

1 **Host-parasite interaction explains variation in prevalence of avian**  
2 **haemosporidians at the community level.**

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4 L. Garcia-Longoria<sup>1\*</sup>, A. Marzal<sup>1</sup>, F. de Lope<sup>1</sup> and L. Z. Garamszegi<sup>2</sup>

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6 <sup>1</sup> Departamento de Anatomía, Biología Celular y Zoología, Universidad de  
7 Extremadura, E-506071 Badajoz (Spain).

8

9 <sup>2</sup> Department of Evolutionary Ecology, Estación Biológica de Doñana-CSIC, E-41092  
10 Seville, Spain

11

12 \*Corresponding author: e-mail: luzlongoria@unex.es

13 Tel.: 0034924289412

14 Fax: 0034924289417

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16 **ABSTRACT**

17 Parasites are a selective force that shape host community structure and dynamics, but  
18 host communities can also influence parasitism. Understanding the dual nature from  
19 host-parasite interactions can be facilitated by quantifying the variation in parasite  
20 prevalence (i.e. the proportion of infected host individuals in a population) among host  
21 species and then comparing that variation to other ecological factors that are known to  
22 also shape host communities. Avian haemosporidian parasites  
23 (e.g. *Plasmodium* and *Haemoproteus*) are abundant and widespread representing  
24 an excellent model for the study of host-parasite interactions. Several geographic and  
25 environmental factors have been suggested to determine prevalence of avian  
26 haemosporidians in bird communities. However, much remains to be known regarding  
27 whether host and parasite traits, represented by phylogenetic distances among species  
28 and degree of specialization in host-parasite relationships, can influence parasite  
29 prevalence. The aims of this study were to analyze factors affecting prevalence in a bird  
30 community and to test whether the degree of parasite specialization on their hosts is  
31 determined by host traits. Our statistical analyses suggest that prevalence is mainly  
32 determined by the interaction between host species and parasite lineages where  
33 tolerance and/or susceptibility to parasites plays an essential role. Additionally, we  
34 found that although some of the parasite lineages infected a low number of bird species,  
35 the species they infected were distantly related and therefore the parasites themselves  
36 should not be considered typical host specialists. Prevalence was higher for generalist  
37 than for specialist parasites in some, but not all, host species. These results suggest that  
38 prevalence mainly results from the interaction between host immune defences and  
39 parasite exploitation strategies wherein the result of an association between particular  
40 parasite lineages and particular host species is idiosyncratic.

41 Keywords: bird community, *Haemoproteus*, host-parasite interaction, *Plasmodium*,  
42 prevalence.

## 43 INTRODUCTION

44 Parasites have been suggested as a selective force since they might shape host  
45 community dynamics [1], alter interspecific competition and influence energy flow [2].  
46 An essential trait in studies of host-parasite interactions is prevalence (i.e. the  
47 proportion of individuals infected by a parasite or pathogen in a population at one point  
48 in time) [3]. The importance of prevalence is reflected in the amount of studies showing  
49 how major ecological factors shaped its intra- and interspecific variation [4,5]. In bird  
50 communities, geographic areas, environmental issues or host species/population have  
51 been suggested as factors determining prevalence [6]. However, more studies are still  
52 needed in order to understand mainly factors affecting parasite prevalence at the  
53 community level.

54 Avian *Plasmodium* and *Haemoproteus* spp. represent a well-studied host-  
55 parasite system. These parasites are vector-transmitted organisms that can cause host  
56 mortality or morbidity during the acute phase of infection impacting the life histories of  
57 their hosts [7]. Their life-cycle is complex, involving sexual stages in their dipteran  
58 vectors and asexual stages in their vertebrate hosts [7]. Prevalence of these parasites  
59 may be affected by different factors concerning the vector, hosts and the parasites  
60 themselves [7]. Some authors have focused their attention on the possibility that host  
61 traits might determine prevalence of these parasites [8–10]. For instance, blood parasite  
62 susceptibility of both bird individual [11] and bird species [12,13] have been recently  
63 suggested as factors affecting prevalence. In this sense, some bird species may have  
64 developed both tolerance and resistance mechanisms [14] underlying the importance of  
65 bird species in prevalence studies.

