Host migration strategy and blood parasite infections of three sparrow species sympatrically breeding in Southeast Europe

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Abstract

Mobile hosts like birds occupy a wide array of habitats in which they encounter various vector and parasite faunas. If the infection probability for vector-borne parasites varies among seasons and biomes, a migratory life can critically influence the infections of a host. The growing body of literature on avian blood parasites suggests that host migrations do not only influence prevalence of infection but can also evoke higher infection intensities and increased parasite diversity in migrant compared to resident host species.

We investigated the prevalence, intensity and diversity of *Plasmodium* and *Haemoproteus* infections in three closely-related and sympatrically breeding sparrow species with different migration strategies ranging from residential house sparrow and partially migratory tree sparrow to the obligate migratory Spanish sparrow. With a prevalence of 49%, the migratory Spanish sparrows were significantly less frequently infected than the resident house sparrows (82%). The partially migratory tree sparrow showed an intermediate prevalence of 60%. The parasitaemias were similar in all three host species and indicated mostly chronic but also few acute infections. While we found *Plasmodium* parasites in all three sparrow species, only Spanish sparrows were infected with *Haemoproteus* parasites in our study. With nine clearly identified parasite lineages in our study and the highest number of lineages per infected individuals (i.e. relative diversity) Spanish sparrows harboured the most diverse parasite fauna.

Our results suggest that migration strategies can affect *Plasmodium* and *Haemoproteus* infections of sparrows resulting in a lower parasite prevalence and higher parasite diversity in migratory hosts – at least during our host's breeding period. A general scope for all annual cycle periods and across various bird taxa remain to be elucidated.

Keywords: Bird migration, *Haemoproteus*, host-parasite interaction, *Passer domesticus*, *Passer hispaniolensis*, *Passer montanus, Plasmodium*.

Introduction

Host migration strategy – ranging from fully resident to obligate migratory – and habitat use of hosts are considered prime factors in shaping vector-borne parasite infections (Altizer et al. 2011; Sehgal 2015). The diversity of current migration strategies, in turn, is considered to be the result of differential parasite pressures in various habitats across the globe (O'Connor et al. 2018). The net effect of host migration on parasite prevalence is ambiguous: migration can facilitate transmission through dense multi-species aggregations on hotspots resulting in higher prevalence year-round (Altizer et al. 2011). Alternatively, a migratory lifestyle can also hamper transmission and reduce prevalence when migratory hosts move away from parasite-rich areas for periods of the annual cycle (Loehle 1995), when infected migrants experience a higher mortality compared to non-infected conspecifics (Bradley and Altizer 2005) or when infected individuals migrate spatially or temporally separated from uninfected individuals (Johns and Shaw 2016). Endurance flights have also been associated with changes in physiology and immune function (Eikenaar and Hegemann 2016), which could ultimately affect the susceptibility of avian migrants.

The effect of habitat on vector-borne parasite infections is more straightforward: Ambient humidity and temperature jointly set the developmental conditions of parasites and vectors (Valkiūnas 2005; Pérez-Rodríguez et al. 2013). Insect vectors vary greatly in their developmental requirements and therefore differ in their distribution. For instance, if high humidity and temperature favour the larval development of a certain dipteran vector (Jarošík et al. 2011), it would occur in higher abundance and result in a higher probability of transmission of the associated parasites in humid and warm habitats (Loiseau et al. 2012). As migration increases the annual geographic range, migration amplifies habitat effects: Migratory hosts encounter a greater diversity of habitats and vectors within the annual cycle and, depending on the adaptability of the host-parasite systems, can harbour a more diverse parasite fauna compared to resident species (Figuerola and Green 2000; Koprivnikar and Leung 2015).

Here, we investigate the influence of host migration strategy on the prevalence, intensity and diversity of infections with two genera of haemosporidian parasites in South-eastern European sparrows. We used comparable sample sizes from a single area during the breeding season of three closely related species: the house sparrow *Passer domesticus* (Linnaeus, 1758), the tree sparrow *P. montanus* (Linnaeus, 1758) and Spanish sparrow *P. hispaniolensis* (Temminck, 1820), in order to minimize confounding effects of sampling effort, breeding habitat, season and host phylogeny. The house sparrow is a globally distributed rural and urban breeder with a resident lifestyle throughout Europe (Cramp 1994). Haemosporidian parasites in the house sparrow are

