Detecting transmission areas of malaria parasites in a migratory bird species

LUZ GARCIA-LONGORIA[1]*, OLOF HELLGREN[2], STAFFAN BENSCH[2], FLORENTINO DE LOPE[1] *and* ALFONSO MARZAL[1]

[1] *Departamento de Biología Animal, Universidad de Extremadura, E-06071 Badajoz, Spain*
[2] *Department of Biology, Molecular Ecology and Evolution Lab, Ecology Building, Lund University, SE- 22362 Lund, Sweden*

(*Received 20 February 2015; revised 31 March 2015; accepted 8 April 2015; first published online 13 May 2015*)

SUMMARY

The identification of the regions where vector-borne diseases are transmitted is essential to study transmission patterns and to recognize future changes in environmental conditions that may potentially influence the transmission areas. SGS1, one of the lineages of *Plasmodium relictum*, is known to have active transmission in tropical Africa and temperate regions of Europe. Nuclear sequence data from isolates infected with SGS1 (based on merozoite surface protein 1 (MSP1) allelic diversity) have provided new insights on the distribution and transmission areas of these allelic variants. For example, MSP1 alleles transmitted in Africa differ from those transmitted in Europe, suggesting the existence of two populations of SGS1 lineages. However, no study has analysed the distribution of African and European transmitted alleles in Afro-Palearctic migratory birds. With this aim, we used a highly variable molecular marker to investigate whether juvenile house martins become infected in Europe before their first migration to Africa. We explored the MSP1 allelic diversity of *P. relictum* in adult and juvenile house martins. We found that juveniles were infected with SGS1 during their first weeks of life, confirming active transmission of SGS1 to house martins in Europe. Moreover, we found that all the juveniles and most of adults were infected with one European transmitted MSP1 allele, whereas two adult birds were infected with two African transmitted MSP1 alleles. These findings suggest that house martins are exposed to different strains of *P. relictum* in their winter and breeding quarters.

Key words: avian malaria, *Delichon urbica*, juveniles, MSP1, *Plasmodium relictum*.

INTRODUCTION

Parasites can regulate their host populations by reducing the fecundity or the survival of their host population, but our current knowledge of population regulation of hosts by parasites is still limited (see reviews in Møller, 2005; Schmid-Hempel, 2011). In the case of malaria and related haemosporidian parasites, most of the mortalities of infected birds normally occur during the acute phase of the parasite infection, which usually happens several days after the parasite transmission (Valkiūnas, 2005). Juvenile birds are especially susceptible for infection because they are immunologically naïve, which may drive to population decline (Samuel *et al*. 2011). However, the areas of transmission of these vector-borne diseases remain a key knowledge gap in our understanding of these pathogens. The use of new molecular tools could provide an essential knowledge to identify the regions where vector-borne diseases are transmitted in order to study the host population dynamics and to recognize future changes in environmental conditions that may potentially influence the transmission areas. Haemosporidians are among the most well studied blood parasites of reptiles, mammals and birds (Valkiūnas, 2005). Avian *Plasmodium* species show a cosmopolitan distribution, being found in all continents except Antarctica (Valkiūnas, 2005). To date, more than 50 morphospecies of avian malaria parasites of the genus *Plasmodium* have been described worldwide (Valkiūnas, 2005; Palinauskas *et al*. 2007). *Plasmodium relictum* is one of the most widespread and harmful parasite species of avian malaria, being responsible for mass mortality, population declines and even extinctions of many bird species (Van Riper III *et al*. 1986; Valkiūnas, 2005). For all these reasons and due to its devastating effects, the International Union for Conservation of Nature classifies *P. relictum* as one of the worst invasive species in the world (Lowe *et al*. 2000). Therefore, it becomes essential to identify the geographical distribution of *P. relictum* lineages and to assess their infection prevalence in birds in order to develop appropriate management strategies to promote biodiversity conservation policies worldwide.

