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Population- and age-specific patterns of haemosporidian assemblages and infection levels in European bee-eaters (*Merops apiaster*)

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ABSTRACT

Amongst other factors, host behaviour critically determines the patterns with which blood parasites occur in wild host populations. In particular, migratory hosts that sequentially occupy distant sites within and across years are expected to show distinct patterns of blood parasitism depending on their population-specific schedules and whereabouts. Here, we monitored haemosporidian parasitism in two populations of European bee-eaters (*Merops apiaster*), breeding in Portugal and Germany, with fundamentally different spatiotemporal migration patterns and colonisation histories. We describe and compare the composition of their parasite fauna as well as host population-, age- and sex-specific patterns in the frequency and intensity of infections. We found haemosporidian prevalence to be higher in Portugal compared with Germany and the prevalence generally increased with host age in both populations. Bee-eaters breeding in Portugal and wintering in western Africa mostly hosted parasites of the genus *Haemoproteus*, while *Plasmodium* lineages prevailed in birds breeding in Germany and wintering in central Africa. We found 18 genetic lineages, of which nine uniquely occurred in Germany, three uniquely in Portugal and six occurred in both breeding populations. The infection intensities (= % infected per inspected erythrocytes) ranged from 0.002% up to maximally 2.5% in Portugal and 9.6% in Germany. The intensity was higher in Germany compared with Portugal, vastly varied between the parasite genera (*Haemoproteus* > *Plasmodium*), but also differed between lineages of the same genus. Our results suggest that populations from different parts of a host's breeding range differ in prevalence and the composition of their haemosporidian assemblages, rather than in the intensity of their infections. Whether these patterns are mainly caused by differential habitat use throughout the annual cycle and/or the population-specific co-evolutionary backgrounds of a host species in range expansion remains to be elucidated.

1. Introduction

The composition of parasite assemblages, as well as the occurrence and severity of infections, in wild bird populations are influenced by a multitude of factors including the life cycle strategy of hosts (Altizer et al., 2011) and the co-evolutionary history of the host–parasite system (Lutz et al., 2016). Describing fundamental infection parameters (e.g. parasite prevalence, the composition of assemblages and the severity of infections) and determining factors which influence these parameters, are fundamental to understanding host–parasite systems and their reactions to changing environments (Lafferty, 2009).

Particularly for indirectly transmitted parasites, the patterns of parasitism might predominantly be influenced by environmental conditions. For example bloodsucking insects, the vectors of avian haemosporidian parasites, often have specific temperature and humidity requirements for their development (Jarošík et al., 2011). Long-distance migratory birds, which sequentially encounter a larger variety of sites and potential vectors, tend to host richer parasite assemblages than resident birds (Koprivnikar and Leung, 2015; Emmenegger et al., 2018). While the influence of migratory behaviour on infection prevalence is ambiguous (Altizer et al., 2011), coloniality and social lifestyle of hosts clearly facilitate the transmission of pathogens and therefore increase their prevalence (Gabaldon and Ulloa, 1980).

In addition to a species' life cycle strategy, individual life cycle stages can influence infection parameters. Blood parasitism may

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vary according to age (Thul et al., 1980; Davidar and Morton, 1993) or between sexes, if these groups differ in their probability of becoming infected (Valkiūnas, 2005) or in their ability to combat infections (Norris et al., 1994; McCurdy et al., 1998).

Here, we aimed to describe the fundamental patterns in (i) blood parasite prevalence and (ii) the composition of blood parasite assemblages, and to assess (iii) the intensities of blood parasite infections (henceforth referred to as 'parasitaemia') of European bee-eaters (*Merops apiaster*; henceforth-referred to as 'bee-eaters'), an obligate long-distance migratory near-passerine. To this aim, we monitored blood parasitism in two bee-eater populations, one situated on the Iberian peninsula in Portugal, belonging to the traditional Mediterranean breeding range, and one in central-eastern Germany, founded in the course of the recent range expansion of this species in central Europe (Kinzelbach et al., 1997). Bee-eaters from these distant breeding regions differ in their core non-breeding ranges, with Portuguese birds mainly wintering in the western part of sub-Saharan Africa and German birds mainly in the Congo region and Angola (Hahn et al., 2019). Over a total of six consecutive years, we assessed the composition of assemblages, the hosts' sex- and age-specific prevalences and the intensities of infections with haemosporidian parasites of the genera *Plasmodium* and *Haemoproteus* (henceforth referred to as 'haemosporidian parasites'). After being transmitted from the dipteran vector to a susceptible host, these intraerythrocytic protozoa cause symptomatic infections for a few days to several weeks and afterwards, most infections become chronic (Valkiūnas, 2005).