66 In addition to characteristics of hosts, characteristics of parasites themselves can  
67 affect their prevalence in wild communities. Haemosporidians parasites present high

68 plasticity and versatility reflected in the number of parasite lineages found among bird  
69 species. Thus, within haemosporidian parasites there are more than 3000 parasite  
70 lineages [15] infecting more than 1500 bird species. Each of these lineages may have  
71 diverse virulence [16] which could result in an array of different negative effects inside  
72 the hosts. The plasticity that malaria lineages may have [17,18] allow these parasites to  
73 fully exploit hosts reacting even to changes in the physiological state of the host or the  
74 environment [19]. Thus, prevalence displayed by parasite lineage in a determined host  
75 species or individual is simply the interaction between host immune defences  
76 (susceptibility or tolerance) and parasite strategy (virulence). Additionally, malaria  
77 parasites must find the correct host in order to complete its life cycle and achieve a high  
78 transmission rate [3,20]. To find the precise host is crucial for specialist parasites that  
79 need a particular bird species unlike generalist parasites that infect a wider range of bird  
80 species [21]. However, and despite the large number of studies focused on  
81 haemosporidian parasites, there are a scarce number of them dealing with factors  
82 affecting prevalence in wild communities from the perspectives of both the host and  
83 parasites.

84 *Plasmodium* and *Haemoproteus* spp. might be detected in several host species  
85 that can be distantly or closely related to each other [22–24]. Mechanisms allowing  
86 parasite to switch host is a central issue nowadays in avian malaria studies [25,26]. Host  
87 switching mechanism is a strategy that malaria parasites may use, in order to infect  
88 larger number of bird species [27,28], where closely parasite lineages may be detected  
89 in bird species with similar immune defences [29–31]. Thus, parasite lineages infecting  
90 close related bird species may exhibit the same exploitation strategy for avoiding host  
91 defences [32]. Conversely, more generalist parasites would be able to infect a broad  
92 range of bird species that might be distantly related [33]. Analysing phylogenetic

93 distances between bird species sharing the same parasite lineages become essential in  
94 community studies in order to determine parasite strategies and how specialist and  
95 generalist parasites exploit different host species. However, little is know about how  
96 haemosporidian parasite lineages infect bird host species depending on the host  
97 phylogenetic distance.

98         The fact that haemosporidian parasites need a vector for completing their cycle  
99 life extends the number of elements affecting prevalence of these parasites. Although  
100 some studies have shown limited or no relationship between climate and prevalence  
101 [34], some have showed that vector populations might increase their number due to  
102 climate change and, therefore, vector-borne infectious diseases may increase every year  
103 [35]. Vector availability might also change among seasons, thus, during spring the  
104 amount of vectors in the wild increase [36] raising the probabilities to become infected  
105 [37]. During this season the probability of becoming infected increases significantly not  
106 only because of vector availability but also because of the secretion of sexual hormones  
107 that may alter immune system and, therefore, facilitate the entrance of blood parasite in  
108 the vertebrate host [38]. These studies emphasize the importance of including both year  
109 and season of sampling for studies focused in prevalence of haemosporidians  
110 prevalence when analysing communities.

111         Taken as a whole, studies focused on prevalence at the community level have  
112 analysed certain factors affecting the presence of prevalence separately. However, to  
113 date there is no study dealing with the main factors (i.e. host species, parasite lineage  
114 and vector conditions) that might affect the presence of haemosporidians parasites or  
115 with the parasite strategy depending on the host phylogenetic distance. Hence, the aims  
116 of this study are (i) to analyse factors affecting prevalence of haemosporidian parasites  
117 in a wild bird community such as phylogenetic distances, parasite phylogeny, host ID,

118 parasite lineage or seasonal effects and (ii) compare the phylogenetic distances among  
119 bird species infected with the same parasite lineage in order to determine whether the  
120 parasite infects phylogenetic close host species. For this purpose, factors such as year,  
121 day of sampling, bird species, parasite lineage, season of sampling, host-parasite  
122 interaction and phylogenetic relationships among avian hosts were included in the  
123 analyses and related with prevalence of haemosporidian parasites.

124

## 125 **MATERIAL AND METHODS**

### 126 **Data collection and database**

127 The study was carried out from February to October during a 9-year period (2002-2010)  
128 in the surroundings of Badajoz (SW Spain) (38°52'N, 705'W). Birds were collected  
129 always during the sunrise and during the early hours of the morning. Using a mist-net  
130 system, we captured a total of 815 individuals from 21 bird species belonging to nine  
131 different families. We obtained one microcapillary of blood (70µl) from the brachial  
132 vein of each individual and stored it in 500 µl of 96% ethanol until analysis.