With the use of mtDNA cytochrome b gene (cyt b) to barcode the parasites more than 500 avian *Plasmodium* parasite lineages have been identified (MalAvi database 2015-01-15) (Bensch *et al*. 2009). Moreover, four different cyt b lineages have been described within the morphologically described species of *P. relictum* (Palinauskas *et al*. 2007; Valkiūnas *et al*. 2007; Ilgunas *et al*. 2013; Kazlauskiene *et al*. 2013). Two of the

* Corresponding author: Departamento de Biología Animal, Universidad de Extremadura, E-06071 Badajoz, Spain. E-mail: luzlongoria@unex.es

doi:10.1017/S0031182015000487

P. relictum cyt b lineages (SGS1 and GRW4) are some of the most abundant and geographically widespread of all bird *Plasmodium* lineages. Both lineages are host generalists infecting 95 species of 28 families (SGS1) and 60 species in 19 families (GRW4) (MalAvi database 2015-01-15) (Bensch *et al*. 2009), respectively. The lineages SGS1 and GRW4 exhibit different transmission areas (Hellgren *et al*. 2007), with GRW4 being transmitted in New Zealand, Africa, Asia and America (Beadell *et al*. 2006; Marzal *et al*. 2011), whereas SGS1 shows a widespread distribution in Europe, Africa and Asia (Palinauskas *et al*. 2007). Recently, SGS1 was also detected in Oceania (Howe *et al*. 2012) and South America (Marzal *et al*. 2015). In consequence, SGS1 was suggested to be one of the few *Plasmodium* lineages with active transmission in both tropical Africa and temperate regions of Asia and Europe (Hellgren *et al*. 2007).

Investigations based on multiple nuclear loci of *P.relictum* have provided new insights into allelic variation, geographical structure and parasite transmission (Hellgren *et al*. 2013). The merozoite surface protein 1 (MSP1) is a gene which shows a high variability (Miller *et al*. 1993) and encodes a protein involved in the attachment of the malaria parasite to the red blood cell (Gerold *et al*. 1996). Because of its high variability, this gene is a good candidate for investigating population structure and phylogeography of malaria lineages. For example, the SGS1 lineage transmitted in tropical Africa has a different set of MSP1 alleles compared with those transmitted in Europe, suggesting the existence of separate SGS1 populations along the European-African migratory flyways (Hellgren *et al*. 2015). This pattern implies the existence of transmission barriers (e.g. vector communities or abiotic factors) limiting transmission between regions, but further studies are required to confirm this geographical distribution.

The house martin is a migratory species with a high fidelity to its area of hatching and nesting (Cramp and Perrins, 1994; Lope and Silva, 1998) and also to its wintering grounds (Ambrosini *et al*. 2011). This species migrates from Africa to Europe for breeding. Once the breeding is completed, adult house martins and new-born individuals migrate back to their African wintering quarters (Tumer and Rose, 1989; Cramp and Perrins, 1994). Previous studies in different localities of Europe and Northern Africa have found haemosporidian infections in more than 70% of adults (Marzal *et al*. 2008, 2013*a*, *b*; Piersma and van der Velde, 2012; Van Rooyen *et al*. 2014). Additionally, different *P. relictum* cyt b lineages such as SGS1, GRW4 and GRW11 have been found infecting adult house martins in these populations (Marzal *et al*. 2008, 2013*b*; Piersma and van der Velde, 2012; van Rooyen *et al*. 2014). These blood parasite infections are thought to be transmitted on the African wintering grounds or during migration. This assumption is based on the absence of haemosporidian infection in the single study analysing haemosporidian infections in 112 fledgling and juvenile house martins before their first migration (Piersma and van der Velde, 2012). However, the confirmation of transmission areas of haemosporidian parasites in house martins requires further investigation. Therefore, the first goal of our study was to determine whether haemosporidian transmission in house martins occurs at European sites by sampling juvenile birds. Additionally, the second objective of this study was to analyse the MSP1 alleles in *P. relictum* lineages infecting adult and juvenile house martins in order to identify their potential areas of transmission.