European bee-eaters are common colonial breeders in southern Europe and central Asia and, compared with other aspects of natural history and ecology (Fry, 1984), there is little known about their haemosporidian parasites. Three lineages of *Haemoproteus* had been described to infect this host species to date (MalAvi database for avian haemosporidian parasites: http://130.235.244.92/Malavi, accessed on the 20 February 2020; Bensch et al., 2009). Adult bee-eaters in two breeding colonies near the Black Sea coast were more often infected with the lineage H-MEAPI01 (21%) than with H-MEAPI02 (2%) (n = 48; Dimitrov et al., 2016). Additionally, one bird sampled during the non-breeding season in Malawi was infected with QUERY01 (n = 3; Lutz et al., 2015). In resident bee-eater species of the tropics and subtropics, four other *Haemoproteus* lineages, as well as three lineages of the genus *Plasmodium,* have been detected (Ishtiaq et al., 2007; Beadell et al., 2009; Lutz et al., 2015). Although some have partially overlapping non-breeding distributions with migratory bee-eaters, none of these lineages had been reported for European bee-eaters. Moreover, data about the haemosporidian infection intensities are completely lacking for European bee-eater populations, except for single type specimens (Dimitrov et al., 2016).

Based on temporally similar annual cycles but different spatial organisations of the two host populations, we had the following hypotheses for the three infection parameters (parasite prevalence, assemblage composition and parasitaemia). Firstly, prevalence might differ between the populations, as migrants experience site- (and route-) specific vector abundances and thus differential transmission probabilities (Yohannes et al., 2009) at distinct breeding sites and in their non-breeding grounds (Hahn et al., 2019). Regarding host age, we hypothesised that infection probabilities accumulate over an individual's lifetime and thus prevalence increases with age (Davidar and Morton, 1993), because avian blood parasite infections are usually benign and mostly persist in a chronic stage (Valkiūnas, 2005). Particularly, in species such as bee-eaters with a relatively long lifespan compared with many other migratory landbirds, the effect of accumulating infections over a lifetime should exceed the effect of selective mortality of infected individuals. With regard to host sex, the transmission rate, and therefore the prevalence, was not expected to differ between the sexes, as bee-eater pairs share incubation and parental care (Cramp and Simmons, 2004), and they migrate and spend the non-breeding period in sex-mixed flocks (Dhanjal-Adams et al., 2018).

Secondly, we expected to find (dis-) similarities between the sampled populations regarding the composition of parasite assemblages on several levels. If vectors feeding on bee-eaters transmit specific parasite lineages and have small distribution ranges, then we might find population-specific lineage compositions. With decreasing parasite specificity and increasing distribution ranges of vectors, the parasite lineage compositions of the two populations were expected to become more similar, but the two populations could still vary in the frequency of parasite lineages and/or genera.

Finally, parasitaemia was not expected to differ between host populations or between age classes of hosts. However, we expected to find varying levels of parasitaemia between the two sexes if hormonal differences during the breeding season influence the susceptibility and/or immune function of adult hosts (Owen and Moore, 2006).

2. Materials and methods

2.1. Study sites and field work

We sampled adult bee-eaters in breeding populations in Portugal (PT, 39°N, 7.15°W; 2015–2018) and Germany (DE, 51.3°N, 12.0°E; 2013–2018). After the birds were caught with walk-in traps in breeding burrows, they were ringed, sexed and aged, based on plumage characteristics (according to the online species file "European Bee-eater (*M. apiaster*)" from the Identification Atlas of Aragon's Birds: http://blascozumeta.com/species-files/, accessed on 9 March 2020). During the breeding period, adult European bee-eaters could be assigned to two age classes: second-year birds, which have fledged in the previous calendar year (EURING age code 5) and older birds which have fledged at least 2 years ago (EURING age code 6).

We sampled blood by piercing the brachial vein with a hypodermic needle and collected a drop of blood with a heparinized capillary. We prepared two thin blood smears per individual sample. To preserve the DNA for molecular genetic analyses, the remaining blood was transferred to a tube prefilled with storage buffer (0.015 M NaCl, 0.05 M Tris- HCl, 0.001 M EDTA, pH 8) or to an EDTA-treated filter paper (only samples from Germany, 2018). For 149 samples from Germany, we only yielded blood smears, but no blood for genetic analyses. Consequently, the infection status of these individuals was only determined by microscopy, and parasite lineages could be assigned molecularly (n = 13). For two additional samples, we checked for infection and lineage assignment molecularly but had no smears to assess parasitaemia.