133 Methods were approved by Institutional Commission of Bioethics of Univ. of  
134 Extremadura (CBUE 49/2011)

135

### 136 **Prevalence and genetic detection of parasite lineages**

137 DNA from the avian blood samples were extracted in the laboratory using a standard  
138 chloroform/isoamylalcohol method [39]. Diluted genomic DNA (25 ng/µL) was used  
139 as a template in a polymerase chain reaction (PCR) assay for detection of the parasites  
140 using the nested-PCR protocols described [40]. The amplification was evaluated by  
141 running 2.5 µL of the final PCR on a 2% agarose gel. All PCR experiments contained  
142 one negative control for every eight samples. In the very few cases of negative controls

143 showing signs of amplification (never more than faint bands in agarose gels), the whole  
144 PCR-batch was run again to make sure that all positives were true. All samples with  
145 positive amplification were sequenced directly using procedures described [41]. The  
146 obtained sequences of 478 bp of the cyt b were edited, aligned and compared in a  
147 sequence identity matrix using the program BioEdit [42]. Parasites with sequences  
148 differing by one nucleotide substitution were considered to represent evolutionary  
149 independent lineages [43]. Five new sequences were deposited in GenBank under the  
150 accession numbers JQ749720 – JQ749724.

151

## 152 **Phylogenetic analyses and statistical procedures**

153 For our statistical models (see below) we created two different phylogenetic trees  
154 (supporting information Figs S3 and S4) to control for the common descent of different  
155 parties: one for the host of bird species and one for the parasite lineages. The first one  
156 relied on the 1000 trees generated by the birdtree.org website [44], from which a  
157 consensus tree was created for the sampled 21 species by using Geneious v5.4 [45]. The  
158 second tree relied the sequences of the parasite lineages [15] identified in this study.  
159 Then, we created a phylogenetic tree using MrBayes 3.1 [46,47]. We used jModelTest  
160 0.1 [48] in order to determine which parasite tree offered the best fit to our data. The  
161 burn-in was identified through Tracer 1.2.2 [49]. We sampled 10 million generations at  
162 intervals of 1000. Finally we analysed the files generated by Bayesian MCMC runs by  
163 MrBayes in Tracer with the objective of confirm whether the parasite tree generated  
164 was the most adequate to our analyses.

165         These phylogenetic trees were entered in the subsequent statistical models  
166 sequentially to evaluate the same list of predictors (first, we run the model using the  
167 bird phylogenetic tree and then we run the same model but we used the parasite



168 phylogenetic tree). The general strategy for our modelling was to incorporate different  
169 factors that might be related to: (i) vector effects (breeding season or non-breeding  
170 season, year and date of sampling), (ii) host effects (host species, phylogenetic  
171 relatedness), (iii) parasite effects (lineage identity and parasite phylogenetic  
172 relatedness), and (iv) host-parasite interaction (the combination of bird species and  
173 parasite lineage). Given that our parasite screening method allowed us to detect  
174 prevalence (yes or no) at the individual level for each parasite lineage screened, we  
175 could also incorporate effects due to host individual identity. We used the Bayesian  
176 framework for generalized mixed models (GLMM) incorporating Markov chain Monte  
177 Carlo (MCMC) estimation available in the package ‘MCMCglmm’ [50]. Because  
178 dependent variable (a given parasite lineage detected or not in a given individual of a  
179 given species) has a binomial distribution, we adopted the “categorical” family of  
180 distribution. The fixed variables we considered were the date of sampling and the  
181 breeding status of individuals (breeding or not), as these factors may affect infection  
182 status due to mosquito abundance/activity and susceptibility of hosts during the  
183 demanding chick-feeding period [7]. The year of sampling, parasite lineage, bird  
184 individual identity, host species and phylogenetic relationships (parasite or host) were  
185 used as random factors. Additionally, we also included the interaction between host  
186 species and parasite lineage as a random factor [11,51]. Plots were made with the R  
187 package ggplot2 (v. 2.1.0) [52]. All the statistical analyses were carried out with the  
188 program R v.1.1.383 [53].

