Host-parasite associations and host-specificity in haemoparasites of reed bed passerines

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(*Received 28 September 2011; revised 26 October 2011; accepted 26 October 2011; first published online 5 January 2012*)

SUMMARY

The host specificity and host sharing of avian haemoparasites (genera *Haemoproteus* and *Plasmodium*) is still poorly known, although they infect a large proportion of several studied bird populations. This study used molecular techniques to detect haemoparasites in marsh warblers and in other passerines that feed in reed beds, at 4 sites in Portugal. The host-specificity of the parasite lineages was analysed and compared with other cases described in the literature to assess whether apparent host specificity changes according to the studied system. Nine lineages of *Haemoproteus* and 15 of *Plasmodium* were found, of which only 10 *Plasmodium* were proven to have local transmission. Each lineage was confined to a distinct set of host species. The distribution of parasites in the host species was non-nested, meaning that specialist lineages did not always share hosts with generalists. The most prevalent lineages were those with a wider host range, indicating that the ability to infect more hosts will enhance a parasite's prevalence in its entire host range. We also found that in our areas, a specialist parasite (*H*. MW1) appears to have a more generalist character than described in the literature, suggesting that a parasite's apparent specialization can depend on the type of host species sampled.

Key words: avian malaria, *Haemoproteus*, haemosporidian, host–parasite association, host range, local transmission, nestedness, *Plasmodium*, specialists versus generalists.

INTRODUCTION

Parasites obtain food, habitat and dispersal from their hosts (Valkiūnas, 2005). A generalist parasite is one that is capable of infecting and completing its life cycle in many host species, while a specialist will be found in only a few host species. Specialist parasites may be very well adapted to particular hosts, but will be unable to infect other closely related species if they come into contact with them.

A parasite's probability of infecting a suitable host depends on many factors, including the host-parasite compatibility and rate of encounter (Combes, 1997). The probability of physical contact with a susceptible host is influenced by the host and parasite's behaviour, life-cycle, population density, etc. Vector-transmitted parasites have complex systems of interactions, which also include the vector's behaviour and population dynamics. If the vectors contact with many possible host species, then the parasites present in the vector might end up in incompatible or suboptimal hosts, which reduces the probability of successful infections (Dobson, 2004). Therefore, a vector-transmitted parasite in a host-rich community has advantages in being host-generalist, that is, maintaining compatibility with a wide set of hosts, even if some of them are not optimal. This should increase its encounter rate with suitable hosts and, therefore, its overall prevalence in the community (Dobson, 2004; Hellgren *et al*. 2009).

But does a parasite always appear as a specialist or as a generalist, or will parasites be considered more or less generalist according to the conditions that they face? In different parts of their distribution range, parasites will find different assemblages of possible hosts, vectors and even other competitor parasites. According to the different communities that they find, they might appear to be more or less host-specialist. A parasite that is a generalist in one community may be unable to infect most of the hosts present in a different place, thus appearing to be more specialist; and a parasite that is fully adapted to few hosts may encounter a new community of naive hosts and be able to infect many of them, therefore becoming a generalist in that community.

At a community level, the interactions between parasites and their hosts define an antagonistic network. Determining the general ecological pattern of these interactions may help to understand and predict the spread of parasites and diseases in general (Graham *et al*. 2009). Nestedness is a particular structure reported for many networks, in which specialists only interact with subsets of the species that interact with more generalist organisms. The

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doi:10.1017/S0031182011002083

nested pattern has been described for many mutualistic webs (Bascompte *et al*. 2003; Bascompte and Jordano, 2007) and, although it has been suggested that it should not apply to most antagonistic networks (Thompson, 2006), nestedness was also found in host-ectoparasite networks (Graham *et al*. 2009). Applied to host-parasite networks, a nested pattern would mean that specialist parasites should only be able to infect a few of the host species that generalist parasites can infect – or, in other words, that more resistant hosts would only be infected by a few generalist parasites, while the less-resistant hosts would be infected both by generalist and specialist parasites (Graham *et al*. 2009).