2.2. Molecular detection of haemosporidian parasites

We analysed 370 blood samples from 322 birds from the breeding sites in Portugal as well as 671 blood samples from 510 birds from breeding sites in Germany. To assess the infection status, we extracted and purified DNA from whole blood with spin-column kits and performed a nested PCR (primer pairs by Hellgren et al. (2004); nested 1: HaemNF1/HaemNR3, nested 2: HaemF/HaemR2). The PCR products were visualised in stained 2% agarose gel. Positive samples showed a clear band at approximately 500 bp. Samples with ambiguous PCR results (weak bands) were rerun and, together with all positive samples, additionally screened by microscopy to reduce the risk of false negative results.

To calculate age- and sex-specific prevalence (= number of infected/tested individuals), we excluded samples from individuals that had already been sampled in previous years, to avoid pseudo-replication.

2.3. Haemosporidian lineage assignment

The products of parasite-positive and unclear PCRs were purified, entered a single-pass sequencing (with HaemF and HaemR2) and the fluorescent-labelled fragments were analysed by electrophoresis. We checked chromatograms and edited sequences in BioEdit (Hall, 1999). The chromatograms of 16 samples showed mixed template patterns to a degree that only parasite genus (n = 1) or neither genus nor lineage could be assigned (n = 15). The chromatograms of 242 samples from 209 individuals were clean or with only single mixed bases, which did not hamper lineage assignment. For these, we used a multi-sequence analysis (msa function and package; Bodenhofer et al., 2015) which compared our derived sequences with already described lineages registered in the MalAvi database, so we could detect known lineages (if a sequence 100% matched a known lineage) or describe a new lineage (if sequences did not fully match known lineages). We compared the proportion of *Plasmodium* and *Haemoproteus* lineages, to describe the parasite composition for the two breeding populations at the genus level. Additionally, we assessed which lineages are unique and which are shared between the populations. Finally, we calculated the relative diversity (= number of different lineages found/infected individuals). To avoid pseudo-replication and bias in the relative frequencies of parasite lineages/genera, a certain lineage/genus was only counted once per individual bird even if it has been found repeatedly in several study years.

2.4. Estimation of the intensity of infection

We determined the parasitaemia (= number of infected/number of examined erythrocytes) of samples with parasite-positive and unclear PCR results by examining 100 microscopic fields of the Giemsa-stained blood smears with 1000× magnification under a light microscope (Primo Star, Carl Zeiss AG). The parasites were counted, and the total number of inspected erythrocytes was estimated by extrapolation from five representative pictures (Boone et al., 2010). In case we did not find parasite-infected erythrocytes (n = 18) or only extracellular stages (n = 2) in blood smears of PCR-positive samples with a clear chromatogram, we set the parasitaemia to half of the minimum detectable intensity (i.e. ½ infected erythrocytes/total inspected erythrocytes; following Hahn et al. (2018)). If we did not find any parasite in samples with unclear PCR results and unclear chromatograms, we set the infection status to 'uninfected'.

2.5. Statistical analyses

We tested for prevalence patterns by fitting a Generalised Linear Model (GLM; function glm of the R package stats; R Core Team, 2019: https://cran.r-project.org) with infection status as the dependent variable (family = binomial) as well as age, sex and population as independent variables. To correct for variation in the relative frequency of parasite lineages and to adjust the number of lineages found in our study for rare lineages (Heck et al., 1975), we calculated rarefaction (function rarefy of the R package vegan; Oksanen et al., 2018: https://cran.r-project.org/package=vegan). Similar to the model for prevalence, we fitted a linear model to test for factors influencing parasitaemia (LM; function lm of the R package stats) with log-transformed parasitaemia (Stahel, 2017: https://rdrr.io/rforge/regr0/man/logst.html) as the dependent variable as well as parasite genus, population, sex and age as independent variables. For all models, estimates with P values below 0.05 were considered significant. Details on the model construction and outputs as well as the rarefaction calculations are given in the Supplementary Data S1. All calculations and statistical analyses were performed in R (R Core Team, 2019: https://cran.r-project.org).