189         We used uninformative priors with a low degree of belief in all parameters. The  
190 model was run for 130000 iterations preceded by a burn-in of 30000 iterations, and  
191 sampling every 100 iterations to avoid autocorrelation. We evaluated model  
192 convergence visually by plotting the chains and checking that they had mixed properly

193 and by plotting the autocorrelation (supporting information Figs S1 and S2). Once both  
194 model were finished we rescaled our parameters by estimating the marginal parameter  
195 modes using Kernel Density Estimation [50].

196 We examined phylogenetic relatedness of bird species infected by the same  
197 lineages, and determined if species with non-zero prevalence are more closely related to  
198 each other than could be expected by chance. The significance of this metric was tested  
199 by comparison to a null distribution derived from 999 random permutations among the  
200 tips of the phylogenetic tree followed by calculation of the main pairwise distance  
201 (MPD). Calculations were carried out with the `ses.mpd` function of the R package  
202 Picante [54]. When running a main pairwise distance calculation different value comes  
203 out. Thus, MPDobs = observed mean pair-wise phylogenetic distance between all  
204 species pairs infected with the parasite. The mean and standard deviation of MPD in the  
205 null distribution were obtained by randomization of species in the phylogenetic distance  
206 matrix (`taxa.labels` method in Picante).  $Z = (\text{MPDobs} - \text{mean MPD of the null}$   
207  $\text{distribution})/\text{SD of the null distribution}$ . Negative values of  $Z$  indicate greater  
208 phylogenetic homogeneity (clustering).  $P$  is the probability of drawing an MPD from  
209 the null distribution at least as extreme as MPDobs, based on 999 randomizations.

210

## 211 **RESULTS**

### 212 **Total prevalence and lineages detected**

213 We determined the presence of 26 parasite lineages belonging to the genera  
214 *Haemoproteus* (N = 13) and *Plasmodium* (N = 13) in the analysed host species (Table  
215 1). We found that 63.80% of total individuals and 81 % of species were infected by  
216 haemosporidian parasites. We detected five new parasite lineages that had not been  
217 previously described (Table 2).

218 **Table 1.** Prevalence and genetic identity of haemosporidian parasite lineages in Passeriform host species analyzed in this study. and parasite  
 219 lineage found in each host species.

| FAMILY                    | SPECIES                       | PREVALENCE (%)<br>(N infected / N tested) | PARASITES LINEAGES  |
|---------------------------|-------------------------------|---|---|
| Aegithalidae              | <i>Aegithalos caudatus</i>    | 0 (0 / 4)                                 |   |
| Certhiidae                | <i>Certhia brachydactyla</i>  | 0 (0 / 8)                                 |   |
| Fringillidae              | <i>Carduelis cannabina</i>    | 20 (1 / 5)                                | SGS1  |
|                           | <i>Carduelis carduelis</i>    | 18.75 (3 / 16)                            | CARDUEL1,CARDUEL2   |
|                           | <i>Serinus serinus</i>        | 6.67 (1 / 15)                             | CCF2  |
|                           | <i>Carduelis chloris</i>      | 16.67 (1 / 6)                             | CARDUEL3  |
|                           | <i>Fringilla coelebs</i>      | 10.0 (1 / 10)                             | SGS1  |
| Hirundinidae              | <i>Delichon urbicum</i>       | 68.55 (266 / 388)                         | DURB6, GRW2, GRW4, GRW9, SGS1, RTSR1, DELURB1, DELURB2, DELURB3,<br>DELURB5   |
|                           | <i>Hirundo rustica</i>        | 17.35 (30 / 168)                          | DELURB2, DELURB5, GRW9  |
|                           | <i>Riparia riparia</i>        | 14.28 (4 / 28)                            | CCF2  |
| Paridae                   | <i>Cyanistes caeruleus</i>    | 33.33 (2 / 6)                             | SGS1  |
|                           | <i>Parus major</i>            | 25 (2 / 8)                                | HIPOL1, SGS1  |
| Passeridae                | <i>Passer domesticus</i>      | 64.57 (177 / 275)                         | COLL1, GRW11, PADOM2, PADOM5, PADOM1, SGS1, PADOM8, PADOM22,<br>PAHIS1, GRW11 |
|                           | <i>Passer hispaniolensis</i>  | 67.74 (21 / 31)                           | PADOM5, SGS1, PADOM1  |
| Sturnidae                 | <i>Sturnus unicolor</i>       | 0 (0 / 5)                                 |   |
| Sylviidae                 | <i>Phylloscopus collybita</i> | 7.14 (1 / 14)                             | HIPOL1  |
|                           | <i>Sylvia atricapilla</i>     | 28.57 (2 / 7)                             | SGS1  |
|                           | <i>Sylvia melanocephala</i>   | 12.12 (4 / 33)                            | SGS1, SYMEL1  |
| Turdidae                  | <i>Erithacus rubecula</i>     | 16.67 (1 / 6)                             | ROBIN1  |
|                           | <i>Luscinia megarhynchos</i>  | 0 (0 / 15)                                |   |
|                           | <i>Turdus merula</i>          | 30 (3 / 10)                               | SYAT5, TURDUS3  |
| TOTAL INDIVIDUAL ANALYZED |                               | Mean = 63.80 (520 / 815)                  |   |

