


Extremely low malaria prevalence in a wetland specialist passerine

Eszter Szöllősi¹ , Zsófia Tóth², Katharina Mahr^{2,3}, Herbert Hoi³
and Ádám Z. Lendvai^{2,4}

Research Article

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Author for correspondence:

Eszter Szöllősi,
E-mail: eszter.szollosi@ttk.elte.hu

¹Department of Systematic Zoology and Ecology, Behavioural Ecology Group, Eötvös Loránd University, Pázmány Péter sétány 1/c, 1117 Budapest, Hungary; ²Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary; ³Department of Integrative Biology and Evolution, Konrad Lorenz Institute of Ethology (KLIV), University of Veterinary Medicine, Vienna, Savoyenstraße 1a, A-1160 Vienna, Austria and ⁴Department of Geology, Babeş-Bolyai University, Str. Mihail Kogalniceanu nr. 1, 400084 Cluj-Napoca, Romania

Abstract

Avian malaria (caused by *Plasmodium* spp.) and avian malaria-like infections (caused by *Haemoproteus* spp.) are widespread and can seriously affect the health of their bird hosts, especially of immunologically naïve individuals. Therefore, these parasites have long been in the focus of bird-parasite studies. However, the species richness and diversity of these protozoan species have only been revealed since the use of molecular techniques. Diversity and prevalence of these parasites among different bird species and even between populations of a species show a large variation. Here, we investigated prevalence of avian malaria and avian malaria-like parasites in two distant populations of a non-migratory wetland specialist passerine, the bearded reedling (*Panurus biarmicus*). While previous studies have shown that reed-dwelling bird species often carry various blood parasite lineages and the presence of the vectors transmitting *Plasmodium* and *Haemoproteus* species has been confirmed from our study sites, prevalence of these parasites was extremely low in our populations. This may either suggest that bearded reedlings may avoid or quickly clear these infections, or these parasites cause high mortality in this species. The remarkably low prevalence of infection in this species is consistent with earlier studies and makes bearded reedlings a possible model organism for investigating the genetic or behavioural adaptations of parasite resistance.

Introduction

Avian malaria (*Plasmodium*) and malaria-like (*Haemoproteus*) parasites (Protozoa, order Haemosporidia) are abundant on all continents, except Antarctica (Valkiūnas, 2005) and the infections caused by these parasites can have serious physiological impacts on birds (Atkinson and van Riper, 1991; Merino *et al.*, 2000; Knowles *et al.*, 2010; Asghar *et al.*, 2015). The effects of these parasites can be particularly strong for individuals of immunologically naïve host species and the arising negative fitness consequences may affect entire populations (van Riper *et al.*, 1986; Townsend *et al.*, 2018).

The advent of molecular techniques in blood parasite detection revealed a remarkable species richness, diversity and distribution range of these parasites. To date we know of 2303 *Plasmodium* and *Haemoproteus* lineages detected from 1479 bird species (Malavi database: <http://mbio-serv2.mbioekol.lu.se/Malavi>); however, the number of different parasite lineages is thought to exceed 10 000 (Bensch *et al.*, 2004). Consequently, studies investigating the costs of avian malaria and malaria-like infections need to identify lineages involved in the given host–parasite relationship.

A large variation in avian Haemosporidian diversity and prevalence was found between, but also within a host species, e.g. when inhabiting different geographical regions (e.g. Hellgren *et al.*, 2009; Marzal *et al.*, 2011; Szöllősi *et al.*, 2011). Environmental conditions can influence the prevalence and virulence of different parasite species, both through the abundance and diversity of vector populations and also through the composition of bird host species communities (Scheuerlein and Ricklefs, 2004; Valkiūnas, 2005; Hellgren *et al.*, 2007; Soltész, 2017). In addition, each parasite species has its own transmission period, possibly causing variances in parasite prevalence patterns in different areas and seasons (Valkiūnas, 2005).

