J Ornithol (2012) 153:1253–1259
DOI 10.1007/s10336-012-0860-0

ORIGINAL ARTICLE

Local haemoparasites in introduced wetland passerines

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Received: 7 March 2012 / Revised: 30 April 2012 / Accepted: 2 May 2012 / Published online: 24 May 2012

Abstract When colonizing a new area, introduced species may lose their original haemoparasites. If the local parasites are unable to infect the novel introduced hosts, these may gain a fitness advantage over their local competitors. Alternatively, the introduced species may be susceptible to local parasites and enter the local transmission dynamics. We studied these two possibilities in communities of wetland passerines infected with haemosporidians (genera *Haemoproteus* and *Plasmodium*) in Portugal, southwest Europe. Four introduced and six native (resident and breeding migrant) passerine species were tested for haemosporidians in four reed beds. Our results suggest that the introduced species have lost their original haemoparasites upon colonization and entered the local transmission cycle. Two local *Plasmodium* lineages infected the exotic species: one of them (SGS1) was the most host generalist and prevalent lineage in the native species, so was expected to be present in the exotics at random. The other lineage (PADOM01) was rarer in the sampled community, but was present in native hosts that are phylogenetically close to the infected exotic species; therefore, the colonization of the exotic host by PADOM01 seems to be constrained by the parasite's specialization and by phylogenetic factors. When phylogeny was controlled for, there were no significant differences in infection prevalence and number of lineages between exotics and natives.

Keywords *Plasmodium* · *Haemoproteus* · Haemosporidioses · Avian malaria parasites · Introduced birds · Exotic species

Zusammenfassung

Lokale Blutparasiten bei neu zugezogenen Sperlingsvögeln in Feuchtgebieten

Neu eingebürgerte Arten verlieren möglicherweise ihre originalen Blutparasiten, wenn sie ein neues Gebiet besiedeln. Sind die örtlichen Parasiten nicht in der Lage, die neu zugezogenen Wirte zu infizieren, gewinnen diese womöglich einen Fitness-Vorteil gegenüber ihren ortsansässigen Konkurrenten. Andererseits sind die neuen Arten vielleicht aber auch empfänglich für die örtlichen Parasiten und geraten dann in die örtliche Übertragungs-Dynamik. Wir untersuchten diese beiden Alternativen bei Gruppen von Feuchtgebiets-Sperlingsvögeln in Portugal, Südwest-Europa, die mit Haemosporidien (*Haemoproteus* und *Plasmodium*) infiziert waren. In vier Schilfgürteln wurden vier neu angesiedelte sowie sechs lokale (ortsansässige und brütende Zugvögel) Sperlingsvogelarten auf Haemosporidien getestet. Unsere Ergebnisse legen nahe, dass die neu zugezogenen Arten nach der Besiedelung ihre ursprünglichen Blutparasiten verloren und in die örtlichen Übertragungs-Zyklen gerieten. Zwei lokale *Plasmodium*-Verwandtschaftslinien infizierten die neu

Communicated by F. Bairlein.

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angesiedelten Arten: eine davon (SGS1) war der größere Wirts-Generalist und in den ortsansässigen Arten am weitesten verbreitet; wir erwarteten, dass er in den neu angesiedelten Arten zufällig verteilt war. Die andere Linie, PADOM01, trat in der Testgruppe seltener auf, war aber in denjenigen ortsansässigen Wirten vorhanden, die den angesiedelten, infizierten Arten phylogenetisch nahe standen. Demnach scheint die Kolonisierung der angesiedelten Wirtsvögel durch PADOM01 durch die Spezialisierung des Parasiten sowie durch phylogenetische Faktoren eingeschränkt zu sein. Ein Test der Phylogenie zeigte zwischen angesiedelten und ortsansässigen Tieren keine signifikanten Unterschiede in der Verbreitung der Infektionen und der Anzahl der Verwandtschaftslinien.

Introduction

Most of the exotic species that are introduced to new areas never become naturalized, even if they find suitable environmental conditions (Duncan et al. 2003). Yet some manage to establish self-sustained populations in the area of introduction, increase in numbers and spread. In the absence of its natural co-evolved enemies (such as parasites), they can reach a higher growth rate or survivorship than at their native areas (Torchin et al. 2001, 2003). The exotic species that are so successful as to become invasive may unbalance the ecosystems and drive losses in the biodiversity of native species' populations. Therefore, they can be a major cause of extinction (Vitousek et al. 1997; Mack et al. 2000).