221 **Table 2.** List of parasite lineages found in the current study. New detected parasite  
 222 lineages are marked in bold.

| Parasite lineage | Morphological species          | GenBank acc.<br>Number | Number of host<br>species infected |
|------------------|--------------------------------|------------------------|------------------------------------|
| COLL 1           | <i>Plasmodium sp.</i>          | AY831747               | 1                                  |
| DELURB1          | <i>Haemoproteus hirundinis</i> | EU154343               | 1                                  |
| DELURB2          | <i>Haemoproteus sp.</i>        | EU154344               | 2                                  |
| DELURB3          | <i>Haemoproteus sp.</i>        | EU154345               | 1                                  |
| DELURB5          | <i>Plasmodium sp.</i>          | EU154347               | 2                                  |
| DURB6            | <i>Plasmodium sp.</i>          | EU219392               | 1                                  |
| GRW11            | <i>Plasmodium relictum</i>     | AY831748               | 1                                  |
| GRW2             | <i>Plasmodium ashfordi</i>     | AF254962               | 1                                  |
| GRW4             | <i>Plasmodium relictum</i>     | AF254975               | 1                                  |
| GRW9             | <i>Plasmodium sp.</i>          | EU810681               | 2                                  |
| HIPOL1           | <i>Haemoproteus sp.</i>        | DQ000324               | 2                                  |
| ROBIN1           | <i>Haemoproteus attenautus</i> | AY393807               | 1                                  |
| CCF2             | <i>Haemoproteus sp.</i>        | AF495551               | 2                                  |
| <b>CARDUEL1</b>  | <b><i>Haemoproteus sp.</i></b> | <b>JQ749720</b>        | <b>1</b>                           |
| <b>CARDUEL2</b>  | <b><i>Haemoproteus sp.</i></b> | <b>JQ749721</b>        | <b>1</b>                           |
| <b>CARDUEL3</b>  | <b><i>Haemoproteus sp.</i></b> | <b>JQ749722</b>        | <b>1</b>                           |
| <b>SYMEL1</b>    | <b><i>Haemoproteus sp.</i></b> | <b>JQ749723</b>        | <b>1</b>                           |
| <b>TURDUS3</b>   | <b><i>Plasmodium sp.</i></b>   | <b>JQ749724</b>        | <b>1</b>                           |
| PADOM01          | <i>Plasmodium sp.</i>          | DQ058611               | 2                                  |
| PADOM02          | <i>Plasmodium sp.</i>          | DQ058612               | 1                                  |
| PADOM05          | <i>Haemoproteus passeris</i>   | HM146898               | 2                                  |
| PADOM08          | <i>Plasmodium sp.</i>          | GU065648               | 1                                  |
| PADOM22          | <i>Haemoproteus sp.</i>        | GU065650               | 1                                  |
| PAHIS1           | <i>Haemoproteus sp.</i>        | GU065651               | 1                                  |
| SGS1             | <i>Plasmodium relictum</i>     | AF495571               | 9                                  |
| RTSR1            | <i>Plasmodium sp.</i>          | AF495568               | 1                                  |

223

224 **Factors affecting prevalence**

225 Our results suggest that bird defences and parasite virulence (i.e. host-parasite  
226 interaction) affect infection status stronger than the rest of the variables analysed (Table  
227 3) where this interaction might be the most important factor determining prevalence.  
228 Additionally, two more factors appeared to affect variance in prevalence, although on a  
229 lesser scale. Thus, our models suggested that breeding season and parasite lineage might  
230 affect haemosporidian prevalence.