Protists of the genera *Plasmodium* (also referred to as avian malaria parasites) and *Haemoproteus* (Apicomplexa: Haemosporida) infect the blood cells of birds through the bite of an infected dipteran vector – a mosquito in the case of *Plasmodium* spp., a biting midge or an hippoboscid fly for *Haemoproteus* spp. (Valkiunas, 2005). The use of molecular techniques (Bensch *et al*. 2004; Ricklefs *et al*. 2004; Waldenström *et al*. 2004) has unveiled that this is a very diverse group and has defined mitochondrial lineages, which greatly outnumber the traditional morpho-species and may be considered as separate species (Bensch *et al*. 2004; Pérez-Tris *et al*. 2007). At the lineage level, the host specificity of haemosporidians is still poorly understood, although at the genus level, the *Plasmodium* genus seems to contain more generalist parasites than *Haemoproteus* (Fallon *et al*. 2005). In both genera, while some lineages infect hosts from a wide range of families, others are very host-specific (Waldenström *et al*. 2002). The structure of these host-endoparasite interaction networks is also unknown. Passerine species are known to suffer from relatively high haemoparasite infection rates, but these vary greatly between host species and between geographical areas (Valkiunas, 2005).

This study focused on the presence of *Haemoproteus* and *Plasmodium* lineages in bird assemblages (2 species of sparrows (family Passeridae) and 7 species of Old World warblers (4 families of the superfamily Sylvioidea)) at 4 reed beds in Portugal. The bird's parasite fauna was analysed using molecular techniques. The structure of the host-parasite interaction network was assessed and the host specificity of each parasite lineage was compared with other cases reported in the literature. Overall, this study evaluated the degree of specialization of haemoparasite lineages in a rich community of bird hosts.

MATERIALS AND METHODS

Study area

This study took place in 4 Portuguese wetlands: Taipal (N 40°11′, W 8°41′), Tornada (N 39°27′, W 09°3′) Santo André (N 38°4′, W 8°48′) and Vilamoura (N 37°04′, W 8°07′). Populations of several species of mosquitoes, possible vectors of avian haemosporidians, reproduce here. All 4 wetlands have vast extensions of common reed bed (*Phragmites australis*), which attracts a wide variety of ducks, waders and other waterbirds, both resident and migratory. They are important breeding and refuelling areas for migrating passerines such as the reed warbler (*Acrocephalus scirpaceus*), the great reed warbler (*Acrocephalus arundinaceus*) and the Savi's warbler (*Locustella luscinioides*) and also harbour important populations of resident passerines, such as the Cetti's warbler (*Cettia cetti*).

Field work

Passerines were captured with mist nets from March 2007 to November 2008 in all areas and from July to September 2009 in the Tornada site only. The most abundant species in these sites were sampled: the reed and the great reed warblers, the Savi´s warbler, the willow warbler (*Phylloscopus trochilus*), the common and the Iberian chiffchaffs (*Phylloscopus collybita* and *P. ibericus*), the Cetti's warbler, the Eurasian tree sparrow (*Passer montanus*) and the house sparrow (*Passer domesticus*, although this sparrow spends a great part of the day outside the reed bed). The 2 sparrows and the Cetti's warbler are residents, the willow warbler is a passage migrant, the common chiffchaff winters in the study area and all the other species reproduce in these Portuguese marshes. Less-abundant species that were present (of finches, thrushes, tits, warblers, etc.) were not sampled.

Individuals were ringed, weighed, measured and then sexed and aged according to Svensson (1995). A blood sample (around 40 μl) was collected from the jugular or brachial veins using a 25 G or 30 G needle and stored in 96% ethanol, after which the birds were released.

Laboratory methods

Total DNA was extracted using a standard ammonium acetate protocol. Birds were sexed by a polymerase chain reaction (PCR) amplifying a CDH gene's fragment, using the primers 0057F (CGTCAATTTCCATTTCAGGTAAG) and 002R (TTATTGATCCATCAAGTCTC). Resulting products were run in 2% agarose gels for band visualization. The successful sexing of a sample confirmed that the extracted DNA was in good enough condition to be amplified by PCR.

Samples were diagnosed for haemoparasite infections using a nested PCR developed by Waldenström *et al*. (2004). A portion of the parasite's mitochondrial cytochrome *b* gene was amplified using the primers HaemNF/HaemNR2 (for pre-amplification) followed by HaemF/HaemR2 (Bensch *et al*. 2000),

https://doi.org/10.1017/S0031182011002083 Published online by Cambridge University Press

which are specific to *Haemoproteus* and *Plasmodium* spp. Each PCR included approximately 25 ng of genomic DNA, 1·5 mM $MgCl_2$, 2·5 μl of 10× PCR buffer II, 400 mM of each deoxynucleoside triphosphates, 0·6 mM of each primer, and 0·625 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, California), in a total volume of 25 μl. The thermal profile started with 3 min of denaturation at 94 °C, followed by cycles of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 45 sec, and ended with an elongation step at 72 °C for 10 min. One μl of the products of the pre-amplification PCR was used as template for the second PCR. This final reaction used the same reagents in the same concentrations and the same thermal profile, the only difference being that the pre-amplification PCR ran for 20 cycles while the final PCR ran for 35 cycles (Waldenström *et al*. 2004). Final amplification products (479 bp) were run in a 2% agarose gel.