The parasite community of an exotic species should change during the colonization of a new area. The original parasites are often lost before or during their hosts' introduction (Torchin et al. 2003; MacLeod et al. 2010). Even if the parasites arrive in the founder hosts' population, they can fail to colonize the new region for three reasons: (1) propagule pressure: too few parasites or too few infected hosts may have been introduced (MacLeod et al. 2010); (2) absence of competent vectors in the new region, in the case of vector-transmitted parasites (Torchin et al. 2003); and (3) transmission rates can be too low to sustain the parasite's population (Anderson and May 1978; MacLeod et al. 2010).

Upon arrival, an introduced host will also be exposed to the local parasites of the new area. Two different outcomes are possible: (1) if the host is susceptible to these local parasites, it will join the local dynamics of host–parasite transmission and endure whatever fitness cost these parasites may have; or (2) the local parasites may not be able to infect or have more difficulty infecting the introduced species (Torchin et al. 2003; Marzal et al. 2011). In this case, the resulting absence of these parasites or lower parasite load can give the introduced species an advantage over their competitors in the new area (Torchin et al. 2001).

Avian haemosporidians of the genera *Haemoproteus* and *Plasmodium* have a broad geographical distribution and infect bird species of a wide range of families (Waldenström et al. 2002; Valkiūnas 2005). Nevertheless, individual haemoparasite species and lineages infect distinct host species and have different host-specificity (Hellgren et al. 2007): while some infect only a few closely related host species, others can be found in numerous birds belonging to several families. Therefore, when a parasite community encounters an exotic host, the host–parasite compatibility and the infection outcome is somewhat unpredictable. Due to the potential deleterious effect of these parasites on host health and reproduction (Merino et al. 2000; Marzal et al. 2005; Norte et al. 2009; Knowles et al. 2010), an introduced bird species could gain a fitness advantage over the native birds if it avoids the local haemoparasite's pressure.

In Portugal, one of the most successful introduced avian species is the Waxbill *Estrilda astrild* (family Estrildidae, superfamily Passeroidea). This species, originally from Sub-Saharan Africa, was released in coastal Portugal in the 1960s and has quickly spread to most of continental Portugal and part of Spain (Silva et al. 2002). The Red Avadavat *Amandava amandava* (Estrildidae: Passeroidea), from tropical South Asia, was introduced in Italy in the 1980s and has also colonized Spain and Portugal (Matias 2002). The Black-headed Weaver *Ploceus melanocephalus* and the Yellow-crowned Bishop *Euplectes afer* (Ploceidae: Passeroidea) were introduced from Africa in the 1980s. All these species arrived in Portugal after accidental escapes from the pet trade, and have successfully colonized wetlands and riverine areas (Matias 2002; Matias et al. 2007). This study compares the haemosporidian infections of these exotic passerines with those of native marsh warblers and sparrows in the reed beds colonized by the exotics. We aimed to find if the introduced birds were free of local parasites, or had been included in the local cycle of host–parasite interactions. To our best knowledge, it is the first time that haemosporidians from these exotic bird species were investigated.

Methods

Field work

Birds were captured using mist nests in regular ringing sessions (109 sessions) from March 2007 to September 2009. These took place in four coastal wetlands of Portugal, where nesting of exotic species was previously confirmed: Paul do Taipal (40°11′N 8°41′W), Paul de Tornada (39°26′N, 9°08′W), Lagoa de Santo André (38°4′N, 8°48′W) and Vilamoura (37°04′N, 8°07′W). All these wetlands provide good conditions for the development of

mosquitoes such as *Culex pipiens* and *Culex theilerii* (Ventim et al. 2012) and other biting insects, potential haemosporidian vectors. Its reed beds are important stop-over, wintering and nesting sites for migrants (such as Reed and Great Reed Warblers, *Acrocephalus arundinaceus* and *A. scirpaceus*) and resident birds (such as Cetti's Warbler *Cettia cetti*).