231 **Table 3.** Variance of prevalence in relation to fix and random factors. Results of both models are showed. Bolded values represent factors that  
 232 were statistically significant.

|                            | Bird             |                               |               |               | Parasite      |                           |               |               |
|----------------------------|------------------|-------------------------------|---------------|---------------|---------------|---------------------------|---------------|---------------|
|                            | Mean             | Lower/upper 95% CI            | <i>p</i> MCMC | Rescaled mean | Mean          | Lower/upper 95% CI        | <i>p</i> MCMC | Rescaled mean |
| <i>Fixed factors</i>       |                  |                               |               |               |               |                           |               |               |
| Day                        | -4.055e-03       | -8.653-03 / 7.416e-04         | 0.108         | -0.004        | -3.503e-03    | -8.545e-03 / 7.867e-04    | 0.152         | -0.035        |
| <b>Breeding season</b>     | <b>1.825e+03</b> | <b>-3.292e-01 / 4.2623+00</b> | <b>0.092</b>  | <b>2.071</b>  | <b>1.7373</b> | <b>-1.212e-01 / 3.842</b> | <b>0.074</b>  | <b>1.775</b>  |
| <i>Random factors</i>      |                  |                               |               |               |               |                           |               |               |
| Year                       | 1.616            | 0.243 / 4.364                 |               | 0.415         | 1.367         | 0.181 / 3.374             |               | 0.578         |
| <b>Lineage</b>             | <b>2.208</b>     | <b>0.0002 / 4.897</b>         |               | <b>1.335</b>  | <b>1.436</b>  | <b>0.0002 / 3.600</b>     |               | <b>1.009</b>  |
| Individual                 | 0.006            | 0.0001 / 0.018                |               | 0.005         | 0.007         | 0.0002 / 0.021            |               | 0.007         |
| Species                    | 0.126            | 0.0003 / 0.567                |               | 0.007         | 0.166         | 0.0001 / 0.845            |               | 0.006         |
| <b>Linage*<br/>Species</b> | <b>13.26</b>     | <b>8.318 / 21.97</b>          |               | <b>7.007</b>  | <b>11.030</b> | <b>6.785 / 15.590</b>     |               | <b>7.076</b>  |
| Phylo                      | 0.008            | 0.0002 / 0.028                |               | 0.007         | 0.1629        | 0.0002 / 0.747            |               | 0003          |

233

234

## 235 **Infected bird species and phylogenetic distances**

236 The random effects for parasite lineage shows that there are considerable differences  
237 among parasite lineages in their host exploitation strategies. We found that the most  
238 prevalent parasite lineage was *Plasmodium relictum* SGS1 as it was detected in 9 out of 21  
239 host species. However, the prevalence in particular host species was quite variable (see  
240 Fig. 1). In contrast, the other lineages seem to be more specialists, at the present study,  
241 as they were detected in fewer species.

242

243 **Figure 1:** Prevalence of each parasite lineage in every host species found in the  
244 community. Bird species with no infection are not present in the figure. Lineages  
245 infecting more than one bird species have been colored marked while parasite lineages  
246 infected only one host species remain in grey. Noted that depending on the sample size  
247 the dot showing the prevalence change its magnitude.

248

249

250 We detected 8 different lineages infecting, at least, two host species (Fig. 1).  
251 Thus, we analysed the phylogenetic distance between the hosts species infected by one  
252 relative to the average phylogenetic distance between two species on the entire tree. Our  
253 results showed that *Haemoproteus* spp. CCF2 lineage, *Plasmodium* spp. HIPOL1,  
254 GRW9 and *P. relictum* SGS1 infected non-closely related bird species. Moreover, we  
255 detected that *Haemoproteus passeris* PADOM05 lineage; *Haemoproteus* spp.  
256 DELURB2 lineage and *Plasmodium* spp. DELURB5 and PADOM01 lineages tended to  
257 infect closely related bird species (Table 4).