well studied, with global prevalence of around 40% – ranging from 0% in island populations and in the north of Europe to over 80% in the south of continental Europe (Marzal et al. 2011). In the public reference database about haemosporidian parasites of birds one can find 12 genetic lineages of *Plasmodium* and *Haemoproteus* identified so far for house sparrows in Southeast Europe (MalAvi database accessed on the 08/06/2018; Bensch et al. 2009). Tree sparrows are generally partial short-distance migrants (Cramp 1994), i.e. when a fraction within a local population is resident whereas another fraction migrates short distances. Prevalences range from 4% in Bulgaria (Shurulinkov 2005) to 37% in Spain (Ventim et al. 2012b) and, so far, four lineages of *Plasmodium* and *Haemoproteus* have been identified in South-eastern European tree sparrows. Finally, the Spanish sparrow shows a more complex migration strategy with fully resident populations in Western Europe and obligate migratory eastern populations (Summers-Smith 1988). Prevalence varied between 33% in Bulgaria (Dimitrov et al. 2010) and 80% in a mixed colony with Indian house sparrows (*Passer d. indicus*) in Kazakhstan (Valkiūnas 2005) and seven lineages have been recorded in Spanish sparrows in Southeast Europe. Data on intensity of haemosporidian infections (i.e. the percentage of infected erythrocytes, henceforth referred to as 'parasitaemia') of sparrows are generally scarce, with few exceptions for house and tree sparrows (Ventim et al. 2012a; Bichet et al. 2014).

If migration strategy affects blood parasite infections, we expect little differences between the resident house sparrows and the partially migratory tree sparrow but pronounced differences between them and the obligate migratory Spanish sparrow (Figure 1). In particular, we hypothesise that i) **prevalence** of haemosporidian infections in Spanish sparrows differs from those in house and tree sparrows: prevalence would be higher if migration increases susceptibility to blood parasite infections, or prevalence would be lower if migration hampers parasite transmission. Furthermore, we expect that ii) **parasitaemia** would be higher in Spanish sparrows than in the other two species, if parasite intensities resulting from flight-induced relapses (Gylfe et al. 2000) or infections transmitted during migration remain elevated until the breeding season. Finally, we expect iii) parasite **diversity** to be higher in Spanish sparrows compared to house and tree sparrows, provided migrant species do not only encounter but also get infected by more diverse parasites on their journeys.

Materials and Methods

Study site and periods

We analysed haemosporidian infections in house sparrows, tree sparrows and Spanish sparrows breeding in the Danube river flood plain in Southeast Europe. We captured the sparrows using mist nets in the vicinity of the Biological Experimental Station 'Kalimok' and the village of Nova Cherna (Bulgaria; 44.00°N 26.45°E) in three

98 consecutive periods in 2015, covering mid-breeding (July 1 - 18), late-breeding (July 25 - August 10) and post-

99 breeding (August 17 - 30).

100

101 Blood sampling

102 All birds were sexed (based on sex-specific plumage and cloacal protuberance), aged, weighed and ringed. To

103 avoid misclassifications of juveniles and females between house and Spanish sparrows, we only sampled adults

104 and preferably males, yielding sex ratios (males/females/unknown) of 39/7/1 for house sparrows, 18/5/27 for

105 tree sparrows and 83/0/0 for Spanish sparrows (see Appendix A in ESM 1). In total, we sampled approximately

106 30µl blood from 180 individuals (sample sizes per species and period: 16/16/15 for house sparrows, 16/26/8 for

107 tree sparrows and 21/28/34 for Spanish sparrows) from the brachial vein with a heparinized capillary. We

108 immediately prepared two to three thin blood smears per individual. The smears were dried fast using a fan,

109 fixed on the same day for 5min in 100% methanol as well as stained within one month for 1h in 10% Giemsa's

110 solution.

111

112 Extraction and PCR

113 For 177 birds, there was residual blood in the capillary to be stored in SET buffer (0.015M NaCl, 0.05M Tris,

114 0.001M EDTA, pH8) at -20°C for genetic analysis. From these samples, we extracted DNA (DNeasy blood and

115 tissue kit, Qiagen) and, to test for the presence of parasites of the genera *Plasmodium* and *Haemoproteus*, we

116 performed a nested PCR developed by Hellgren et al. (2004). In brief, the product of a first PCR (3min at 94°C

117 + [30s at 94°C +30s at 50°C + 45s at 72°C] for 20 cycles + 10min at 72 C) using the primer pair HaemNF1 and

118 HaemNR3 (detecting several haemosporidian genera) was used as a template in the second PCR (identical

119 thermal profile but with 35 cycles instead of 20 cycles) with the primer pair HaemF and HaemR (detecting

120 *Haemoproteus* and *Plasmodium* only). The samples were not molecularly tested for the presence of

121 *Leucocytozoon* infections. Further, the PCR products were visualized in stained (GelRed, Biotium Inc.) 2%

122 agarose gels. Parasite-negative samples and samples with unclear results of the first nested PCR (weak bands)

123 were analysed twice to avoid false-negative results. Finally, we yielded positive PCR results for 102 samples

124 which were used to calculate species specific prevalence and determine parasitemia.