Four exotic species from the superfamily Passeroidea were sampled: the Black-headed Weaver and the Common Waxbill were present in great numbers and could be sampled during the 3 years, while the Yellow-crowned Bishop and the Red Avadavat were uncommon, so only opportunistic samples were taken. Among the native passerines, the six most abundant species in the study areas were sampled: Reed and Great Reed Warblers, Cetti's Warbler, Savi's Warbler *Locustella luscinioides* (superfamily Sylvioidea), House Sparrow *Passer domesticus* and Tree Sparrow *Passer montanus* (superfamily Passeroidea). For each species (exotic and native), both juvenile and adult individuals were sampled whenever possible. After the birds were ringed, a blood sample (around 40 µl) was collected from their jugular or brachial vein using a 25 G or 30 G needle and stored in 96 % ethanol.

Molecular analysis

DNA was extracted from the blood samples by a standard ammonium acetate protocol (Sambrook and Russell 2001). To confirm the good condition of the extracted DNA, all samples were tested using a universal bird sexing protocol that amplifies a CDH gene's fragment by polymerase chain reaction (PCR), using the primer pair 0057F/002R (Round et al. 2007). The reaction products were run in 2 % agarose gels for band visualization; the appearance of one or two bands confirmed the successful DNA amplification.

The samples were diagnosed for infections by amplification of a portion of the parasite's cytochrome *b* gene. Variation in this genetic sequence defines parasite lineages, which may be considered as separate species (Bensch et al. 2000; Pérez-Tris et al. 2007). We used a nested PCR protocol (Waldenström et al. 2004) with primers that are specific for the genera *Haemoproteus* and *Plasmodium*: HaemNF/HaemNR2 (Waldenström et al. 2004) for the pre-amplification PCR, followed by HaemF/HaemR2 (Bensch et al. 2000) for the specific PCR. A sample of 1 µl of the products of the pre-amplification PCR was used as template for the second PCR. False positives (contaminations) were controlled for by including a negative control per each 24 samples during extraction and a negative control (water) for each 12 samples during PCR. None of these controls ever showed amplification.

Samples that were negative for infection were confirmed by a second nested PCR, while all samples showing positive amplification were precipitated with ammonium acetate and ethanol and sequenced using primer HaemF. The obtained sequences were aligned and edited with BIOEDIT (Hall 1999) and compared with the sequences stored at GenBank and the MalAvi database (Bensch et al. 2009) for identification of the parasite's lineage. New host–parasite associations were confirmed by repeating the whole process, from extraction to sequence identification.

Statistical analysis

The Red Avadavat and Yellow-crowned Bishop were excluded from these analyses because their sample size was too small to be representative of the species' prevalence and number of infecting lineages (Table 1). All other sampled bird species were classified according to their host type (as exotic or native), and the effect of this binary variable on prevalence and on the number of parasite lineages per species was tested performing two ANOVAs on STATISTICA 7 (Statsoft, 2002). The values obtained for the *F* statistics were then used to perform a phylogenetic ANOVA using the ''Phenotypic Diversity Analysis Programs'', PDAP (Garland et al. 1993).

In a conventional ANOVA, the obtained value of the *F* statistics is compared with the standard distribution of *F*, with degrees of freedom determined by the number of groups being compared and the total number of observations (in this case, species) in the dataset. However, the phylogenetic relationships between species prevent them from being statistically independent data points and make it difficult to know how many degrees of freedom should be considered (Garland et al. 1993). Therefore, the obtained *F* values cannot be directly compared with the conventional tabulated distributions of the *F* statistics to find the associated probability value and significance. Phylogenetically correct significance values must be obtained from empirical null distributions of *F* statistics, which can be created using computer simulation models of traits evolving along known phylogenetic trees (Garland et al. 1993).

The phylogenetic tree of the sampled species was built following Johansson et al. (2008), Treplin et al. (2008) and Arnaiz-Villena et al. (2009). Using PDSIMUL (Garland et al. 1993), 1,000 sets of tip values were simulated for the two traits under study (infection prevalence and number of lineages per host species), assuming a gradual Brownian motion model of evolutionary change. Prevalence was bounded to vary between 0 and 1, while the number of lineages was bounded between 0 and 30 (the highest number registered for a passerine host in the MalAvi database; Bensch et al. 2009). We used the between-species means of real data as both starting values and expected means of simulated tip values. The expected variances of the simulated tip data were set equal to the variances of the

Table 1 Sample size, number of infections and number and name of the parasite lineages found per bird species