MATERIALS AND METHODS

Study site and collecting samples

The study was carried out in a colony of house martins in the surroundings of Badajoz (38° 50′ N, 6° 59′ W), southwest Spain, during a 6-year period (2006–2012) as part of a longer study. For the present study we captured 422 house martins, 310 of them were classified as juveniles according to the morphological characteristics established by Svensson *et al*. (2009) and Lope (1986). Most of the individuals were caught in July, at the end of their breeding season (Pajuelo *et al*. 1992). Therefore, the all of the juveniles included in this study were between 2 and 3 months old. All birds were individually identified with numbered metal rings. One microcapillary of blood (70 μL) was obtained from the brachial vein of each individual and stored in 500 μL of SET buffer (0·15 M NaCl, 0·05 Tris, 0·001 M EDTA, pH 8·0) until DNA extraction.

Molecular detection of blood parasite infections

Haemosporidian parasites (*Plasmodium* spp.) were detected from blood samples using molecular methods (Bensch *et al*. 2000; Waldenström *et al*. 2004). DNA from the avian blood samples were extracted in the laboratory using the standard phenol/chloroform/isoamylalcohol method (Sambrook *et al*. 2002). Diluted genomic DNA (25 ng/μL) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using nested PCR-protocols described by Waldenström *et al*. (2004). The amplification was evaluated by running 2·5 μL of the final PCR on a 2% agarose gel. All PCR experiments contained one negative control for every eight samples. In the very few cases of negative controls showing signs of amplification (never more than faint bands in agarose gels), the whole PCR-batch was run again to make sure that all positives were true. All positive amplifications were precipitated and sequenced in

https://doi.org/10.1017/S0031182015000487 Published online by Cambridge University Press

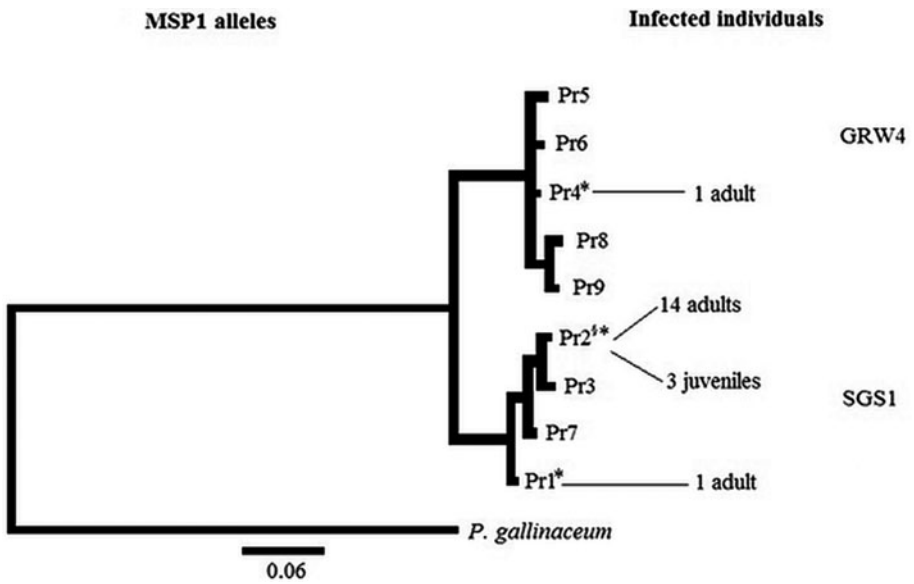

Fig. 1. Phylogenetic relationship between all the MSP1_b14 alleles detected to date (Hellgren *et al*. 2015) and number of individuals (adults or juveniles) infected by these alleles. * and § represent confirmed active transmission in Africa and Europe, respectively (Hellgren *et al*. 2015).

order to identify the species and lineage in each infection. The obtained sequences were edited, aligned and compared in a sequenced matrix using the program Bioedit (Hall, 1999). We selected SGS1 and GRW4 infected house martins for further analyses of the MSP1 gene (270 nucleotides, block 14) and detect the MSP1 allele following the protocol described by Hellgren *et al*. (2013) and using the primers MSP1_3F, MSP1_3R, MSP1_3FN and MSP1_3RN.