We controlled for contamination by including a negative control per each 24 samples during extraction and a negative control (water) for each 8 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 genus and lineage. New parasite lineages and new host-parasite associations were confirmed by repeating the whole process.

Data analysis

In order to test for a nested distribution of parasite lineages in the host species, a matrix of presences/absences of all lineages in all host species was built and then ordered from the more parasitized hosts (lines filled with more presences) to the less parasitized hosts (less filled lines). The matrix's nestedness metric NODF (Nestedness based on Overlap and Decreasing Fill; Almeida-Neto *et al*. 2008) was calculated using ANINHADO 3 (Guimarães and Guimarães, 2006). The same software performed a nestedness analysis, which compares the filling structure of our matrix with the structure of a random matrix. Nestedness was tested for using χ^2 tests against the null hypothesis that the parasites present in less parasitized hosts are a subset of the parasites in more infected hosts. The programme's CE null model builds random matrices by filling cells in proportion to the row and column totals of each lineage and species, against which our matrix was compared (Graham *et al*. 2009). This analysis was made first for each site, then for all birds from the 4 locations pooled together.

Each parasite's host-specificity was calculated considering the number of host species it could infect, the prevalence in all the infected species and also the taxonomic distance between such hosts. Two different specificity indices were calculated: the host breadth index (HB; Fallon *et al*. 2005) and the standardized host range index S_{TD*} (Poulin and Mouillot, 2005). With both indices, low values indicate parasite lineages that primarily infected closely related hosts, while high values reflect parasite lineages that were found across divergent host species. The higher the indices are, the more generalist is the parasite. However, the two indices have slightly different behaviours.

The HB is based on the phylogenetic distance between a parasite's hosts, weighted by the parasite's prevalence in the different hosts (Fallon *et al*. 2005):

$$\mathrm{HB} = \sum_{i=1}^{n}\sum_{j=1}^{n}\omega_{ij}(p_i p_j)$$

p_i and p_j being the prevalence of the parasite in host species i and j. Because not all the phylogenetic distances between hosts were available, in this study they were replaced by the taxonomic distinctness (Clarke and Warwick, 1998): ω_{ij} is the number of taxonomical steps (from the species to the genera, family, infra-order or order level) needed to get to the common ancestor of any pair of hosts. This modification also allows a more direct comparison with the S_{TD*} index, which originally uses taxonomic distinctness (Clarke and Warwick, 1998; Poulin and Mouillot, 2005). For parasites with only 1 host (i), the HB was calculated as p_i^2. This index increases whenever the number of host species increases, but can be greatly influenced by the prevalences in each host, giving out very different results for parasites with a similar number of hosts (for example, for parasites with only 1 host species).

The S_{TD*} (Poulin and Mouillot, 2005) shows the mean taxonomic distinctness among the host species used by a parasite, weighted for the parasite's prevalence in the different hosts:

$$S_{TD^*} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{i<j}\omega_{ij}(p_i p_j)}{\sum_{i=1}^{n}\sum_{j=1}^{i<j}p_i p_j}$$

p_i and p_j being the prevalence of the parasite in host species i and j and ω_{ij} the taxonomic distinctness between 2 host species. Whenever there was only 1 host, the S_{TD*} was considered to equal 1. This index has a narrower variation range, allowing easier interpretation of values, and is more stable when hosts with a similar number of hosts are compared; but it does not necessarily grow as the number of hosts increases (the addiction of closely related hosts will actually lower the index, by reducing the average distinctness between hosts).

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To assess whether parasites always show the same degree of apparent host specificity in all the studied systems, we compared our own data with previously reported cases. For each parasite lineage found in this study, a list of prevalences in all the reported passerine hosts was compiled from the MalAvi database (Bensch *et al*. 2009). We assume that the sampling effort was the same for all parasite lineages in the total tested individuals, as all lineages can potentially be detected every time a blood sample is analysed by this method. However, the sampling effort for all host species is unavoidably not constant across all the consulted studies, which is a frequent problem in comparative studies.