The prevalence and virulence of a parasite species may also vary due to mutual adaptations between vectors, birds and the parasites. For example, vector species may differ in their host breadth so that some of the vectors have wide host preference and suck blood from a range of bird species with a diverse array of parasite species while others are host specialists (Valkiūnas, 2005; Malavi). Similarly, the parasites may also vary in their ability to complete their development in a range of distantly related vector and bird host species (generalist parasites) or only in a smaller group of closely related species (specialist parasites). Further, the strength of the immune response of the hosts affects the prevalence and intensity of parasite infections (reviewed in Westerdahl, 2007; Marzal *et al.*, 2018; Antonides *et al.*, 2019).

Avian malaria and malaria-like parasites show a complex life cycle. They reproduce asexually in birds (intermediate hosts) and sexually in insect vectors (definitive hosts; vectors of *Plasmodium* are species from the family *Culicidae*; vectors of *Haemoproteus* are species from the family *Ceratopogonidae* and *Hipposcidae*). Larvae of the vectors of these parasites develop in a wide variety of aquatic habitats (Soltész, 2017) and freshwater wetlands with reed beds particularly represent an ideal environment because of the high humidity and low salinity of the habitat (Cox, 1993). These conditions may result in high vector abundance and diversity, and in turn, a high prevalence of blood parasites in birds nesting in these habitats.

In this study, we investigated a specialized reed dwelling species, the bearded reedling *Panurus biarmicus*. They spend their entire life in freshwater wetlands, and considering the optimal developmental conditions for the vector species, we predicted a very high risk of blood parasite infestation in bearded reedlings. Consequently, we expected a high prevalence as well as high parasite diversity.

Previous studies focused mainly on the description of the parasite fauna of various bird communities breeding in freshwater wetlands (Ventim et al., 2012a, 2012b; Neto et al., 2015; Nilsson et al., 2016; Dimitrov et al., 2018). However, less is known about the parasites that are transmitted locally (but see Ventim et al., 2012a, 2012c; Nilsson et al., 2016; Dimitrov et al., 2018) or about the consistency of parasite communities across populations of a host species.

For this reason, in our descriptive study, we investigated two geographically distinct bearded reedling populations and compared our findings with parasite data reported previously in other wetland passerine populations.

Materials and methods

Study species and field methods

The fieldwork was carried out at Hortobágy-Halastó (47°37'N, 21°04'E, Hungary) and at Lake Neusiedl (47°56'N 16°46'E, Austria) (Fig. 1). The reed bed area in Hortobágy-Halastó is 4.52 km² and in Lake Neusiedl is 180 km² in size. The bearded reedling is a small (~13 g), resident, wetland specialist passerine common throughout Eurasia. It is dependent on extended reed beds throughout its life, and uses this habitat for breeding, foraging and wintering (Cramp and Perrins, 1993). We caught a random sample of 47 male and female individuals with mist nets between mid-April and mid-July 2016 in three sampling points in Hungary and 51 male and female individuals between mid-August and mid-October 2016 in two sampling points in Austria (Fig. 1). Individuals sampled in the Hungarian population consisted of both adults ($n = 35$, sampled from mid-April onwards) and juveniles ($n = 12$, sampled from mid-June onwards), whereas most individuals sampled in Austria had already completed their post-juvenile moult and were in adult plumage by the time of sampling.

Sample collection and parasite detection

Blood samples were taken from the brachial vein and stored on ice in the field and separated to plasma and cell fractions (used in the present analysis) by centrifuging the samples on 2200 g for 5 min. Thereafter the samples were kept at -20 °C until further analyses in the laboratory. DNA was extracted by an ammonium-acetate method (Nicholls et al., 2000) then the concentration of genomic DNA was adjusted to ca. 35 ng μL^{-1} by quantifying genomic DNA on a BioTek Epoch Microplate Spectrophotometer. A highly sensitive polymerase chain reaction (PCR) based method was used for the detection of parasite prevalence (Waldenström

et al., 2004). In all PCRs, both negative (ddH₂O) and positive controls (DNA samples from individuals of other bird species that were previously confirmed to be infected) were used to control for possible contaminations and failures during PCRs, respectively. None of the negative controls ever showed any amplification of parasite DNA, and parasite DNA was always amplified correctly from positive controls. To ascertain that none of the samples went through degradation between sample collection and parasite investigation and that the samples were in a good condition for PCR analyses, all samples were checked for DNA quality by amplifying the CHD (chromo-helicase-DNA-binding) genes of bird host DNA (using the primer pairs described by Griffiths et al., 1998). To reduce the risk of losing infections because of sampling error, negative samples were screened twice for blood parasites.