125

126 Prevalence estimation from the records on the MalAvi database

127   We also used MalAvi database entries to estimate an average prevalence for each host species, to compare these

128   to the prevalences in our study. Therefore, we used entries of all lineages which have been detected in the three

129   sparrow species in Southeast Europe (i.e. Albania, Bosnia, Bulgaria, Greece, Croatia, Macedonia, Moldavia,

130   Montenegro, Romania, Serbia and Turkey (Eastern Thrace)). As the number of tested and infected individuals

131   are entered per lineage and not all studies explicitly declare the number of mixed infections in the respective

132   article, we conservatively estimated ranges of prevalence for each study, with a lower limit resulting from the

133   assumption of the maximal possible number of co-infections and an upper limit resulting from the assumption of

134   the absence of co-infections. Finally, we calculated a weighted mean (weighted by the sample size of the

135   studies) among all lower and among all upper limits within each host species to get one average range for each

136   sparrow species.

137

138   Microscopic measurement of parasitemia

139   We microscopically determined parasitaemia (% infected erythrocytes) in PCR-positive birds by counting

140   infected erythrocytes in 100 microscopic fields with 1000x magnification (Primo Star and AxioCam ERc5s,

141   Carl Zeiss AG). The total number of inspected erythrocytes was extrapolated from five microscopy pictures (i.e.

142   every 20th field, following Boone et al. 2010). No blood parasites of the genus *Leucocytozoon* was found in any

143   of the blood smears we screened. In case we found no infected erythrocytes in the blood smears of PCR positive

144   samples which showed no signal in the chromatogram, we did not only exclude those from the analysis of

145   lineage diversity, but also considered those false-positive and corrected the infection status to uninfected (n=3)

146   for the calculation of prevalence. In case we found no infected erythrocytes (n=5) or only extracellular stages

147   (n=4) in blood smears of samples with clear chromatograms, we set the parasitaemia to half the minimum

148   detectable intensity (i.e. ½ infected erythrocytes / total inspected erythrocytes; see Hahn et al. 2018).

149

150   Sequencing and lineage determination

151   Parasite-positive PCR products were purified by enzymatic clean-up and hydrolysis of excess reagents

152   (ExoSAP-IT kit, Thermofisher), sequenced with the forward primer (HaemF) using a sequencing kit (ABI

153   BigDye® Terminator v3.1 Cycle, Applied Biosystems) for single-pass sequencing and the fluorescent-labelled

154   fragments were analysed by electrophoresis in an DNA analyzer (ABI 3730xl, Applied Biosystems) following

155   the manufacturers protocols. Samples which yielded clear chromatograms with the forward primer were

156   sequenced with the backward primer (HaemR). The sequences were checked, edited (BioEdit software; Hall

157   (1999)) and assigned to known lineages from the MalAvi database (Bensch et al. 2009) by a multi-sequence

158   analysis (msa function and package; Bodenhofer et al. (2015)). From the 102 samples we skipped lineage

159   assignment for 10 which repeatedly showed no signal. From the 92 sequences with signal, we yielded 46 reads

160   of full length (479bp). Four of the full-length sequences did not match 100% with known lineages. These

161   sequences are most similar (1-3 base-pairs difference) to two very closely related and widespread lineages, and

162   the sequences of these potentially new lineages can be found in the MalAvi database and in GeneBank

163   (accession no. MH909229- MH909232). Therefore 42 sequences entered the analysis of lineage diversity, while

164   50 sequences (18 from house sparrows, 14 from tree sparrows and 18 from Spanish sparrows) had to be

165   excluded from the analysis of lineage diversity. These comprise of 43 sequences with 70-99% of the full length

166   and seven samples with only 28-50% of the full length. But, in brief, 29 of 50 sequences showed 90-95%

167   accordance with the most similar lineage from MalAvi database. None of these samples shared the unique

168   *Haemoproteus* sequence between 400 and 430 base pairs and 25 of 29 were most similar to two ubiquitous

169   lineages. The remaining 21 of 50 sequences contained larger gaps and therefore reach maximal similarities

170   between 90 and 100%, often with the same degree of similarity with several lineages. Therefore, we also could

171   not resolve mixed infections, even if sophisticated methods exist (Pérez-Tris and Bensch 2005). As our

172   procedure occasionally reads mixed infections as single infections, the frequency of certain lineages might be

173   underestimated, (Valkiūnas et al. 2006), but prevalence and parasitaemia are not affected by this imprecision.