Assemblages of 9 bird species were simulated: a subset of 9 birds was randomly selected from the compiled host list and each parasite's host range index was calculated for that subset of hosts. In this way, all the selected hosts had at least 1 parasite, but some parasites could be absent from all 9 hosts (in this case, their indices were considered to be zero). This simulation was repeated 1000 times. The probability of a lineage appearing to be more specialist in this particular system than is generally described is the probability of finding lower indices in these simulations than in the real case under study.

Table 1. Sample size of all bird species and number of detected infections in each species

Bird species	Sample size	No. of infections	% infections
Cetti's warbler, *Cettia cetti*	309	172	55·66
Great reed warbler, *Acrocephalus arundinaceus*	37	20	54·05
Reed warbler, *Acrocephalus scirpaceus*	421	104	24·70
Savi's warbler, *Locustella luscinioides*	46	7	15·22
Common chiffchaff, *Phylloscopus collybita*	116	0	0·00
Iberian chiffchaff, *Phylloscopus ibericus*	27	1	3·70
Willow warbler, *Phylloscopus trochilus*	36	2	5·56
House sparrow, *Passer domesticus*	121	45	37·19
Tree sparrow, *Passer montanus*	53	16	30·19
Total	1166	367	31·48

RESULTS

Prevalence of Haemoproteus *and* Plasmodium

In total, 1166 birds from 4 species were sampled (Table 1), out of which 367 (31·5%) revealed infections (5·6% by *Haemoproteus* spp. and 25·9% by *Plasmodium* spp.). However, infection rates varied considerably between species, from 55·7% for the Cetti's warbler to zero infections for the common chiffchaff. These 9 bird species hosted 24 parasite lineages, 9 of *Haemoproteus* and 15 of *Plasmodium* (Table 2). Two lineages of *Haemoproteus* and 2 of *Plasmodium* were identified for the first time: *H*. GRW16 and *P*. GRW17 in the great reed warbler, *H*. PADOM23 in a house sparrow and *P*. CET01 in a Cetti's warbler (GenBank Accession numbers HQ262948 to HQ262951); these were named following the guidelines proposed by Bensch *et al*. (2009). Only 3 mixed infections were detected: 1 of *P*. PADOM01 and an unidentified *Plasmodium* lineage (in a house sparrow) and 2 of a pair of unidentified *Plasmodium* lineages (one in a Cetti's warbler, the other in a reed warbler). This is surely an underestimation of the real number of mixed infections, which is a known limitation of the used technique (Valkiunas *et al*. 2006).

Most of the host-parasite associations found in this report had already been described in previous studies (Bensch *et al*. 2009 and references therein), except for 6 parasites in the reed warbler and for the newly identified lineages. One lineage of *Haemoproteus* (MW1) and 4 of *Plasmodium* (GRW04, GRW06, GRW11 and SGS1) infected more than one host.

Haemoproteus lineages were only present in migrant species, with 1 exception: an adult house sparrow, a species known to spend limited time in the reed bed. Almost all the migrants infected with *Haemoproteus* spp. were adults; only 2 willow warblers sampled during migration, in autumn 2007, were juveniles. This suggests that *Haemoproteus* lineages are not transmitted locally and that the infected birds probably acquired the parasite elsewhere. In the *Plasmodium* genus, there were also some lineages that were only present in adult individuals of migratory species, hinting for non-local transmission: GRW4, GRW6, RTSR1 and WW4. On the other hand, lineages COLL1, GRW11, GRW17, SGS1, SW2, SW5 and SYAT05 occurred in resident species and/or were detected in juveniles that were still attached to their birth reed bed, which shows local transmission.

Lineage host specificity

The nestedness analysis revealed a non-random and non-nested pattern of parasites in each host, for each site as well as for the 4 areas pooled together (Coimbra: NODF = 6·74, null model's NODF = 10·05, p = 0·80. Santo André: NODF = 11·62, null model's NODF = 14·09, p = 0·68. Vilamoura: NODF = 6·13, null model's NODF = 9·15, p = 0·73.

https://doi.org/10.1017/S0031182011002083 Published online by Cambridge University Press

Table 2. Number of infections of each parasite lineage found in each host species, followed by the host range indices HB and S_{TD*} for each lineage

(*H*. GRW16, *H*. PADOM23, *P*. GRW17 and *P*. CET01 were found for the first time. Host species are: *A aru, A. arundinaceus; A sci, A. scirpaceus; C cet, C. cetti; L lus, L. luscinioides; P ibe, Phylloscopus ibericus; P troc, P. trochilus; Pa do, Passer domesticus; Pa mo, P. montanus*.)