All samples with positive amplification for parasite DNA were sequenced directly using the BigDye[®] Terminator v3.1 cycle sequencing kit. Products from the sequencing reactions were purified and sent to a capillary electrophoresis platform (Biological Research Centre, Hungary). Sequences were edited and aligned using the program BioEdit (Hall, 1999) and identified to genus (*Haemoproteus* or *Plasmodium*) and lineage level by comparing sequence data with those of the previously identified parasites reported in MalAvi database.

Data analyses

Mean prevalence (No of infected individuals/No of tested individuals \times 100) of *Haemoproteus* and *Plasmodium* parasites in each host species; Species evenness (E) and Simpson's index (D) for measure parasite diversity in each host population were calculated. Species evenness (E) was calculated from the formula $E = D/D_{\text{max}}$ (where D_{max} means the number of parasite species/population). Simpson's index (D) was calculated using the formula as follows (where p_i is the relative abundance of the lineages in a population):

$$D = \frac{1}{\sum_{i=1}^s p_i^2}$$

The few sampled populations combined with the number of effects to control for (i.e. species, populations, migratory status, locality, phylogeny) prevented us to perform statistical analyses on the indices describing prevalence patterns in the different species.

Results

Detected lineages and parasite prevalence

None of the 47 Hungarian bearded reedling samples and only two out of the 51 Austrian samples showed a positive amplification for either *Plasmodium* or *Haemoproteus*. This means 0 and 3.9% prevalence in the Hungarian and the Austrian populations, respectively. Both birds that showed positive amplification for malaria in the Austrian population were infected with the same *Plasmodium* lineage, pSGS1 (see also Table 1). Mean prevalence; Species evenness (E) and Simpson's index (D) are listed in Table 1.

Discussion

We found extremely low Haemosporidian prevalence and diversity in the two distinct bearded reedling populations in Hungary and Austria. Prevalence in the Hungarian population was 0%, while in the Austrian population only two out of the



Fig. 1. Sampling points of bearded reedlings (*Panurus biarmicus*). We collected blood samples from two distinct populations, one from Hungary, Hortobágy-Halastó ($n=47$ individuals from three sampling points, HU1, HU2 and HU3) and one from Austria, Lake Neusiedl ($n=51$ individuals from two sampling points, AT1 and AT2).

51 sampled individuals (3.9%) were infected with avian malaria and with only one *Plasmodium* lineage (pSGS1). This consistent and remarkably low infection level is in contrast to our predictions namely that due to the environment, prevalence, as well as diversity, should be high. This result hence suggests that bearded reedlings are either very sensitive to malaria causing the selective disappearance of infected individuals or that the species has special adaptations for malaria resistance.

We can rule out the possibility that the low prevalence found is due to the weaknesses of the techniques used in parasite detection. We used a highly sensitive PCR-based method that can detect infections in as low intensities as 0.001% and sometimes in intensities as 0.0001% (Waldenström *et al.*, 2004) which is much below the detected parasitaemia in wild-caught birds during the breeding season (0.01–1%, Valkiunas, 2005). In addition, this method has already proved to successfully detect various Haemosporidian lineages from different bird species also in our laboratory (e.g. from collared flycatchers, see Szöllösi *et al.*, 2016; Jones *et al.*, 2018; from house sparrows, see Marzal *et al.*, 2011). Furthermore, all negative samples were screened twice and our positive controls were always amplified correctly from the samples. We also used a reasonable sample size for both populations ($n=47$ and 51 in the Hungarian and the Austrian populations, respectively). Though the sampling period was different for the Hungarian (samples were collected between mid-April and mid-July 2016) and the Austrian populations (samples were collected between mid-August and mid-October 2016), both populations were screened during the most active period of the vectors. Though the Hungarian population consisted of both juvenile and adult individuals, also juveniles in the Hungarian population had time to develop infections as they were sampled from June onwards, while the first fledglings appear in early April. This makes it unlikely that differences in the sampling periods would have been responsible for the pattern reported. We therefore suggest that the genuinely low prevalence in our populations has an ecological explanation.