174   For gaining insight on potential transmission areas of the parasites, we screened the MalAvi database for the

175   lineages we detected in our three host species and made an overview on whether or not the lineage had been

176   found in resident and/or migrant bird species in Southeast Europe, the rest of Europe and/or Africa, in order to

177   identify presumed transmission areas.

178

179   Statistical analysis

180   We tested for differences in prevalence between hosts and sampling periods by fitting a Generalized Linear

181   Mixed-Effects Model (function glmer of the R package lme4; Bates et al. (2015)) with infection status as

182   dependent variable (family=binomial) and both species and periods as independent variables. To explicitly

183   allow for species-specific intercept and slopes we included species and periods in a random term (for modelling

184   details, see Appendix B in ESM 2). Additionally, we calculated average prevalence per host species from the

185   MalAvi database entries weighted for the varying sample sizes of the different studies.

186 Similar to the model for prevalence, we fitted a Linear Model to test for differences in parasitaemia (function `lm`

187 of the R package `stats`; R Core Team (2016)) with the log of individual parasitaemia as dependent variable as

188 well as species and period as independent variables (see Appendix B in ESM 2).

189 We tested for host-specific differences in lineage diversity by calculating relative diversity (= number of

190 lineages / number of infected hosts) for both our study (with the actual number of infected hosts) and the

191 lineages in the MalAvi database (with the estimated number of infected hosts i.e. the mean of the possible range

192 of infected individuals). Relative diversity values depict the average number of lineages per infected host

193 individual and account for differences in sample size per host species. Additionally, we calculated rarefaction

194 (function `rarefy`), which corrects the number of found lineages accounting for rare lineages in our study and

195 Chao dissimilarity indices (function `vegdist(method="chao")`, both of the R package `vegan`; Oksanen et al.

196 (2018)) which integrates the information about unique and shared lineages to compare parasite assemblages.

197

198 **Data accessibility**

199 All accompanying data on parasites are on the MalAvi database and along with the appendices on

200 10.17632/5gj8hkt4n2.1.

201

202 **Results**

203 Prevalence

204 In total 82% of the house sparrows, 60% of the tree sparrows and 49% of the Spanish sparrows were infected

205 with *Plasmodium* or *Haemoproteus* parasites (Figure 2a). We detected *Plasmodium* infections in all three

206 sparrow species but *Haemoproteus* infections only in Spanish sparrows. Thus, the prevalence was significantly

207 higher in house sparrows compared to tree sparrows (GLME: slope = -1.25[±0.55]; z-value = -2.29; p-

208 value = 0.02) and Spanish sparrows (GLME: slope = -1.54[±0.48]; z-value = -3.22; p-value = 0.001), but not

209 significantly different between the latter two species (GLME: z=-0.29, p=0.46). Prevalence did not significantly

210 change over the three periods (GLME: z-values = 0.09-1.43, p-values = 0.15-0.86). The prevalence deduced

211 from the MalAvi database entries from Southeast Europe range from 35 to 60% in house sparrows, from 41 to

212 56% in tree sparrows and from 15 to 23% in Spanish sparrows.

213

214 Parasitemia

215 The infection intensities varied between 0.001 and 2% with a median of 0.01% (25th to 75th percentile: 0.003-

0.02%; n=31) for house sparrows, 0.02% (0.004-0.03%; n=27) for tree sparrows and 0.01% (0.003-0.03%; n=41) for Spanish sparrows (Figure 2b). Parasitaemia did not differ between host species (LM: t-values = -0.1-1.2, p-values = 0.3-0.9) nor between the three periods (LM: t-values = -0.1-0.9, p-values= 0.3-0.9).