2.6. Data accessibility

All accompanying data to this article can be found online on Zenodo (DOIs: R script https://doi.org//10.5281/zenodo.3968360, Data1 (i.e. infection data sheet) https://doi.org//10.5281/zenodo.3968354, Data 2 (i.e. FASTA file of lineages) https://doi.org//10.5281/zenodo.3968358).

3. Results

3.1. Age-specific prevalence patterns

The total blood parasite prevalence over all study years, populations and age classes was 21.2%. The prevalence was higher for Portuguese birds (33.3%) compared with German birds (14.5%; GLM: slope $_{PT \rightarrow DE}$ = 0.89 [± 0.18], z = 4.92, P < 0.001; see Fig. 1).

Prevalence did not differ between males and females of the same age class (slope $_{m \rightarrow f}$ = −0.03 [± 0.17], z = −0.17, P = 0.86; see Fig. 1). However, birds older than the second calendar year were significantly more often infected (26.6% overall, 18.2% in Germany and 33.7% in Portugal) than second-year birds (15.9% overall, 13% in Germany and 25.7% in Portugal; slope $_{6 \rightarrow 5}$ = 0.38 [± 0.19], z = 2.01, P = 0.04; see Fig. 1).

3.2. Population-specific parasite composition

Most of the infections in Germany were caused by *Plasmodium* parasites (62% of infected individuals; 18% were not assignable to a parasite genus/lineage). Conversely, in Portugal most of the infections involved parasites of the genus *Haemoproteus* (61% of

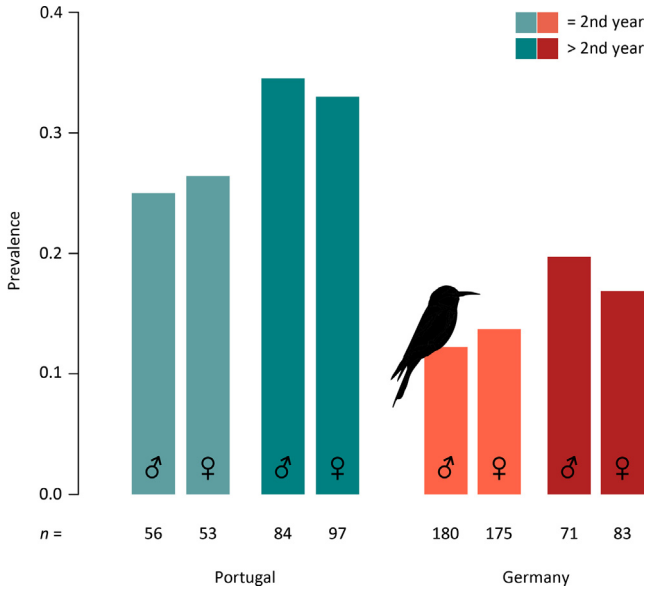

Fig. 1. The prevalence of avian blood parasite infection was higher in Portuguese compared with German bee-eaters. In both breeding populations, the prevalence was lower in birds which were in their second calendar year (EURING code 5) compared with birds which were older than the second calendar year (EURING code 6), but it did not differ significantly between sexes (♂ = males, ♀ = females).

infected individuals; 7% were not assignable to a parasite genus/lineage).

We detected H-MEAPI01 and H-MEAPI02 in both study populations, thus confirming two of three known *Haemoproteus* lineages which are already registered in the MalAvi database for European bee-eaters. Additionally, we detected six *Plasmodium* lineages currently known from other avian hosts (P-ACCTAC01, P-AEMO01, P-GRW02, P-GRW06, P-GRW09, P-MILANS06). Furthermore, we found six new *Haemoproteus* (H-MEAPI03-8) and four new *Plasmodium* lineages (P-MEAPI09-12) in our study.

The five most prevalent lineages P-GRW02 (n = 86), H-MEAPI03 (n = 70), H-MEAPI02 (n = 11), P-GRW09 (n = 8) and H-MEAPI01 (n = 6) were all found in both breeding populations (Fig. 2). Detailed and population-specific numbers of incidences for all lineages detected in this study can be found in the MalAvi database, and the new lineages are additionally registered in GenBank (accession no. **MT419905–MT419914**).

We found fewer parasite lineages in Portuguese compared with German birds: The rarefied lineage richness (i.e. after correcting for rare parasite lineages; Heck et al., 1975) was 8.5 (±0.64) lineages for birds from Portugal and 15 (±0.00) lineages for birds breeding in Germany. Even when accounting for the different number of incidences, it still results in a lower relative diversity of 0.09 parasite lineages per infected individual in Portugal, compared with 0.16 parasite lineages per infected individual in Germany.