Parasite prevalence varies considerably among host species and sometimes also between different populations of a species. One straightforward explanation for these differences in Haemosporidian prevalence is that the abundance and the diversity of the suitable vectors differ between locations due to differences in local climatic and environmental conditions necessary for vector and parasite development (Burkett-Cadena *et al.*, 2013; Golding *et al.*, 2015).

From the year of sampling, we have information about the mosquito fauna (family *Culicidae*) of the Austrian study site (Führer *et al.*, unpublished data), while mosquitoes at the

Hungarian site were sampled in previous years (in 2011 and in 2012, Soltész, 2017). These studies show that both sites harbour a vast array of blood sucking vectors. The mosquito species found at Hortobágy-Halastó included *Culex modestus*, *C. pipiens* and *Ochlerotatus caspius* from which altogether more than 40 *Haemoproteus* and *Plasmodium* lineages were detected, out of which 21 were also proved to be transmitted in Europe. At Lake Neusiedl, Führer *et al.* reported the presence of *C. modestus*, *C. pipiens* and *Uranotaenia unguiculata* (unpublished data). From these species 42 *Haemoproteus* and *Plasmodium* lineages were detected and out of which 22 were proved to be transmitted in Europe (MalAvi). These studies and our personal experience in the field sites suggest that a highly diverse and abundant vector fauna is available for parasite transmission. Based on the potentially available vector and parasite species, one would expect a high rather than low blood parasite prevalence and diversity in the sampled bearded reedling populations.

However, none of the sampled birds was infected with either *Haemoproteus* or *Plasmodium* at Hortobágy-Halastó, and the prevalence and diversity were also very low in the Austrian population (3.9% prevalence, $D=1$). These results are consistent with the low parasite prevalences and diversities reported from other populations of bearded reedlings (Table 1). Among the three populations tested previously across Europe, at Lake Durankulak, Bulgaria, two out of the 35 adult and none of the 18 juvenile individuals sampled during spring and summer (in year 2006–2008, 2013–2016) were detected to be infected with Haemosporidian parasites, making a 3.8% overall prevalence in this population. However, for some reason, the authors were not able to sequence their samples; therefore, no information is available about the lineages infecting this population (Dimitrov *et al.*, 2018). Similarly, in a Swedish population at Lake Kvismaren, where Nilsson *et al.* (2016) screened juvenile bearded reedlings during summer (year 2004), only four out of the 40 individuals were proved to be infected with *Haemoproteus*. Out of these four individuals, three were infected with hWW2 (7.5% prevalence) and one with hTURDUS2 (2.5% prevalence). In addition, in a Turkish population of the species sampled at Sultan Marshes, Yildirim *et al.* (unpublished) screened altogether 58 adult and juvenile individuals and found that five out of them were infected with hGRW5 (8.6% prevalence) and one with hWW1 (1.7% prevalence) (see also MalAvi database). While zero prevalence in our Hungarian population is the lowest reported, consistent reports of low infection levels suggest that low Haemosporidian prevalence is a general phenomenon in this species.