	Lineage	*C cet*	*A aru*	*A sci*	*L lus*	*P ibe*	*P tro*	*Pa do*	*Pa mo*	HB	S_{TD*}
Haemoproteus	GRW01		12							$1{\cdot}1\times10^{-1}$	1
	GRW16		1							$7{\cdot}3\times10^{-4}$	1
	HIPOL1			1						$5{\cdot}6\times10^{-6}$	1
	MW1			40	1					$2{\cdot}6\times10^{-2}$	3·00
	PADOM23							1		$6{\cdot}8\times10^{-5}$	1
	RW1			5						$1{\cdot}4\times10^{-4}$	1
	SW1			2						$2{\cdot}3\times10^{-5}$	1
	WW1						1			$7{\cdot}7\times10^{-4}$	1
	WW2						1			$7{\cdot}7\times10^{-4}$	1
Plasmodium	BT6					1				$1{\cdot}4\times10^{-3}$	1
	CET01	1								$1{\cdot}0\times10^{-5}$	1
	COLL1				1					$4{\cdot}7\times10^{-4}$	1
	GRW02		1							$7{\cdot}3\times10^{-4}$	1
	GRW04		3	25	2					$8{\cdot}0\times10^{-2}$	2·12
	GRW06			12	1					$6{\cdot}2\times10^{-3}$	3·00
	GRW11	8		2				5		$1{\cdot}6\times10^{-2}$	3·91
	GRW17		1							$7{\cdot}3\times10^{-4}$	1
	PADOM01							2		$2{\cdot}7\times10^{-4}$	1
	RTSR1			3						$5{\cdot}1\times10^{-5}$	1
	SGS1	159	2	9				37	16	4·7	3·35
	SW2			1						$5{\cdot}6\times10^{-6}$	1
	SW5			3						$5{\cdot}1\times10^{-5}$	1
	SYAT05	3								$9{\cdot}4\times10^{-5}$	1
	WW4				2					$1{\cdot}9\times10^{-3}$	1

Tornada: NODF = 2·56, null model's NODF = 5·78, p = 0·81; all areas: NODF = 20·61, null model's NODF = 19·70, p = 0·39).

The parasites in the studied community had HB indices between $5{\cdot}6\times10^{-6}$ and 4·7, and S_{TD*} indices between 1 (when only 1 host species was found) and 3·91 (Table 2). When compared with the host range indices obtained from MalAvi with Monte Carlo simulations, the lineage *H*. MW1 appeared as significantly more generalist in our study than in studies reported in MalAvi: the probability of finding a smaller index in the random simulations than in the studied system was 0·028 using HB and 0·049 using S_{TD*}. Two other lineages, BT6 and WW4, also showed as being significantly more generalist in the studied system, with the HB index ($P = 0{\cdot}005$ and 0·002, respectively). Because these lineages only had 1 host species in this study, they did not give significant results with STD* (the significant result with HB being due only to higher prevalences in our study than in the simulations).

DISCUSSION

Haemoproteus lineages were mostly found in migrants, and almost always in adults, with 3 exceptions: 1 resident house sparrow and 2 juvenile willow warblers during their post-breeding migration. All these individuals had certainly spent plenty of time outside the studied reed beds and could have been infected elsewhere. This suggests that there is no transmission of *Haemoproteus* lineages in our study areas. However, this is not the case for other European areas; for example, transmission of *H*. WW2 to the willow warbler has been proved to happen in Swedish woodlands (Bensch and Akesson, 2003). This agrees with the fact that *Haemoproteus* main known vectors, the biting midges (genus *Culicoides*, Ceratopogonidae), prefer forested habitats and seem to be absent from the studied reed beds (R. Ventim, *unpublished data*). Also, *Haemoproteus* spp. appears to have high affiliation to a single transmission area and a single bird fauna, despite the vast numbers of infected birds that perform annual migrations between Africa and Europe (Hellgren *et al*. 2007). Therefore, it is not expected that African-transmitted *Haemoproteus* lineages would be able to adapt to the European conditions and vectors and be able to be transmitted to new hosts in their breeding quarters. *Plasmodium* parasites do this more often, as is the case for SGS1 (Hellgren *et al*. 2007); so some of the *Plasmodium* lineages that were present in this community are expected to be transmitted locally as well as in Africa. Local transmission of *Plasmodium* lineages COLL1, GRW17, GRW11, SGS1, SW2, SW5 and SYAT05 was proven in our studied reed beds, because these parasites were found in birds that should have spent

https://doi.org/10.1017/S0031182011002083 Published online by Cambridge University Press

most of their lives in those areas (resident species or juveniles from migrant warbler species, all still attached to their birth reed bed).