The two breeding populations shared six out of 18 lineages, including the five most frequent lineages described above. Three lineages where unique to the birds breeding in Portugal and nine were only found in birds breeding in Germany.

3.3. Parasite-specific infection intensities

Infection intensity ranged from a minimum of 0.002% to maximally 2.5% in Portugal and 9.6% in Germany. Birds breeding in Portugal were less intensively infected than birds breeding in Germany (slope $_{DE\rightarrow PT}$ = −0.26 [± 0.12], t = −2.35, P = 0.03), and *Plasmodium* parasite infections were less intense than infections with *Haemoproteus* parasites (LM: slope $_{H\rightarrow P}$ = −0.28 [± 0.12], t = −2.60, P = 0.01). However, parasitaemia differed by several orders of magnitude between the lineages within the parasite genera (Fig. 3).

We did not find significant differences in parasitaemias between the sexes (slope $_{m\rightarrow f}$ = 0.04 [± 0.11], t = 0.37, P = 0.71) nor between age classes (slope $_{5\rightarrow 6}$ = 0.08 [± 0.12], t = 0.68, P = 0.50).

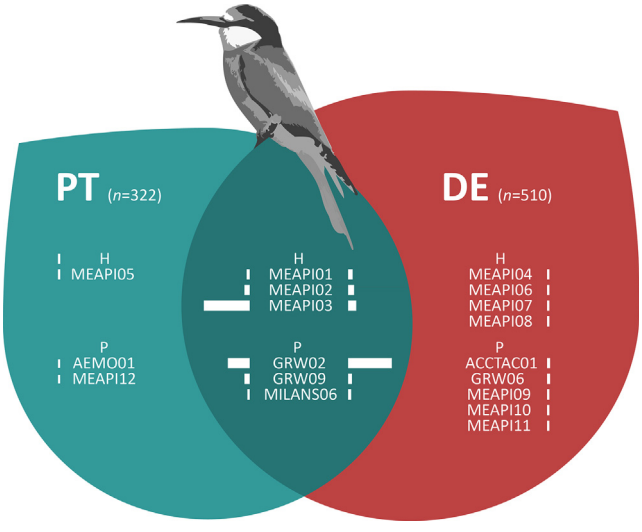

Fig. 2. The 18 haemosporidian lineages detected in bee-eaters breeding in Portugal (PT, 2015–2018) and Germany (DE, 2013–2018). Some of the lineages, belonging to the genera *Haemoproteus* (H) and *Plasmodium* (P) occurred in only one of the two populations (PT = 3, DE = 9), or in both breeding populations (n = 6). Sample sizes are given in parentheses and the number of incidences is indicated by bars (shortest bar ≙ 1, longest bar ≙ 61).

4. Discussion

This is the first known study describing population-specific frequency, diversity and intensity of avian haemosporidian parasites in European bee-eaters. Besides fundamental knowledge about patterns of parasitism in different age cohorts, our study also provides insights into natural history aspects of the host–parasite relationships between bee-eaters and their haemosporidians. We confirmed several known lineages reported in the global database on avian haemosporidian parasites and their hosts (Bensch et al., 2009). We also added many new genetic lineages, which is highlighted by the fact that the second most prevalent haemosporidian lineage in our study is a newly described one.

We showed for the first time that bee-eaters in their second calendar year, i.e. after one complete migration and non-breeding cycle, were approximately 10% less often infected than older birds after two or more complete annual cycles. We are confident that our results are robust, as sampling included many individuals, extended across several years and results were consistent in two distinct breeding populations (Fig. 1). This age effect indicates that an infection acquired over the course of a lifetime rarely undergoes complete clearance, but rather persists chronically in the host, resulting in increasing prevalence with increasing host age (Davidar and Morton, 1993). It also suggests that the accumulation of infection in old birds is not fully counterbalanced by an accelerated senescence and increased mortality of infected birds (Atkinson and Van Riper, 1991; Asghar et al., 2015). If infections were not accumulating and infected individuals on average age faster and die earlier, this would result in no age effect or lower prevalence in higher age classes.