Phylogenetic reconstruction

The obtained sequences were edited, aligned against the SGS1_MSP1 gene (KC969175) and compared in a sequence identity matrix using the program BioEdit (Hall, 1999). The quality of the alignment was checked by manual inspection. Genetic differences between the MSP1 alleles were calculated using a Jukes-Cantor model as implemented in MEGA 5·2. We used MEGA 5·2 for phylogenetic reconstruction of the MSP1 alleles where the homolog sequence of *P. gallinaceum* (AJ809338·1) was used as an out-group. The phylogenetic tree for all the alleles found was constructed in the programme MEGA 5·2 and using a Maximum Likelihood model. Bootstrap values were used in order to obtain a consensus phylogeny using 200 iterations.

RESULTS

Prevalence of infection and genetic parasite diversity (Cyt b gene analyses)

We analysed 422 blood samples from adult ($N = 112$) and juvenile ($N = 310$) house martins in search for haemosporidian parasites. Among adults 80 (71%) individuals were infected with haemosporidian parasites. In juveniles only three were found to be infected (0·96%).

Of the 80 infected adult birds, 20% were infected with *Plasmodium* spp. and 80% were infected with *Haemoproteus* spp. We found five different blood parasite lineages infecting adult house martins, of which three were of the genus *Haemoproteus* (DELURB1: 32 infected birds; DELURB2: 29 infected birds; DELURB3: 3 infected bird), and two of them from the genus *Plasmodium* (SGS1: 15 infected birds; GRW4: 1 infected bird). The three infected juveniles were all infected with the *P. relictum* lineage SGS1.

Genetic parasite diversity (MSP1 gene analyses)

All the samples infected with *P. relictum* lineages ($N = 19$; 16 adults and 3 juveniles) were selected for further molecular analyses. From each sample, we obtained a 268 bp MSP1_ b14 fragment of high quality. We used a SGS1_MSP1 gene (KC969175) in order to confirm the amplification of the MSP1 block 14 (MSP1_b14). Within adults, 14 out of 16 individuals showed the allele Pr2 (SGS1), whereas one individual was infected with Pr1 (SGS1) and the other one was infected with Pr4 (GRW4) (Fig. 1). Moreover, we found the same allele (Pr2; SGS1) in all the juvenile house martins infected with malaria (Fig. 1).

DISCUSSION

In this study we analysed blood samples from adult and juvenile house martins in search for haemosporidian

https://doi.org/10.1017/S0031182015000487 Published online by Cambridge University Press

parasites. We showed, for the first time, that juvenile house martins become infected with *Plasmodium* parasites already before their first migration to Africa, thus confirming that active transmission of *Plasmodium* spp. to house martins occur in Europe. By analysing the MSP1 alleles in *P. relictum* lineages, we were able to get a more detailed view of the likely transmission areas for the infections found in the adult birds. Below, we will discuss the biological meaning of these results in detail.

House martins have been used in several studies to analyse life-history consequences of haemosporidian infections (e.g. Piersma and van der Velde, 2012; Marzal *et al*. 2013*a*, *b*; Van Rooyen *et al*. 2014). In our study, we show that 70% of adults were infected with haemosporidian parasites. This prevalence is similar to what has been reported from previous studies in this house martin population (Marzal *et al*. 2008, 2013*a*, *b*). Moreover, the prevalence of haemosporidian parasites among adult house martins greatly exceed the prevalence in juveniles. This difference may be explained by a higher mortality of young individuals during the infection, before being captured for sampling, due to their näive immune system (Sol *et al*. 2003) and/or the maintenance of haemosporidian infection in infected birds that survived the acute phase of infection (Valkiūnas, 2005). Alternatively, the juveniles may not yet have been exposed to infections, or have only recently been infected and in the phase when *Plasmodium* cryptozoites are developing in reticuloendothelial cells, and therefore absent in the blood stream (Valkiūnas, 2005).