Host type	Bird species (Superfamily)	*n*	No. infected (prevalence)	No. of lineages	Lineage names
Exotic	Red Avadavat (P)	4	0	0	–
	Common Waxbill (P)	104	1 (0.96 %)	1	SGS1
	Yellow-crowned Bishop (P)	2	1	1	PADOM01
	Black-headed Weaver (P)	65	5 (7.7 %)	1	SGS1
Native	House Sparrow (P)	121	45 (37.2 %)	4	PADOM01, ^{H}PADOM23, GRW11, SGS1
	Tree Sparrow (P)	53	19 (35.8 %)	1	SGS1
	Great Reed Warbler (S)	37	20 (54.1 %)	6	^{H}GRW01, GRW02, GRW04, ^{H}GRW16, GRW17, SGS1
	Reed Warbler (S)	410	103 (25.1 %)	11	GRW04, GRW06, GRW11, ^{H}HIPOL01, ^{H}MW1, ^{H}RW1, ^{H}SW1, RTSR1, SGS1, SW2, SW5
	Savi's Warbler (S)	46	7 (15.2 %)	5	COLL1, GRW04, GRW06, ^{H}MW1, WW4
	Cetti's Warbler (S)	305	171 (56.1 %)	4	CET01, GRW11, SGS1, SYAT05

Bird superfamily, in parentheses: *P* Passeroidea, *S* Sylvioidea. Prevalence, in brackets, is given as a percentage. Lineage names marked ^{H} belong to the genus *Haemoproteus*, unmarked ones are *Plasmodium* lineages

real data. The program PDANOVA (Garland et al. 1993) calculated the F values for the two traits in the 1,000 simulations, generating the empirical null distributions of F. The upper 95 % percentile of these distributions, calculated with STATISTICA 7, was the critical value against which the F ratios of our real dataset were compared (Garland et al. 1993).

Results

A total of 1,120 individual birds were sampled, from which 945 belonged to native species and 175 were exotic (Table 1). The sampled birds harboured 21 different parasite lineages. All the native species were found to be infected by at least one lineage of *Plasmodium*, and four of them were also infected by at least one *Haemoproteus* lineage (Table 1). The *Plasmodium* SGS1 was the lineage infecting a larger number of native host species. Among the exotic species, no infection was found in the Red Avadavat, while the other three species were infected by one *Plasmodium* lineage each (Table 1). No *Haemoproteus* parasites were found in the exotic species.

All parasite lineages that were found in the exotic species were also present in the native species. The *Plasmodium* SGS1, found in the Waxbill and Black-headed Weaver, was the most prevalent lineage in the native species (causing 61.9 % of all infections in native individuals) and infected 5 of the 6 sampled native species. PADOM01, found in one Yellow-crowned Bishop, also infected House Sparrow; but it was present only in 4.5 % of the infected individuals of that species. Moreover, in the set of native species, PADOM01 accounted for only 0.55 % of all infections.

On average, each exotic host species was infected by fewer lineages than a native host species (Table 1), but this effect was not statistically significant either in a conventional ANOVA ($F_{1,8} = 2.85$, $p = 0.142$) or in the phylogenetic ANOVA (critical value of the empirical null distribution = 8.81, much higher than the real case's F ratio).

Native species had total infection prevalences ranging from 15 to 56 % of infected individuals (Table 1), with a mean of 37.6 %. In the exotic species, prevalences were of 1 % in the Common Waxbill and 8 % in the Black-headed Weaver. The host type (exotic/native) seemed to have a significant effect on the infection prevalence by species using a conventional ANOVA ($F_{1,8} = 6.74$, $p = 0.031$). However, this F ratio was not higher than the critical value of the empirical null distribution ($F = 9.64$), so this effect was not significant in the phylogenetic ANOVA. This discrepancy may be explained by the fact that species' status (exotic or native) has an important phylogenetic component in our study, in which all exotics belong to the same superfamily.

Discussion

Three of the introduced bird species (all except the Red Avadavat) have entered the local haemoparasite transmission dynamics, having acquired two local lineages, PADOM01 and SGS1. These two lineages were also found in the native bird species at these sites and were previously found in native hosts in several other European sites

(Bensch et al. 2009). Therefore, these can be considered local lineages, well implemented in the local transmission cycle and probably present long before the arrival of the exotic hosts. It is very unlikely that these lineages infected the exotic hosts at their original home range. PADOM01 was never found in any host at the original home range of the exotic species, while SGS1 was found in Sub-Saharan Africa (Hellgren et al. 2007), but not in Ploceids or Estrildids, despite the fact that some species of these families were sampled.