258

259 **Table 4:** Phylogenetic dispersion of parasite lineages infecting more than one host  
 260 species.  $N$  = number of bird species infected. Bolded values represent parasite lineages  
 261 that were detected in close phylogenetic bird species.

|  | $N$      | MPD <sub>obs</sub> | Mean $\pm$ SD                       | $Z$           | $P$          |
|--|----------|--------------------|-------------------------------------|---------------|--------------|
| SGS1 <i>P. relictum</i>                | 9        | 79.976             | 81.829 $\pm$ 2.609                  | -0.710        | 0.222        |
| <b>PADOM05 <i>H. passeris</i></b>      | <b>2</b> | <b>5.546</b>       | <b>82.695<math>\pm</math>17.199</b> | <b>-4.485</b> | <b>0.002</b> |
| CCF2 <i>Haemoproteus</i> sp.           | 2        | 91.536             | 80.881 $\pm$ 18.287                 | 0.582         | 0.853        |
| <b>DELURB2 <i>Haemoproteus</i> sp.</b> | <b>2</b> | <b>33.574</b>      | <b>82.087<math>\pm</math>17.088</b> | <b>-2.838</b> | <b>0.038</b> |
| <b>DELURB5 <i>Plasmodium</i> sp.</b>   | <b>2</b> | <b>33.574</b>      | <b>82.111<math>\pm</math>17.851</b> | <b>-2.713</b> | <b>0.047</b> |
| GRW9 <i>Plasmodium</i> sp.             | 2        | 33.574             | 81.479 $\pm$ 18.615                 | -2.573        | 0.054        |
| HIPOL1 <i>Haemoproteus</i> sp.         | 2        | 86.983             | 81.817 $\pm$ 17.714                 | 0.291         | 0.304        |
| <b>PADOM1 <i>Plasmodium</i> sp.</b>    | <b>2</b> | <b>5.546</b>       | <b>81.385<math>\pm</math>18.107</b> | <b>-4.188</b> | <b>0.003</b> |

262

## 263 DISCUSSION

264 In this study of haemosporidian parasites infecting a community of wild birds  
 265 breeding/migrating in the same area, we found that detected prevalence is mostly  
 266 determined by the interaction between host and parasite identity. We also found that  
 267 variation in prevalence might be affected by breeding season and parasite lineage.  
 268 Additionally, we showed that *P. relictum* SGS1 is present in most of the bird species  
 269 but with variable prevalence, while other parasites are more specialist parasites  
 270 infecting fewer and close related host species as *H. passeris* PADOM05. Next, we  
 271 discuss these main results in detail.

272 Virulence of *Plasmodium* and *Haemoproteus* spp. might be highly variable  
 273 between species and lineages. Some malaria parasites might affect host fitness [55,56]  
 274 and others might even influence survival [57–59]. Conversely, host bird species and  
 275 bird individual might also differ in the prevalence displayed due to the tolerance or



276 susceptibility of each organism [11]. Thus, the interaction between parasite lineage and  
277 host bird species ultimately determines infection severity and therefore prevalence. Our  
278 results support this idea where variation in prevalence was mainly affected by the  
279 interaction between parasite lineage and bird host species. In this sense, it has been  
280 suggested that avian malaria parasites (*Plasmodium ashfordi*) express a particular host-  
281 specific expression pattern depending on the infected bird [11]. This study highlighted  
282 the role that the interaction between host immune defence (susceptibility or resistance to  
283 parasites) and parasite strategy (virulence) may play in the prevalence displayed. The  
284 mechanisms behind this host-parasite interaction are still limited where only some target  
285 genes related, for instance, with red blood cell invasion have been deeply analysed  
286 [60,61].

287         With regard to the previous results, prevalence was also affected by parasite  
288 lineage. These results emphasize the importance not only of the host-parasite interaction  
289 per se but also the parasite exploitation strategy that might vary depending on the bird  
290 identity [11]. In this sense, it has been shown that avian malaria parasites might develop  
291 a wide range of different strategies in order to avoid host defences. For example,  
292 *Plasmodium* spp. can display some evasive mechanisms evading host defences by  
293 clonal antigenic switches [62] or even avoid consequences of recognition by the  
294 immune system [63]. Additionally, it has been shown that, during mix infections, these  
295 parasite might compete with one another for access to the available hosts, thus, some  
296 lineages show more prevalence in certain bird species simply because an specific  
297 lineage may eliminate other parasite [23]. Hence, parasite lineage might play an  
298 essential role determining the prevalence displayed in a host rather the host bird species  
299 itself.