Migratory birds are exposed to at least two different parasite communities during their annual cycle. Therefore, migratory species such as house martins could become infected by blood parasites during their breeding season in Europe and/or in their African winter quarters and at stop-over sites. Moreover, the parasites could be transported within the migratory bird and be able to infect resident birds in the new area. However, Hellgren *et al*. (2007) investigated the degree of geographical shifts of transmission of 259 haemosporidian parasite lineages. They showed that most of the parasite lineages are restricted to a specific area and thus dispersing from one biogeographical zone to another is a rare and slow evolutionary process. In line with the data presented by Hellgren *et al*. (2007), all the recent studies of haemosporidian parasites in house martins have assumed that these parasites are only actively transmitted on the African wintering grounds or during migration (Marzal *et al*. 2008, 2013*a*, *b*; Piersma and van der Velde, 2012; Van Rooyen *et al*. 2014). This assumption was supported by Piersma and van der Velde (2012) in a population of house martins in the Netherlands, where none of the analysed juveniles were infected with haemosporidian parasites. In contrast, we detected the presence of malaria parasites in juvenile house martins. As far as we know, this is the first documented case of active transmission of *Plasmodium* parasites in house martins in Europe. These findings give rise to new questions about the transmission areas of malaria parasites in this migratory bird species such as how many house martins can complete the migration despite the infection by malaria parasites.

The data on the distribution of nuclear MSP1 alleles across the cyt b lineages may facilitate the investigation on the distribution of these malaria parasites across geographical regions (Hellgren *et al*. 2013, 2015). A recent study has explored the global phylogeography of the *P. relictum* based on MSP1 allelic diversity, showing several different MSP1 alleles within the cyt b lineages of SGS1 and GRW4. In this study, we identified two MSP1 alleles from two cyt b lineages that are throught to be transmitted in Africa (allele Pr1 from lineage SGS1, and allele Pr4 from lineage GRW4), and one allele from a cyt b lineage thought to be confined to temperate regions (allele Pr2 from lineage SGS1) (Hellgren *et al*. 2015). This pattern suggests the existance of barriers limiting the tramission areas of these parasites. In the present study, we have shown that most of the adult birds infected with SGS1, as well as all the infected juveniles, carried the MSP1 allele with European transmission (Pr2) . Only one out of 15 adults was infected with a tropically transmitted SGS1 allele (MSP1 allele Pr1), while another one was infected with GRW4 (MSP1 allele Pr4) which is known to have transmission in the Afrotropics. These results indicate the existence of two different areas of transmission of malaria parasites for house martins population: one in the African winter range (Pr1 and Pr4), and the other in the European breeding range (Pr2). Moreover, the high number of house martins infected with the MSP1 allele Pr2 compared with the low number infected with the MSP1 African-transmitted alleles (Pr1 and Pr4) suggest that most of the malaria transmission takes place in Europe during the breeding season. This finding agrees with previous studies indicating that haemosporidian parasites are usually transmitted during the breeding season in temperate regions, because biotic and abiotic factors are optimal for the transmission of vector-borne diseases such as malaria (Githeko *et al*. 2000; Valkiūnas, 2005; Cosgrove *et al*. 2008; but also see Dunn *et al*. 2014). However, we cannot exclude that house martins frequently become infected in tropical Africa during the winter or at stop-over sites, but that such infected individuals fail to reach their European breeding quarters because they die during migration. In support of this hypothesis, several studies have shown that blood parasites may increase mortality in their avian hosts during stressful and

https://doi.org/10.1017/S0031182015000487 Published online by Cambridge University Press

energy-demanding periods such as migration (Davidar and Morton, 1993; Valkiūnas, 2005; Garvin *et al*. 2006).