Diversity

We assigned 42 sequences with 100% identity to 10 distinct cytochrome b lineages in the MalAvi database – seven lineages of *Plasmodium* and three of *Haemoproteus* (Figure 3a). Two lineages (P-SGS1 and P-GRW11) are shared among all three sparrow species. House and Spanish sparrows additionally share one lineage (P-COLL1). Furthermore, house sparrows carry one (P-GRW06) unique lineages and Spanish sparrows six (H-PADOM05, H-PAMON01, H-PAHIS1, P-DELURB4, P-PADOM01 and P-PAGRI02). Many of the lineages have already been recorded for these three sparrow species in Southeast Europe (Figure 3b). The Chao estimates which integrate total, shared and unique lineages indicate that the parasite assemblages of the house and tree sparrow are more similar to each other (Chao-dist $_{PasDom-PasMon}$ = 0.3) than to those of the Spanish sparrow (Chao-dist $_{PasDom-PasHis}$ = 0.63, Chao-dist $_{PasMon-PasHis}$ = 0.68). When dividing the number of lineages (4 for the house sparrow, 2 for the tree sparrow and 9 for the Spanish sparrow) by the number of infected individuals for each host species, we obtain relative diversities of 0.13 lineages per infected house sparrow, 0.07 lineages per infected tree sparrow and 0.22 for the Spanish sparrow. The relative diversities from the MalAvi database amount to 0.09 lineages per infected house sparrows, 0.07 lineages per infected tree sparrow and 0.30 lineages per infected Spanish sparrow. When correcting the total number of lineages per host for rare parasites, we obtain similar rarefied number of lineages (4±0 for the house sparrow, 1.96±0.2 for the tree sparrow and 6.4±1 for the Spanish sparrow).

Discussion

The migratory Spanish sparrows showed a significantly lower prevalence than the resident house sparrows. The intermediate prevalence of the partially migratory tree sparrow only differed from the house sparrow's but not from the Spanish sparrow's prevalence. In contrast, parasitaemia levels were similar in all three host species and Spanish sparrows are presumably infected with a higher diversity of haemosporidian parasites.

The absolute prevalence levels were generally higher in our study compared to those deduced from the MalAvi database, but the relative proportions are very similar. Parasite prevalence in Spanish sparrow was 40% lower than in house sparrows and 18% lower than in tree sparrows, which supports our hypothesis, that the prevalence

246 of the house and tree sparrow will not differ to such a degree, compared to the prevalence of the Spanish

247 sparrow (see Figure 1). We acknowledge that our approach allows for a relative comparison but not for an

248 absolute description of species-specific prevalence and its variability. As prevalence can vary between regions

249 and years, spatial and temporal replicates of the sampling scheme are needed for such an endeavour.

250 Unfortunately, we cannot disentangle if the lower prevalence in the Spanish sparrow is caused by increased

251 mortality of infected compared to uninfected individuals on migration (known as "migratory culling", Bradley

252 and Altizer 2005) or if the migratory sparrows already started the journey with a lower pre-selective prevalence,

253 which would mean that physiological or behavioural processes related to the host's migratory lifestyle either

254 changed the transmission probability of pathogens or the hosts' susceptibility to parasites. Increasing migration

255 distance along with an increasing time spent away from the breeding site has been shown to reduce the

256 prevalence of locally transmitted pathogens in theoretical models of the transmission dynamics in animal

257 populations (Hall et al. 2014).

258 Our data do not support the hypothesis of a higher parasitaemia in migratory hosts. Parasitaemia averages on

259 similar level across the sparrows during the breeding period and seems to be unaffected by the hosts migration

260 strategy. However, it cannot be excluded that endurance flights have induced relapses, but elevated parasitaemia

261 did not persist until the breeding season. Yet, parasitaemia might in fact be mostly driven by seasonal changes in

262 host physiology (Allander and Sundberg 1997), seasonal vector abundance and the influx of immunonaïve hosts

263 after the reproductive season (Altizer et al. 2006).

264 Since our three host species are closely related and breed in close proximity, they probably share many vectors –

265 at least during parts of the breeding period. Thus, the disparities in the sets of parasites might arise from

266 transmission during the non-breeding season: Spanish sparrows have regularly been observed in Egypt and

267 especially in the Nile delta during the non-breeding period (Summers-Smith 1988) and Spanish sparrows from

268 our population likely spend the non-breeding period there. In these regions they encounter habitats with

269 different climatic conditions and landscape elements (Pérez-Rodríguez et al. 2013), therefore parasites which

270 cannot develop or be transmitted by vectors at the temperate breeding grounds (Žiegytė and Valkiūnas 2014).