Some previous studies also found low Haemosporidian prevalence in other bird species. However, these species were sampled

Table 1. Overall *Haemoproteus* and *Plasmodium* prevalence (percentage of infected individuals) and parasite lineages detected in different populations of bearded reedlings and coexisting wetland breeding passerines

Host species	Host population	No of infected individuals	No of tested individuals	Overall prevalence (%)	Mean prevalence (%)	Simpson's index	Species evenness	Lineage (No. of positive samples)	Reference
Bearded reedling <i>Panurus biarmicus</i>	Hortobágy-Halastó, Hungary	0	47	0.0	5.6	–	–	–	this study
	Lake Neusiedler, Austria	2	51	3.9		1.0	1.0	pSGS1(2)	this study
	Lake Kvismaren, Sweden	4	40	10.0		1.6	0.8	hTURDUS2(1) hWW2(3)	Nilsson <i>et al.</i> (2016)
	Sultan Marshes, Turkey	6	58	10.3		1.385	0.693	hGRW05(5) hWW1(1)	Yildirim <i>et al.</i> , unpubl.; MalAvi
	Lake Durankulak, Bulgaria	2	53	3.8		–	–	not known	Dimitrov <i>et al.</i> (2018)
Great reed warbler <i>Acrocephalus arundinaceus</i>	Kalimok, Bulgaria	39	52	75.0	42.7	3.507	0.390	hACTSE1(1) hGRW01(18) ● pGRW02(2) hGRW03(1) pGRW04(9) ■ hGRW05(4) ○ pGRW11(1) pRTSR1(1) ♥ pSGS1(2) ♣	Zehtindjiev <i>et al.</i> , 2008
	Sultan Marshes, Turkey	12	41	29.2		2.117	0.423	hGRW01(1) ● pGRW05(8) ○ pGRW10(1) □ hPAHIS1(1) pSYBOR10(1)	Yildirim <i>et al.</i> , unpubl.; MalAvi
	Kvismeren, Sweden	323	782/470*	41.3		3.256	0.250	hGRW01(123) ● pGRW02(45) pGRW04(121) ■ pGRW09(4) pGRW10(8) □ hGRW13(1) pGRW14(1) hPHSIB1(1) pRTSR1(3) ♥ hRW2(1) pSGS1(10) ♣ pSW5(1) hWW2(4)	Bensch <i>et al.</i> , 2007

Eurasian reed warbler <i>Acrocephalus scirpaceus</i>	Lake Durankulak, Bulgaria	14	77	18.2	23.2	2.277	0.759	pGRW10(7) hRW1(1) ● hRW2(6)	Dimitrov <i>et al.</i> (2018)
	Santo André, Portugal	37	124	29.8		4.952	0.550	pGRW04(9) ■ pGRW06(5) ♣ pGRW11(1) ♠ hMW1(12) ○ pRTSR1(1) □ hRW1(1) ● pSGS1(4) ◆ hSW1(2) ♥ pSW5(2) ✨	Ventim <i>et al.</i> (2012b)
	Sultan Marshes, Turkey	12	50	24.0		9.018	0.902	hACDUM2(1) hARW1(2) hGRW01(1) hMW1(2) ○ hPADOM05(1) pRTSR1(1) □ hRW1(1) ● hRW4(1) hSW1(1) ♥ hWW1(1)	Yildirim <i>et al.</i> , unpubl; MalAvi
	Taipal, Portugal	38	123	30.9		2.510	0.418	pGRW04(13) ■ pGRW06(2) ♣ pGRW11(1) ♠ hMW1(20) ○ hRW1(1) ● pSW2(1)	Ventim <i>et al.</i> (2012b)
	Tornada, Portugal	3	52	5.8		3.006	1.0	hMW1(1) ○ hRW1(1) ● pSGS1(1) ◆	Ventim <i>et al.</i> (2012b)
	Vilamoura, Portugal	23	122	18.9		5.470	0.684	pGRW04(3) ■ pGRW06(5) ♣ hHIPOL1(1) hMW1(7) ○ pRTSR1(2) □ hRW1(2) ● pSGS1(2) ◆ pSW5(1) ✨	Ventim <i>et al.</i> (2012b)
Cetti's warbler <i>Cettia cetti</i>	Tornada, Portugal	32	64	50.0	55.0	1.296	0.324	pCET01(1) pGRW11(2) ○	Ventim <i>et al.</i> (2012b)

(Continued)

Table 1. (Continued.)