Once the host's phylogeny was controlled for, there were no significant differences in prevalence or number of lineages between the exotic Black-headed Weaver and Waxbill and the native hosts. No evidence was found that exotic species were less affected by haemoparasites than native hosts in the study area; therefore, the most extensively sampled exotics do not seem to have a competitive advantage over the natives in respect to haemosporidian infections. However, a bigger sample of Yellow-crowned Bishops and Red Avadavats would be needed to generalise this affirmation.

On the other hand, there is no evidence that exotic parasites established in the local community. The haemosporidian infections of these birds at their original home range have not yet been thoroughly studied. However, three *Plasmodium* lineages (BT8, STASTR01 and ZEMAC1) were found for the Red Avadavat in India (Ishtiaq et al. 2007), one (GRW09) was found for the Waxbill in Tanzania (Beadell et al. 2006) and four *Haemoproteus* lineages (PLOMEL01, PLOMEL02, PLOMEL03 and RBQ11) were identified in the Black-headed Weaver in Uganda (Iezhova et al. 2011). None of those lineages were present in the Portuguese study sites. Although nothing is known about the original infections of the Yellow-crowned Bishop, there are two reasons to believe that this species is also infected by some haemosporidian lineages in their original home range: (1) the genera *Haemoproteus* and *Plasmodium* have a wide distribution in the majority of the sampled bird species around the globe (Valkiūnas 2005); and (2) several other Ploceid species were found to be infected by lineages of both haemoparasite genera (Beadell et al. 2006; Hellgren et al. 2007; Durrant et al. 2007; Iezhova et al. 2011). Whichever haemoparasites infected this species in their original home range, they seemed to be absent from the study area. Therefore, the original haemosporidian infections of the studied exotic species were probably eliminated from the studied populations, either because they were absent in the arriving individuals or because they were lost after arrival (Torchin et al. 2003; MacLeod et al. 2010). The same was observed in another invasive passerine, the House Sparrow, which also lost its original haemoparasite fauna upon colonizing America (Lima et al. 2010; Marzal et al. 2011).

From the local lineages that could have colonized the exotic hosts, SGS1 was the most expected because this is the most abundant parasite lineage that was sampled in the study area, and also the most host-generalist. Indeed, it is one of the most generalist lineages worldwide, infecting a great number of birds of different orders (Bensch et al. 2009 and references therein). The most host generalist parasites can also be the most prevalent in single host species and in a community (Hellgren et al. 2009). Because of its abundance, SGS1 is very likely to be transmitted to the exotic hosts by chance, and, because of its generality, it was expected to be able to adapt to these new hosts and succeed in infecting them.

However, PADOM01 is a rare lineage in the studied native community (causing only 0.55 % of all infections), so its presence in the Yellow-crowned Bishop is not easily explained by random processes. In previous studies, this lineage has been found in few host species, all from the superfamily Passeroidea (Johansson et al. 2008): House Sparrow (Bonneaud et al. 2006; Dimitrov et al. 2010), Spanish Sparrow *Passer hispaniolensis* (Marzal et al. 2011) and Yellow Wagtail *Motacilla flava* (Hellgren et al. 2007). In all these cases, this lineage's prevalence in the host was similar to that of the present study. Therefore, globally, this parasite lineage seems to be more host specialist than SGS1, only being able to infect a group of closely related hosts. Its presence in the Yellow-crowned Bishop can be explained by the parasite's affinity to Passeroids. A larger sample of Yellow-crowned Bishops would be needed to know if this species also hosts other parasite lineages.

In summary, in this community of reed bed passerines, the exotic species seemed to have lost the parasites of their original home range and acquired some of the local parasite lineages, entering the local dynamics of host–parasite transmission. The colonization of these new available hosts by local parasites seems to be partially constrained by phylogenetic factors or by the parasite's degree of host-specialization (although other causes, such as ecological or behavioural factors that might limit exposure to vectors, may also play a role in this constraint). However, all available exotic species in this study were phylogenetically related and had a similar establishment success; the haemoparasite scenario may be different for other introduced bird species, with different phylogenies and colonization processes.