271 The Chao estimates suggest that the parasite assemblages of the house and tree sparrow are more similar to each

272 other compared to the assemblage of the Spanish sparrow. Furthermore, already before our study, migratory

273 hosts were repeatedly found to harbour parasites of the genus *Haemoproteus* more often than resident hosts do

274 (Clarabuch and Gonzalez-Solis 1997 and references therein). On the level of parasite assemblage and parasite

275 genus diversity, this also supports our hypothesis, that the differences to the obligate migratory Spanish sparrow

276 are expected to be the most pronounced compared to between the resident house sparrow and the partially

277 migratory tree sparrow (see Figure 1). As the vector taxa of *Haemoproteus* parasites are cosmopolitan, there is

278 no trivial explanation for this pattern. If the higher parasite diversity in the Spanish sparrow would indeed

279 originate from transmissions during the non-breeding period in Africa, we would expect lineages uniquely found

280 in Spanish sparrows to be exclusively recorded in resident hosts in Africa but not in resident host species in

281 Europe (Table 1). Yet, P-PAGRI02 was the only lineage in the MalAvi database that was exclusively detected in

282 resident hosts in North-Africa (Mata et al. 2015). The other lineages unique to Spanish sparrow in our study

283 have also been detected in resident hosts in Southeast Europe and the rest of Europe (Table 1). So, except for P-

284 PAGRI02 the MalAvi database offers little evidence that the surplus diversity of Spanish sparrows was

285 transmitted in the non-breeding sites.

286 Alternative differing parasite assemblages could be explained if parasites require a certain period of time for

287 local adaptation to a host-vector-system. Then, recently established host populations would be expected to carry

288 less locally transmitted parasites than a host species with long historical occupancy. In our study area, house and

289 tree sparrows are traditional members of the avifauna and should thus carry more locally transmitted parasites

290 compared to Spanish sparrows, which colonized the area just 50 years ago. But we cannot describe the complete

291 lineage diversity of the three host species in this region, as a considerable number of sequences could not be

292 assigned because they either did not reach 100% identity with a known lineage (n = 29) or shared maximal

293 similarity with several known lineages (n = 7) or both (n = 14). To describe the complete haemosporidian

294 diversity one would require a larger sample per host species also balanced for sex- and age-classes and coverage

295 of a longer period of the annual cycle over several years to level-out the intra- and inter-annual variation.

296 However, we aimed for a temporal snapshot for the comparison of prevalence, parasitemia and lineage diversity

297 along a gradient of host migration strategies. The following peculiarities indicate that the unidentified lineages

298 do not attenuate our findings of increased lineage diversity in the migratory Spanish sparrows: With 12

299 unidentifiable sequences stemming from Spanish sparrows, 6 from house sparrows and 5 from tree sparrows

300 most of them origin from Spanish sparrows. All unidentified sequences of house and tree sparrows clustered

301 with *Plasmodium* lineages. The majority of all unidentified sequences with maximal similarity with a single

302 known lineage were most similar to the widespread lineages SGS1 (19 of 29) and GRW11 (8 of 29) and can

303 thus already be found in the set of 100% matches (exception is one 94% match with P-ACAGR1 in a house

304 sparrow and one 91% match with P-PADOM07 in a tree sparrow). Finally, we are confident that also the

305 description of the four potentially new lineages would not change our result as three of the four sequences

306   originate from Spanish sparrows and only one from a house sparrow. But to enable the description of the

307   complete lineage diversity in the framework of future studies, the sequences are made available on a repository

308   (see 'Data accessibility' section).

309   In conclusion, our findings of higher diversity, but lower prevalence in the migratory compared to the resident

310   hosts support the idea that migration is a mixed blessing for the hosts of avian blood parasites. Host migration,

311   on one side, incurs the cost of acquiring more diverse parasites, but can also come with benefits for the host:

312   Theoretical work already indicated, that populations with a migratory life-style can profit from lower prevalence

313   (Johns and Shaw 2016). However, the parasitism-related benefits of migration are rarely investigated

314   empirically (but see Teitelbaum et al. 2018). Our study of three sympatrically breeding sparrow species covering

315   a range of migration strategies from resident to obligate migratory provides the basic information for more

316   extensive studies on the interaction of host migration strategy and blood parasites. In how far our findings can

317   be generalized across bird taxa and the causal mechanisms behind such migration-related benefits still remain to

318   be elucidated, for instance by investigating parasitism in populations with differing migratory propensity in a

319   partially migratory species or in species pairs with varying migration strategies covering a broader range of taxa.

320   Parasitism could even act as a selective pressure to increase or uphold migratory propensity, counter-balancing

321   the expected selective pressure towards a reduction in migratory propensity in European bird communities

322   caused by climate change (Schaefer et al. 2007), if the benefit of lower prevalence in migrants outrange the

323   costs of hosting diverse parasites.