In conclusion, we confirmed that active transmission of *P. relictum* (lineage SGS1) occurs in house martins in Europe. Additionally, we detected African and European MSP1 alleles in adult house martins, suggesting two different areas of transmission for the *P. relictum* SGS1 lineage in this migratory bird species. These findings emphasize the importance of using multiple independent loci of avian *Plasmodium* parasites to understand transmission areas of blood parasites. Further studies exploring the transmission and species limits of avian malaria parasites are needed to evaluate the importance of migratory birds in spreading haemosporidian infections.

ACKNOWLEDGEMENTS

We thank the numerous colleagues who contributed with the collection of the samples. In particular, we thank Maribel Reviriego, Carmen Relinque and Nacho García.

FINANCIAL SUPPORT

This study was funded by grants from the Spanish Ministry of Economy and Competition (A.M., F.D.L. and L.G.L., CGL2009-08976 and CGL2012-36665) and the Regional Government of Extremadura (A.M., F.D.L. and L.G.L., GRU: 10134). Luz Garcia-Longoria was supported by a PhD grant from Ministry of Economy and Competition of Spain. The Crafoord foundation (grant 20120630), and the Swedish research council (grant 621-2011-3548) to OH.

REFERENCES

Ambrosini, R., Orioli, V., Massimino, D. and Bani, L. (2011). Identification of putative wintering areas and ecological determinants of population dynamics of common house-martin (*Delichon urbicum*) and common swift (*Apus apus*) breeding in Northern Italy. *Avian Conservation and Ecology* **6**, 3.

Beadell, J. S., Ishtiaq, F., Covas, R., Melo, M., Warren, B. H., Atkinson, C. T., Bensch, S., Graves, G. R., Jhala, Y. V., Peirce, M. A., Rahmani, A. R., Fonseca, D. M. and Fleischer, R. C. (2006). Global phylogeographic limits of Hawaii's avian malaria. *Proceedings. Biological Sciences/The Royal Society* **273**, 2935–2944.

Bensch, S., Stjernman, M., Hasselquist, D., Ostman, O., Hansson, B., Westerdahl, H. and Pinheiro, R. T. (2000). Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. Proceedings. *Biological Sciences/The Royal Society* **267**, 1583–1589.

Bensch, S., Hellgren, O. and Pérez-Tris, J. (2009). MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Molecular Ecology Resources* **9**, 1353–1358.

Cosgrove, C. L., Wood, M. J., Day, M. K. P. and Sheldon, B. C. (2008). Seasonal variation in Plasmodium prevalence in a population of blue tits *Cysnistes caeruleus*. *Journal of Animal Ecology* **77**, 540–548.

Cramp, S. and Perrins, C. M. (1994). *Handbook of the Birds of Europe, the Middle East, and North Africa: The Birds of the Western Palearctic Volume VIII: Crows to Finches*. Oxford, Oxford University Press.

Davidar, P. and Morton, E. S. (1993). Living with parasites: prevalence of a blood parasite and its effect on survivorship in the Purple Martin. *Auk* **110**, 116–119.

Dunn, J. C., Goodman, S. J., Benton, T. G. and Hamer, K. C. (2014). Active blood parasite infection is not limited to the breeding season in a declining farmland bird. *The Journal of Parasitology* **100**, 260–266.

Garvin, M. C., Szell, C. C. and Moore, F. R. (2006). Blood parasites of Nearctic-Neotropical migrant passerine birds during spring trans-Gulf migration: impact on host body condition. *The Journal of Parasitology* **92**, 990–996.