In general, if an introduced species becomes abundant and is infected by some local parasites, it may become a reservoir of infection. This new source of infection may change the local transmission dynamics, increasing infection prevalence in other host species and in the whole system. Given the current increase in the introduction of exotic bird species, further studies are needed to ascertain such patterns and to assess whether there are negative fitness effects for particular native bird species.

Acknowledgments This study was funded by the Fundação para a Ciência e Tecnologia (grant no. SFRH/BD/28930/2006 to R.V.) and by the Spanish Ministry of Science and Innovation (CGL2010-15734, to J.P-T.). The Instituto da Conservação da Natureza e Biodiversidade provided logistic support, permits for bird capture and help in the field work (by Vítor Encarnação, Paulo Encarnação, Nuno Grade and Paulo Tenreiro). The Pato Association also provided logistic support in Tornada. The authors would like to thank the help of Joana Morais in the lab and of several volunteers in the field work.

References

Anderson RM, May RM (1978) Regulation and stability of host–parasite population interactions 1. Regulatory processes. J Anim Ecol 47(1):219–247

Arnaiz-Villena A, Ruiz-del-Valle V, Gomez-Prieto P, Reguera R, Carlos P-L, Serano-Vela I (2009) Estrildinae Finches (Aves, Passeriformes) from Africa, South Asia and Australia: a Molecular Phylogeographic Study. Open Ornithol J 2:29–36. doi:10.2174/1874453200902010029

Beadell JS, Ishtiaq F, Covas R, Melo M, Warren BH, Atkinson CT, Bensch S, Graves GR, Jhala YV, Peirce MA, Rahmani AR, Fonseca DM, Fleischer RC (2006) Global phylogeographic limits of Hawaii's avian malaria. Proc R Soc Lond B 273(1604):2935–2944. doi:10.1098/rspb.2006.3671

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 Lond B 267(1452):1583–1589. doi:10.1098/rspb.2000.1181

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(5):1353–1358. doi:10.1111/j.1755-0998.2009.02692.x

Bonneaud C, Pérez-Tris J, Federici P, Chastel O, Sorci G (2006) Major histocompatibility alleles associated with local resistance to malaria in a passerine. Evolution 60(2):383–389. doi:10.1554/05-409.1

Dimitrov D, Zehtindjiev P, Bensch S (2010) Genetic diversity of avian blood parasites in SE Europe: cytochrome *b* lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from Bulgaria. Acta Parasitol 55(3):201–209. doi:10.2478/s11686-010-0029-z

Duncan RP, Blackburn TM, Sol D (2003) The ecology of bird introductions. Annu Rev Ecol Evol Syst 34:71–98. doi:10.1146/annurev.ecolsys.34.011802.132353

Durrant KL, Reed JL, Jones PJ, Dallimer M, Cheke RA, McWilliam AN, Fleischer RC (2007) Variation in haematozoan parasitism at local and landscape levels in the red-billed quelea *Quelea quelea*. J Avian Biol 38(6):662–671. doi:10.1111/j.2007.0908-8857.04034.x

Garland T, Dickerman AW, Janis CM, Jones JA (1993) Phylogenetic analysis of covariance by computer-simulation. Syst Biol 42(3):265–292

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hellgren O, Waldenstrom J, Pérez-Tris J, Szollosi E, Hasselquist D, Krizanauskiene A, Ottosson U, Bensch S (2007) Detecting shifts of transmission areas in avian blood parasites—a phylogenetic approach. Mol Ecol 16(6):1281–1290. doi:10.1111/j.1365-294X.2007.03277.x

Hellgren O, Pérez-Tris J, Bensch S (2009) A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. Ecology 90(10):2840–2849. doi:10.1890/08-1059.1

Iezhova TA, Dodge M, Sehgal RNM, Smith TB, Valkiunas G (2011) New avian *Haemoproteus* species (Haemosporida: Haemoproteidae) from African birds, with a critique of the use of host taxonomic information in hemoproteid classification. J Parasitol 97(4):682–694. doi:10.1645/ge-2709.1

Ishtiaq F, Gering E, Rappole JH, Rahmani AR, Jhala YV, Dove CJ, Milensky C, Olson SL, Peirce MA, Fleischer RC (2007) Prevalence and diversity of avian hematozoan parasites in Asia: a regional survey. J Wildl Dis 43(3):382–398

Johansson US, Fjeldsa J, Bowie RCK (2008) Phylogenetic relationships within Passerida (Aves: Passeriformes): a review and a new molecular phylogeny based on three nuclear intron markers. Mol Phylogenet Evol 48(3):858–876. doi:10.1016/j.ympev.2008.05.029