324

329 **Authors' contributions**

330 TE, SB and SH designed the study; PZ and DD arranged permissions and licences; TE, DD and SH organized

331 and carried out the field work; JOM carried out the molecular lab work; TE and SH performed the screening by

332 microscopy; TE implemented the data analysis and drafted the manuscript. All authors have revised the draft

333 and agreed on the final version of the article.

339 **Compliance with ethical standards**

340 *Ethical statement* All procedures were in accordance with the local animal ethics

341 guidelines and were permitted by the Bulgarian Ministry of Environment and Waters

342 (licence no. 574/27.03.2014).

343 *Conflict of interest* The authors declare that they have no conflict of interest

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439

Figures

	host migration strategy	resident		partial		migratory
parasite	prevalence	PasDom	≠	PasMon	≠≠	PasHis
	parasitemia	PasDom	=	PasMon	<	PasHis
	diversity	PasDom	<	PasMon	<<	PasHis

Figure 1: Hypothesised influence of host migration strategy on parasite prevalence, parasitaemia and diversity – relative between three sympatrically breeding sparrow species (PasDom = house sparrow, PasMon = tree sparrow, PasHis = Spanish sparrow). We expect more distinctly differing infection parameters (double signs) in the obligate migratory than compared among the partially-migratory or resident sparrows (single signs).
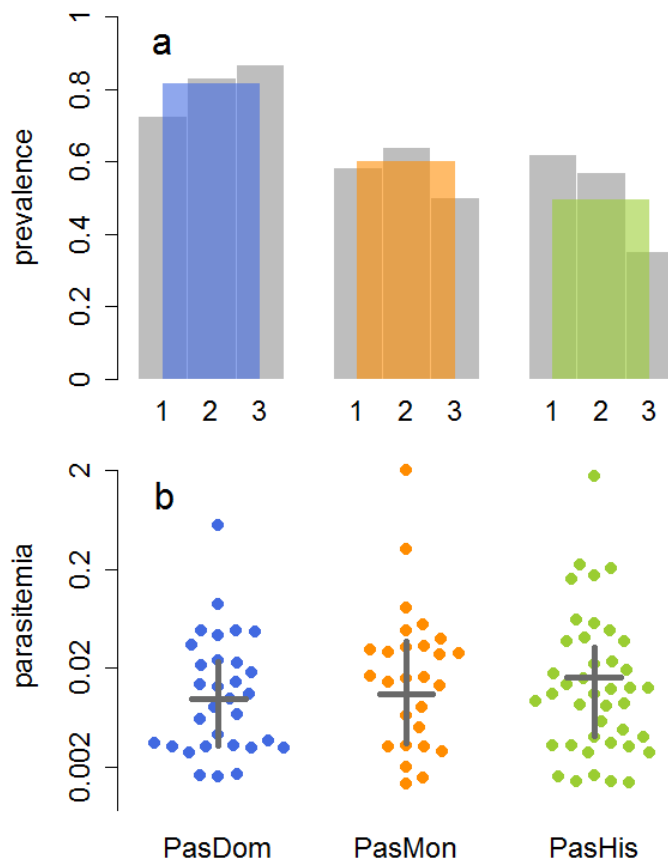
Figure 2: Prevalence and parasitaemia of haemosporidian infections in the three sympatric sparrow species (PasDom = house sparrow, PasMon = tree sparrow, PasHis = Spanish sparrow): The prevalence (a) is significantly higher in house sparrows compared to tree sparrows and Spanish sparrows. Prevalence did not differ significantly between the three periods (grey bars: 1 = July 1-18, 2 = July 25-August 10, 3 = August 17-30, color bars: average over the three periods). The parasitaemia (b) varied from 0.001 to 2% and differs neither between host species nor between periods (dots = individual parasitaemia, horizontal line = median, vertical line = 25-75% quartiles).
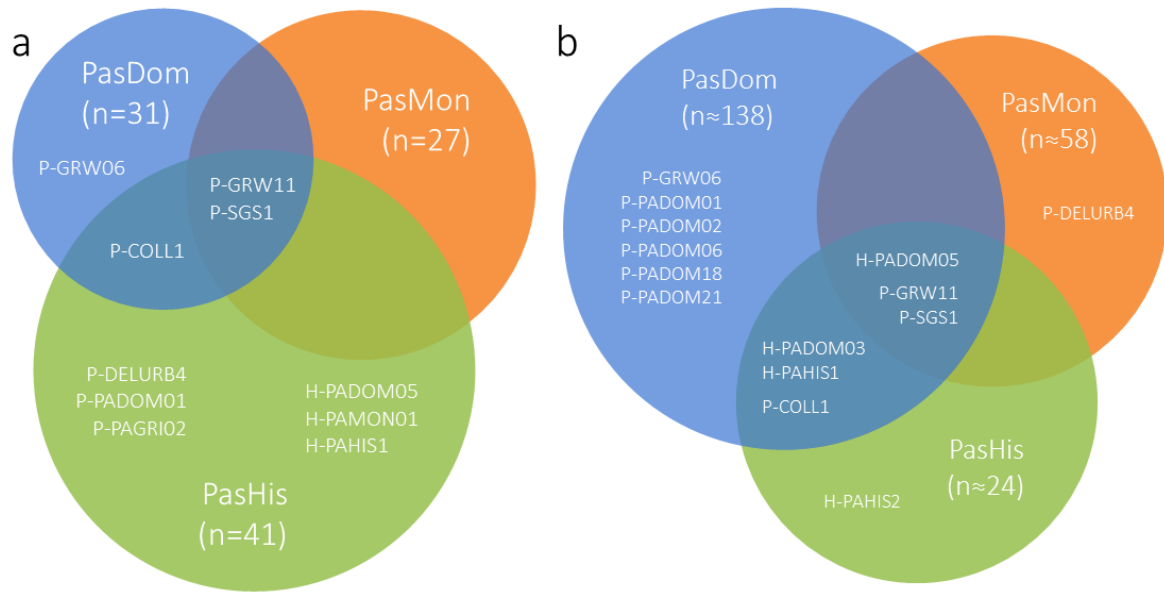
Figure 3: *Plasmodium* and *Haemoproteus* lineages found in house sparrows (PasDom), tree sparrows (PasMon) and Spanish sparrows (PasHis) from Southeast Europe. Panel (a) comprises all the unique and shared cytochrome b lineages identified from the infected individuals in our study (sample sizes in brackets, northern Bulgaria, one year). Panel (b) lists all lineage records from Southeast Europe (i.e. Albania, Bosnia, Bulgaria, Greece, Croatia, Macedonia, Moldavia, Montenegro, Romania, Serbia and Turkey (Eastern Thrace) archived in the MalAvi database (accessed on the 08/06/2018, sample size approximation in brackets). The prefix of the lineage abbreviation signifies the genus *Plasmodium* (P-) or *Haemoproteus* (H-). Note that panel (a) only includes the clearly assigned lineages of our study (sequences with 100% identity with a known lineage from the MalAvi database). The sizes of the bubbles coloured represent the absolute number of lineages found in each host species, but not relative diversity (= the number of lineages / the number of infected individuals).