300 Our results also showed that breeding season affected prevalence displayed in  
301 the community. Previous studies have shown that, during breeding season prevalence of  
302 these parasite significantly increase [64,65] as a consequence of vector availability. The  
303 increase of vectors in the community may expand blood parasites among individuals  
304 and, therefore, increase prevalence [36]. Our results agree with this idea pointing out a  
305 clear connexion between breeding season, vector availability and prevalence of blood  
306 parasites.

307 It has been extensively discussed how specialist and generalist parasites may  
308 have evolved and how host species are selected to be infected [22,66,67]. Although  
309 specialist parasites are supposed to display higher prevalence and parasitemia on their  
310 avian hosts [21], generalist parasite can occasionally cause significant mortalities in  
311 native birds. For instance, in Hawaii birds *Plasmodium relictum* caused the extinction of  
312 native species of honeycreepers [68,69] despite being one of the most generalist parasite  
313 species. In this sense, it has been suggested that generalist parasites (*P. relictum* GRW4  
314 and SGS1) have the ability to infect a broad range of host species but also display a high  
315 prevalence in some of them [70]. Our results agree with this hypothesis, where *P.*  
316 *relictum* SGS1 was detected in most of the bird species infected, and it displayed  
317 different mean prevalence depending on the bird species. Moreover, this  
318 haemosporidian lineage was detected in unrelated host species highlighting the  
319 parasite's ability to adapt to distantly related host species emphasizing the parasite's  
320 generalist strategy. In the case of *P. relictum* SGS1 the number of potential host species  
321 is 113 [15], where every host individual and species provide a different environment  
322 that need to be overcome by the plasticity of parasites [71]. Although the mechanism  
323 underlying this plasticity is still unknown, it has been recently shown that the  
324 reticulocyte binding proteins, a polymorphic gene family involved in host cell invasion

325 and attributed to host specificity [61], is significantly expanded in *P. relictum* [72]. This  
326 discovery could explain the ability of this parasite species to infect a wide range of  
327 avian species and its overwhelming capacity to infect a wide range of host species [72].  
328 Nevertheless, further studies are needed in order to assess mechanisms allowing  
329 generalist parasites to be present in a large number of bird species.

330 Finally, our analysis stated that the number of infected bird species by the same  
331 parasite lineage do not determine whether this parasite may be more or less specialist. In  
332 this sense, *Haemoproteus* spp. CCF2, HIPO11 lineages and *Plasmodium* spp. GRW9  
333 were detected in distantly related bird species suggesting that, although each of these  
334 lineages was only detected in two bird species, they are not strict specialist parasites  
335 [32]. Additionally, *Haemoproteus* spp. PADOM05, DELURB2 lineages and  
336 *Plasmodium* spp. DELURB5, PADOM1 spp. lineages were detected in closely related  
337 bird species suggesting that these lineages may tend to be specialist parasites [32]. The  
338 prevalence displayed by these parasite lineages varied depending on the bird species  
339 infecting. Thus, in house sparrows (*Passer domesticus*) prevalence was higher for  
340 specialist parasites (*Haemoproteus* spp. PADOM05) than for generalist parasites. In  
341 contrast, Spanish house sparrows (*Passer hispaniolensis*) displayed prevalence was  
342 higher for generalist parasites (*P. relictum* SGS1). These results agree with previous  
343 studies suggesting that, in certain cases, specialist parasites might arise higher  
344 prevalence than generalist parasites [73] but, in other cases, generalist manage to reach  
345 high prevalence [70]. Our results can only confirm that depending on the parasite  
346 lineage and the host bird species prevalence might differ, suggesting that is the host-  
347 parasite interaction ultimately the factor that might affect prevalence in a wild  
348 community.

349           In conclusion, we have shown that, at the community level, the interaction  
350 between host and parasite identity might more strongly affect observed variation in  
351 prevalence than bird host species or parasite lineage alone. These results highlight the  
352 importance of studies focusing on the community level and analysing how different  
353 parasite lineages might interact with a variety of host species. The present study also  
354 highlights that even a parasite (such as *P. relictum* SGS1 and *H. passeris*. PADOM05)  
355 that are considered as generalist or a specialist can display both high and low prevalence  
356 per host species. Taken as a whole, these results suggest that prevalence mainly results  
357 from the interaction between host immune defences and parasite exploitation strategy  
358 where parasite lineage might play an important role following an approach depending  
359 on the bird species.

360

361

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