Parasite distribution in the different hosts was not random, indicating that there are specific host preferences for each lineage. This agrees with the fact that most lineages in this study were only detected in 1 of the analysed bird species, supporting our assumption that these are relatively specialized parasites. Nestedness was not detected in this interaction network. In a nested matrix, specialist parasites would concentrate in the most parasitized species of birds, sharing their hosts with generalist parasites (Bascompte *et al*. 2003; Bascompte and Jordano, 2007). Since our matrix is not nested, in our case specialists do not always share hosts with generalist parasites, so they are free from the competitive pressure of generalists. This happened with BT6, WW1 and WW2, lineages that appeared as specialists in the matrix and were present in bird species that were not infected by generalist haemoparasites (the Iberian chiffchaff and the willow warbler). These findings are the opposite from what was found by Graham *et al*. (2009) for ectoparasite-vertebrate host networks in general; this large-scale study analysed networks of mosquitoes, lice, mites, ticks and fleas and mammals, birds, reptiles, amphibians and fish. It found nested structures, meaning that specialized ectoparasites interact with hosts that attract many parasites, while generalist parasites interact with these hosts as well as those that attract fewer parasites. This structure does not seem to apply to all host-parasite interaction networks.

Parasite lineages with higher overall prevalence in the bird community were those infecting a greater number of host species. Moreover, parasites with a broad host range reached high prevalence over a greater number of species, as was also found by Ricklefs *et al*. (2005) and Hellgren *et al*. (2009). Such lineages will be transmitted to vectors more often and, if they are host generalist, a higher proportion of the vectors' bloodmeals will end up in successful transmission. The encounter rate between these parasites and all species in the bird community increases, leading to higher prevalence in all of the hosts. Hence, the prevalence in each host species is amplified due to a wide host range (Hellgren *et al*. 2009).

The most prevalent of all lineages was *P*. SGS1, a lineage of the morpho-species *Plasmodium relictum* (Palinauskas *et al*. 2007). This parasite is known to be very host generalist, infecting hosts from over a dozen different families in distinct continents (Bensch *et al*. 2009 and references therein, such as: Hellgren *et al*. 2007; Beadell *et al*. 2006). In our study, *P*. SGS1 had a high prevalence in the domestic sparrow and Cetti's warbler, but was not as successful infecting reed and great reed warblers, two hosts that had high infection rates by other parasite lineages. These host species had already been described to be infected with SGS1 at similarly low prevalences (Dimitrov *et al*. 2010; Waldenström *et al*. 2002; Zethindjiev *et al*. 2008). This suggests that these are not optimal hosts and that even a generalist parasite can have limited success infecting some hosts.

H. MW1 appeared to be significantly more generalist in our study areas than in most studies reported in MalAvi. Until now, MW1 had only been found in 3 *Acrocephalus* species (Krizanauskiene *et al*. 2006; Waldenström *et al*. 2002), even when many other host families were sampled concurrently. The present study found that it is also capable of infecting the Savi's warbler, a host from a different genus and family that had not been sampled in the previous studies. This lineage seems to have narrow habitat preferences, concentrating in marsh warblers; therefore, it appeared to be more generalist in the studied reed bed communities than in studies (or simulations) involving hosts from other habitats. This exemplifies how the apparent specialization can sometimes depend on the type of host species that are sampled. More research is needed in order to discover more host-parasite associations and thus unveil more details of these complex interaction systems.

ACKNOWLEDGEMENTS

The Portuguese Instituto para a Conservação da Natureza e Biodiversidade provided official permits for bird sampling, as well as logistic support in the field. The authors would like to thank the help of volunteer students and ringers during the field work, as well as the comments of two anonymous referees to earlier versions of the manuscript.

FINANCIAL SUPPORT

This project was supported by the Fundação para a Ciência e Tecnologia (R.V., grant number SFRH/BD/28930/2006; L. M., grant number SFRH/BPD/20682/2004) and the Spanish Ministry of Science and Innovation (J.P.-T., project number CGL2007-62937/BOS).

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