Gerold, P., Schofieldb, L., Blackman, M. J., Holder, A. A. and Schwarz, R. T. (1996). Structural analysis of the glycosyl-phosphatidylinositol membrane anchor of the merozoite surface proteins-1 and -2 of *Plasmodium falciparum*. *Molecular and Biochemical Parasitology* **75**, 131–143.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E. and Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization* **78**, 1136–1147.

Hall, T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hellgren, O., Waldenström, J., Peréz-Tris, J., Szöll Ösi, E., Hasselquist, D., Krizanauskiene, A., Ottosson, U. and Bensch, S. (2007). Detecting shifts of transmission areas in avian blood parasites - a phylogenetic approach. *Molecular Ecology* **16**, 1281–1290.

Hellgren, O., Kutzer, M., Bensch, S., Valkiunas, G. and Palinauskas, V. (2013). Identification and characterization of the merozoite surface protein 1 (msp1) gene in a host-generalist avian malaria parasite, *Plasmodium relictum* (lineages SGS1 and GRW4) with the use of blood transcriptome. *Malaria Journal* **12**, 381.

Hellgren, O., Carter, A., Bensch, S., Albayrak, T., Dimitrov, D., Ewen, J., Kim, K. S., Lima, M., Lynn, M., Palinauskas, V., Ricklefs, R. E., Sehgal, R. N. M., Valkiūnas, G., Tsuda, Y. and Marzal, A. (2015) Global phylogeography of the avian malaria pathogen *Plasmodium relictum* based on MSP1 allelic diversity. *Ecography* **28**, 001–009.

Howe, L., Castro, I. C., Schoener, E. R., Hunter, S., Barraclough, R. K. and Alley, M. R. (2012). Malaria parasites (*Plasmodium* spp.) infecting introduced, native and endemic New Zealand birds. *Parasitology Research* **110**, 913–923.

Ilgunas, M., Palinauskas, V., Iezhova, T. A. and Valkiunas, G. (2013). Molecular and morphological characterization of two avian malaria parasites (Haemosporida: Plasmodiidae), with description of Plasmodium homonucleophilum n. Sp. *Zootaxa* **3666**, 49–61.

Kazlauskiene, R., Bernotiene, R., Palinauskas, V., Iezhova, T. A. and Valkiunas, G. (2013). Plasmodium relictum (lineages pSGS1 and pGRW11): Complete synchronous sporogony in mosquitoes Culex pipiens pipiens. *Experimental Parasitology* **133**, 454–461.

Lope, F. de. (1986). La biometría del avión común (*Delichon urbica urbica L.*) en Badajoz, España. *Ardeola* **33**, 171–176.

Lope, F. de. and Silva, E. da. (1998). La fidelidad al lugar de nidificación o de nacimiento en el avión común (*Delichon urbica urbica L.*) en Badajoz, España. *Ardeola* **39**, 51–58.

Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. (2000). 100 of the world's worst invasive alien species a selection from the global invasive species database. *The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN)* **12**, 12.

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J. and De Lope, F. (2008). Effects of malaria double infection in birds: one plus one is not two. *Journal of Evolutionary Biology* **21**, 979–87.

Marzal, A., Ricklefs, R. E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirják, G. A., Ewen, J., Hellgren, O., Hořáková, D., Iezhova, T. A., Jensen, H., Križanauskienė, A., Lima, M. R., De Lope, F., Magnussen, E., Martin, L. B., Møller, A. P., Palinauskas, V., Pap, P. L., Pérez-Tris, J., Sehgal, R. N. M., Soler, M., Szöllősi, E., Westerdahl, H., Zetindjiev, P. and Bensch, S. (2011). Diversity, loss, and gain of malaria parasites in a globally invasive bird. *PLoS ONE* **6**, 8.

Marzal, A., Reviriego, M., Hermosell, I. G., Balbontín, J., Bensch, S., Relinque, C., Rodríguez, L., Garcia-Longoria, L. and de Lope, F. (2013a). Malaria infection and feather growth rate predict reproductive success in house martins. *Oecologia* **171**, 853–61.