Knowles SCL, Palinauskas V, Sheldon BC (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. J Evol Biol 23(3):557–569. doi:10.1111/j.1420-9101.2009.01920.x

Lima MR, Simpson L, Fecchio A, Kyaw CM (2010) Low prevalence of haemosporidian parasites in the introduced house sparrow (*Passer domesticus*) in Brazil. Acta Parasitol 55(4):297–303. doi:10.2478/s11686-010-0055-x

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10(3):689–710

MacLeod CJ, Paterson AM, Tompkins DM, Duncan RP (2010) Parasites lost—do invaders miss the boat or drown on arrival? Ecol Lett 13(4):516–527. doi:10.1111/j.1461-0248.2010.01446.x

Marzal A, de Lope F, Navarro C, Moller AP (2005) Malarial parasites decrease reproductive success: an experimental study in a passerine bird. Oecologia 142(4):541–545. doi:10.1007/s00442-004-1757-2

Marzal A, Ricklefs RE, Valkiūnas G, Albayrak T, Arriero E, Bonneaud C, Czirják GA, Ewen J, Hellgren O, Hořáková D, Iezhova TA, Jensen H, Križanauskiene A, Lima MR, de Lope F, Magnussen E, Martin LB, Møller AP, Palinauskas V, Pap PL, Pérez-Tris J, Sehgal RNM, Soler M, Szöllosi E, Westerdahl H, Zetindjiev P, Bensch S (2011) Diversity, loss, and gain of malaria parasites in a globally invasive bird. PLoS ONE 6(7):e21905. doi:10.1371/journal.pone.0021905

Matias R (2002) Aves Exóticas que Nidificam em Portugal Continental, 1st edn. ICN, Lisbon

Matias R, Catry P, Costa H, Elias G, Jara J, Moore CC, Tomé R (2007) Systematic list of the birds of Mainland Portugal. An Ornitol 5:74–132

Merino S, Seoane J, de la Puente J, Bermejo A (2000) Low prevalence of infection by haemoparasites in Cetti's warblers *Cettia cetti* from central Spain. Ardeola 47(2):269–271

Norte AC, Araujo PM, Sampaio HL, Sousa JP, Ramos JA (2009) Haematozoa infections in a great tit *Parus major* population in Central Portugal: relationships with breeding effort and health. Ibis 151(4):677–688

Pérez-Tris J, Hellgren O, Krizanauskiene A, Waldenstrom J, Secondi J, Bonneaud C, Fjeldsa J, Hasselquist D, Bensch S (2007) Within-host speciation of malaria parasites. PloS One 2(2). doi:10.1371/journal.pone.0000235

Round PD, Hansson B, Pearson DJ, Kennerley PR, Bensch S (2007) Lost and found: the enigmatic large-billed reed warbler *Acrocephalus orinus* rediscovered after 139 years. J Avian Biol 38(2):133–138. doi:10.1111/j.2007.0908-8857.04064.x

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor

Silva T, Reino LM, Borralho R (2002) A model for range expansion of an introduced species: the common waxbill *Estrilda astrild* in Portugal. Divers Distrib 8(6):319–326

Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: Increased performance of a globally introduced marine crab. Biol Invasions 3(4):333–345. doi:10.1023/a:1015855019360

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421(6923):628–630. doi:10.1038/nature01346

Treplin S, Siegert R, Bleidorn C, Thompson HS, Fotso R, Tiedemann R (2008) Molecular phylogeny of songbirds (Aves: Passeriformes) and the relative utility of common nuclear marker loci. Cladistics 24(3):328–349. doi:10.1111/j.1096-0031.2007.00178.x

Valkiūnas G (2005) Avian malaria parasites and other haemosporidia, 1st edn. CRC, Boca Raton

Ventim R, Ramos JA, Osório H, Lopes RJ, Pérez-Tris J, Mendes L (2012) Avian malaria infections in western European mosquitoes. Parasitol Res. doi:10.1007/s00436-012-2880-3

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. Science 277(5325):494–499. doi:10.1126/science.277.5325.494

Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Mol Ecol 11(8):1545–1554. doi:10.1046/j.1365-294X.2002.01523.x

Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. J Parasitol 90(1):191–194. doi:10.1645/GE-3221RN