Host species	Host population	No of infected individuals	No of tested individuals	Overall prevalence (%)	Mean prevalence (%)	Simpson's index	Species evenness	Lineage (No. of positive samples)	Reference
								pSGS1(28) ● pSYAT05(1) ■	
	Santo André, Portugal	43	65	66.2		1.0	1.0	pSGS1(43) ●	Ventim et al. (2012b)
	Taipal, Portugal	53	96	55.2		1.080	0.360	pGRW11(1) ○ pSGS1(51) ● pSYAT05(1) ■	Ventim et al. (2012b)
	Vilamoura, Portugal	42	84	50.0		1.273	0.424	pGRW11(4) ○ pSGS1(37) ● pSYAT05(1) ■	Ventim et al. (2012b)
Bluethroat <i>Luscinia svecica svecica</i>	Krkonoše, Czech Republic	19	40	47.5	41.9	5.219	0.652	pBT6(4) pDELURB5(1) pGRW04(6) pGRW06(1) pGRW11(1) ● pTURDUS1(1) pSGS1(2) ○ hWW2(3)	Svoboda et al., 2015
<i>Luscinia svecica namnetum</i>	Province of Ávila, Spain (4 sites)	50	101	49.5		7.911	0.396	hARW1(1) pDELURB4(2) hEMSP003(2) pGBCAM1(3) pGRW11(2) ● pLUSVE01(1) hLUSVE02(1) hLUSVE03(1) hLUSVE04(1) hLUSVE05(1) hLUSVE06(1) hLUSVE07(1) hLUSVE08(1) hLUSVE09(1) pRTSR1(2) hRW1(8) pSGS1(14) ○ hWW2(4) pWW3(1) pWW4(2)	Rojo et al., 2014
<i>Luscinia svecica cyanecula</i>	Trebonsko, Czech Republic	11	50	20.0		1.424	0.712	pLINN1(2) pSGS1(9) ○	Svoboda et al., 2015

Only species with at least two populations with a minimum of 40 sampled individuals (i.e. the lowest number of sampled individuals in the bearded reedling populations) and with exact sampling locations are shown. The symbols after certain lineages (e.g. ●, ■, ♥) indicate that the lineage was detected in more than one population of the given species. Lineages shown in bold mean that they would appear also in other population(s) of the given species, but because of the low sample sizes those populations are not indicated in the table. * Indicates that in the Kvismaren population of the great reed warbler some of the individuals were sampled more than once (No of tested samples = 782, No of sampled individuals = 470). This may cause a bias in the estimates of overall prevalence and in the lineage prevalence; however, survival was unrelated to infection status of the individuals in the given species.

Mean prevalence of *Haemoproteus* and *Plasmodium* parasites for each host species, Simpson's index (*D*) and Species evenness (*E*) for the parasites were also calculated in each host population.

in habitats where vectors were either absent or the conditions for parasite development were not favourable [i.e. bird species living on islands far from the continents (Campioni *et al.*, 2018); on coastal areas (Martínez-de la Puente *et al.*, 2015); or in high altitudes (Valkiūnas, 2005)]. Low parasite prevalence and/or diversity of parasites were also found in introduced bird species, which had 'lost' their parasites during the colonization process (Lima *et al.*, 2010; Ventim *et al.*, 2012c). Similar findings apply to host species characterized by small population sizes and fragmented distribution, preventing the maintenance of specialized parasites (Neto *et al.*, 2015; Eastwood *et al.*, 2019). Remarkably low *Haemoproteus* prevalence was reported from the chiffchaff species complex (*Phylloscopus collybita*), although the sample sizes in the sampled populations were moderate (5–30), therefore the prevalence of parasites could not be estimated precisely (Bensch *et al.*, 2012).

If a bird species is utilized by host-specific parasites, the transmission may be hampered by very low population sizes or if the population goes through a bottleneck. Though we have no information about the exact population sizes of the bearded reedlings sampled across Europe, previous bird census data showed that the Hungarian study population consists of ca. 1200 individuals (Lendvai *et al.*, unpublished data) and the Austrian population of approximately 5000–10 000 individuals (Hoi *et al.*, unpublished data). These population sizes cannot be considered to be small. However, we also know from previous studies that the species might be sensitive to the low temperatures and high precipitation during winter, which strongly affect the population size in the following year (Lendvai *et al.*, unpublished data; Wilson and Peach, 2006). This may result in strong fluctuations in the population sizes from year to year which may perhaps provide an unfavourable environment for host specialized parasites and thus results in a lack of such parasites in this species (Neto *et al.*, 2015).