Marzal, A., Asghar, M., Rodríguez, L., Reviriego, M., Hermosell, I. G., Balbontín, J., Garcia-Longoria, L., de Lope, F. and Bensch, S. (2013b). Co-infections by malaria parasites decrease feather growth but not feather quality in house martin. *Journal of Avian Biology* **44**, 437–444.

Marzal, A., García-Longoria, L., Cárdenas Callirgos, J. M. and Sehgal, R. N. (2015). Invasive avian malaria as an emerging parasitic disease in native birds of Peru. *Biological Invasions* **17**, 39–45.

Miller, L. H., Roberts, T., Shahabuddin, M. and McCutchan, T. F. (1993). Analysis of sequence diversity in the *Plasmodium falciparum* merozoite surface protein-1 (MSP-1). *Molecular and Biochemical Parasitology* **59**, 1–14.

Møller, A. P. (2005). Parasitism and the regulation of host populations. *Parasitism and Ecosystems* **75**, 43–43.

Pajuelo, L., de Lope, F. and da Silva, E. (1992). Breeding biology of the House Martin (*Delichon urbica*) in Badajoz W Spain. *Ardeola* **39**, 15–23.

Palinauskas, V., Kosarev, V., Shapoval, A., Bensch, S. and Valkiūnas, G. (2007). Comparison of mitochondrial cytochrome b lineages and morphospecies of twoavian malaria parasites of the subgenera *Haemamoeba* and *Giovannolaia* (*Haemosporida*: Plasmodiidae). *Zootaxa* **1626**, 39–50.

Piersma, T. and van der Velde, M. (2012). Dutch House Martins *Delichon urbicum* gain blood parasite infections over their lifetime, but do not seem to suffer. *Journal of Ornithology* **153**, 907–912.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (2002). *Molecular Cloning: A Laboratory Manual*. Cold Spring, New York.

Samuel, M. D., Hobbelen, P. H. F., Decastro, F., Ahumada, J. A., Lapointe, D. A., Atkinson, C. T., Woodworth, B. L., Hart, P. J. and Duffy, D. C. (2011). The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: a modeling approach. *Ecological Applications* **21**, 2960–2973.

Schmid-Hempel, P. (2011). *Evolutionary parasitology*: *The integrated study of infections, immunology, ecology and genetics*. Oxford, Oxford University Press.

Sol, D., Jovani, R. and Torres, J. (2003). Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. *Oecologia* **135**, 542–547.

Svensson, L., Grant, P. J. and Mullarney, K. (2009). *Collins Bird Guide*. HarperCollins, London.

Turner, A. K. and Rose, C. (1989). *Swallows and Martins: An Identification Guide and Handbook*. (ed Taylor, F. J. R.) Christopher Helm, London.

Valkiūnas, G. (2005). *Avian Malaria Parasites and other Haemosporidia*. CRC Press, Boca Raton.

Valkiūnas, G., Zehtindjiev, P., Hellgren, O., Ilieva, M. and Iezhova, T. B. S., (2007). Linkage between mitochondrial cytochrome b lineages and morphospecies of two avian malaria parasites, with a description of *Plasmodium* (Novyella) ashfordi sp. nov. *Parasitology Research* **100**, 1311–1322.

Van Riper III, C., Van Riper, S. G., Goff, M. L. and Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**, 327–344.

Van Rooyen, J., Jenkins, T., Lahlah, N. and Christe, P. (2014). North-african house martins endure greater haemosporidian infection than their european counterparts. *Journal of Avian Biology* **45**, 001–007.

Waldenström, J., Bensch, S., Hasselquist, D. and Ostman, O. (2004). A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *The Journal of Parasitology* **90**, 191–194.

https://doi.org/10.1017/S0031182015000487 Published online by Cambridge University Press