et al. 2007). In yellow-legged gulls, Martínez-Abraín et al. (2002) found significant differences in *Haemoproteus lari* prevalence between two breeding colonies. However, the birds of both colonies were in equally good body condition and had similar clutch sizes. Further, the intensity of *H. lari* infection was not correlated with body condition or egg volume (Martínez-Abraín et al. 2002), suggesting that *H. lari* parasites had little effect on the health of the gulls under normal conditions.

Studies in other birds also suggest that *Haemoproteus* numbers are normally kept low by natural immunity and only tend to multiply under stress, including other diseases. It has therefore been suggested that increasing *Haemoproteus* parasitemia can serve as a valuable indicator of an underlying disease (e.g., Remple 2004) or stressful conditions (Valkiūnas 2005). However, experimental studies suggest important detrimental effects of *Haemoproteus* blood parasites on bird fitness (Merino et al. 2000; Marzal et al. 2005). In magnificent frigatebirds *F. magnificens*, males infected with *H. iwa* had a less intensely colored red

inflatable gular pouch (Madsen et al. 2007), which is an important ornament used in mate choice. This suggests that even light infections can influence the reproductive success of individuals and thus be subject to intense selection.

Plasmodium

Bennett et al. (1993a) listed 34 species of *Plasmodium* in birds. Although avian *Plasmodium* and their vectors are distributed worldwide except in extreme habitats, their prevalence is much lower than that of other avian blood parasites such as *Haemoproteus* and *Leucocytozoon*. Only one species, *P. relictum*, has been detected in wild seabirds (Fantham and Porter 1944; Laird 1950). *P. relictum* is the most common species of *Plasmodium* in avian hosts, with a wide distribution both geographically and with respect to host phylogeny. Other species, *Plasmodium circumflexum* and *Plasmodium cathemerium*, have been associated with mortality in captive penguins in zoos (Bennett et al. 1993b), but have not been reported in wild seabirds, to our knowledge.

In the present study, we detected a *Plasmodium* infection in one of 28 thin-billed prions, but no parasites were found in blood smears from that bird. On the one hand, the phylogenetic analysis suggested that this lineage belongs to *P. relictum* species because it is included within a monophyletic clade (support of 93%) together with two lines of *P. relictum* isolated in birds belonging to Passeriformes (P22 and P15). In fact, the mean genetic divergence between the *Plasmodium* isolated from thin-billed prion (Anja5p) and the P22 *Plasmodium* isolate is 0%. However, other *P. relictum* isolates displayed on the phylogenetic tree are not clustered into the same monophyletic clade, showing genetic distances as high as 7.6% between isolates. Taking into account that (a) Perkins (2000) suggested that 3% was a reasonable level of specific differentiation, based primarily on differentiation among vertebrate species, and (b) named species of malaria parasite that infect primates differ by as little as 1% sequence divergence (Escalante et al. 1998), the value of genetic distance obtained suggests the occurrence of different genetic species under the same parasite morpho-species. On the other hand, the very low prevalence and intensity of infection suggest this to be a secondary origin, as has been suggested to occur in some ancient birds belonging to Procellariiformes, Pelecaniformes, and Sphenisciformes orders (e.g., Valkiūnas 2005). Thus, the infection might have passed to thin-billed prion from another species and is at present not a well-established parasite for this host species.

Given the highly pelagic distribution of thin-billed prions, an infection would most likely have occurred during the breeding season, when the breeding birds come ashore. There is a range of possible hosts of hemoparasites at New Island, where the source of the *Plasmodium* isolated from thin-billed prions might be found. These include migratory

and resident waders, songbirds, and several duck and goose species, as well as other seabirds breeding at New Island which were not sampled (e.g., kelp gulls *Larus dominicanus* and black-browed albatross *Thalassarche diomedea*).

Outlook

In a recent review regarding the apparent lack of blood parasites in some avian species, Martínez-Abraín et al. (2004) discussed the main hypotheses to explain this absence: (1) the absence or scarcity of proper vectors, (2) a highly specific association between host and parasites with host switching being infrequent (host–parasite assemblage), (3) host immunological capabilities preventing infection by parasites, and (4) competitive exclusion of blood parasite vectors mediated by ectoparasites.

In line with hypothesis (1), blood parasites are generally absent from Antarctic and arctic seabirds, whereas in milder climates, about one third of the seabird species studied so far had hemoparasites. This may be due partly to the absence of appropriate vectors in saline environments (Figuerola 1999). Barbosa and Palacios (2009) noted that the two blood parasites present in sub-Antarctic islands, are absent in Antarctica and suggested that this mirrors the absence of suitable vectors in Antarctica (Merino et al. 1997a). Latitudinal gradients in the prevalence of blood parasites infecting some bird species (Merino et al. 2008) suggest that latitude could also influence the presence of these parasites in seabirds, and more detailed analyses on different geographic areas and bird families should be conducted in this regard.

Although our data are in agreement with previous studies supporting hypothesis (1), the confidence intervals of the computed prevalence suggest that the interpretation of these data should be performed only in a qualitative sense. Further studies should explore the relative importance of extrinsic and intrinsic factors on parasite prevalence, in particular using phylogenetically controlled comparative analyses, systematic sampling and screening of vectors, and within-species comparisons.

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