Tables

Table 1: Potential transmission areas of the parasite lineages deduced from the MalAvi database. For each host species (PasDom = house sparrow, PasMon = tree sparrow, PasHis = Spanish sparrow) we list the lineages clearly identified lineages in alphabetic order. To make an appraisal of the potential transmission areas, we list whether or not a particular lineage has been found in any resident (r), migrant (m) or both (r-m) hosts in Southeast Europe, in the rest of Europe and/or in Africa.

host species	parasite genus	lineage	maximal similarity	found in resident hosts in		
				SE-Europe [b]	Europe [c]	Africa
PasDom	P	COLL1	1.00	r-m	r-m	r
	P	GRW06	1.00	r-m	r-m	r-m
	P	GRW11	1.00	r-m	r-m	r-m
	P	SGS1	1.00	r-m	r-m	r-m
PasMon	P	GRW11	1.00	r-m	r-m	r-m
	P	SGS1	1.00	r-m	r-m	r-m
PasHis	H	PADOM05	1.00	r-m	r-m	r
	H	PAHIS1	1.00	r-m	r	r
	H	PAMON01 [a]	1.00			
	P	COLL1	1.00	r-m	r-m	r
	P	DELURB4	1.00	r	r-m	
	P	GRW11	1.00	r-m	r-m	r-m
	P	PADOM01	1.00	r	r-m	
	P	PAGRI02	1.00			r
	P	SGS1	1.00	r-m	r-m	r-m

[a] No entries of this lineage in the "Host And Sites Table" of the MalAvi database.

[b] Considered as Albania, Bosnia, Bulgaria, Greece, Croatia, Macedonia, Moldavia, Montenegro, Romania, Serbia and Turkey (Eastern Thrace).

[c] All European countries (including the European parts of Russia) except Southeast Europe.