Indeed, neither of the bearded reedling populations studied to date has any common blood parasite. The *Plasmodium* lineage infecting the Austrian population (pSGS1) is highly host generalist, detected previously in 123 other bird species. Also the parasites infecting the previously studied Swedish (hWW2, hTURDUS2) and Turkish (hGRW5, hWW1) populations were detected from a range of 4–21 bird species (MalAvi). The least host generalist among these lineages is the lineage hGRW5 that was detected from three other bird species, however, these bird species are all *Acrocephalus* species (*Acrocephalus arundinaceus*, *A. melanopogon*, *A. palustris*) coexisting in the reed beds with the bearded reedling. The lack of common bearded reedling specialist parasites and the generally low overall prevalence of parasites in this species raise the possibility that the parasites infecting the bearded reedling populations may occasionally come from other coexisting bird species of the sampling areas and that these parasites cannot develop in this bird species. The idea that bearded reedlings may be unsuitable hosts for avian malaria and malaria-like parasites is supported by the fact that prevalence of blood parasites in the coexisting wetland specialist bird species was much higher (Table 1). Even more, each of these other bird species carried some parasite lineages that were present in more than one population of the given species (Table 1) suggesting that these lineages can indeed develop in these bird species.

One caveat though is that all these coexisting species are migratory, because sufficient information (i.e. a reasonable number of individuals/populations) on parasite prevalence was not available for resident wetland specialists (MalAvi). This means that higher infection patterns in coexisting migratory species could also be explained by the fact that these species are exposed to the vectors of Haemosporidian parasites not only during breeding but also during migration and at their wintering sites (e.g. Waldenström *et al.*, 2002; Lemke *et al.*, 2013; Hegemann *et al.*, 2018).

However, high overall Haemosporidian prevalence was also detected in different populations of resident bird species from habitats other than wetlands. In blue tits (*Cyanistes caeruleus*) (Szöllösi *et al.*, 2011) and great tits (*Parus major*) (MalAvi database) from natural forests and urban areas, the average prevalence was much higher [mean prevalence 66.3% (ranging from 30.5 to 100%) and 59.7% (ranging from 32.6–90.0%), respectively] than in bearded reedlings, even though the abundance and diversity of vectors in those habitats were probably lower than in wetlands. Blue tits and great tits were also infected with a range of parasite lineages commonly found in each population (1–5 and 2–3 common lineages/blue tit and great tit populations, respectively), suggesting that host–parasite coevolutionary relationships are probably more important in determining parasite prevalence and diversity than environmental factors. This reasoning is supported by a recent study, where certain parasite lineages were strongly associated with the different host species and prevalence of Haemosporidian parasites was more similar between different populations of a species than across species within a habitat (Pulgarín-R *et al.*, 2018).

It is therefore possible that bearded reedlings are resistant to avian malaria and malaria-like infections and either do not become infected or quickly clear occasional infections. This might either be because bearded reedlings are immunologically adapted or resistant to these infections or apply some unknown behavioural strategies that make them less likely to be exploited by the vectors of *Plasmodium* and *Haemoproteus* parasites. It is also possible that infections by certain pathogens may exclude or reduce the possibility of infections by others (see e.g. Richie, 1988; Nacher, 2004).

The low prevalence and parasite diversity in bearded reedling populations may also indicate that mortality among infected individuals is so high that this manifests in no/only a few infected individuals detected in the population. However, such a high mortality (especially before the first reproduction) should cause very strong selection towards Haemosporidian resistance. To answer these possibilities, further infection experiments and studies on resistance genes are needed. In any case, the remarkably low infection level of bearded reedlings makes them a useful candidate model species for either behavioural or immunological resistance or extreme sensitivity towards avian blood parasite infections.

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