With 33.3% for adult bee-eaters in Portugal and 14.5 % in Germany, the prevalence in our study is similar to adult bee-eaters breeding in Bulgaria, where prevalence reached approximately 30% (Dimitrov et al., 2016). The significant difference in haemosporidian prevalence between the Portuguese and the German breeding populations could have been caused by several mutually non-exclusive factors. According to population genetic studies based on microsatellites, European bee-eaters are thought to be panmictic over much of their breeding range (Ramos et al., 2016; Carneiro de Melo Moura et al., 2019). We thus do not expect differential resistance of breeding populations to cause the differences in prevalence. Considering a spatial perspective, the populations are clearly separated during the breeding season and during migration, but their core non-breeding regions partially overlap (Hahn et al., 2019). Hence, from a spatial point of view, the prevalence differences seem to arise from factors associated with the breeding sites, e.g. the local abundance of the suitable vectors for transmitting the parasites during the breeding period (Okanga and Cumming, 2013). However, this would in turn require that a considerable proportion of the transmissions take place during the breeding period and we would expect to find more acute cases in our samples (also see the discussion of population differences in parasitaemia below). However from a temporal perspective, bee-eaters from both populations spend the vast majority of the year in the non-breeding areas and much less time at their breeding sites (6 months non-breeding residency compared with 3.5–4 months at the breeding sites, Hahn et al., 2019). Even if single birds used similar areas, the vast majority spend the non-breeding season in distant

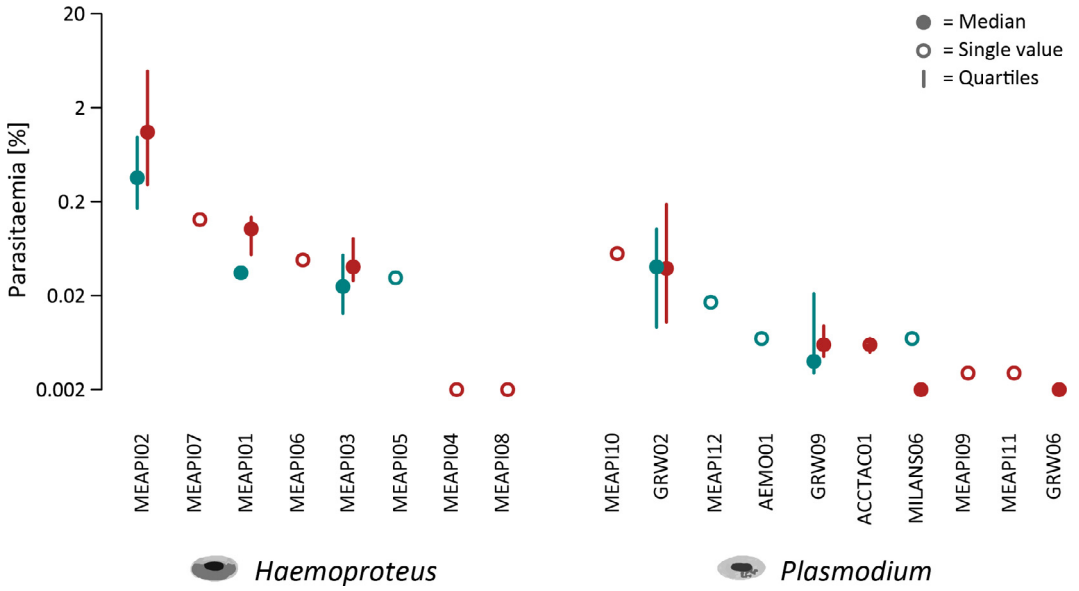

Fig. 3. Median parasitaemia (±quartiles) for *Haemoproteus* and *Plasmodium* lineages found in European bee-eaters breeding in Portugal (blue (dark grey)) and in Germany (red (light grey)), lineages are sorted by parasitaemia within genera. Parasitaemias varied widely, with infections of birds breeding in Portugal and infections with the genus *Plasmodium* being less intense than in Germany and infections with parasites of the genus *Haemoproteus*.

regions with very contrasting habitats. The core non-breeding areas of Portuguese birds is situated in western Africa (including Senegal, Gambia and Guinea-Bissau) dominated by grass- and shrublands, while the core area of German birds is situated in central Africa (including Gabon, Congo and Angola) and is mainly characterised by forests. This makes the distinct vector fauna within these different non-breeding habitats a more probable cause for the pronounced differences in prevalence between the two studied populations.

Interestingly, not only parasite prevalence, but also the composition of the parasite fauna, differed between the two breeding populations. Specific haemosporidian parasite genera are typically transmitted by specific vector taxa (Valkiūnas, 2005). As the different vector taxa rely on specific habitats, these differences in composition could be related to the local vector fauna at the different breeding sites, non-breeding grounds and/or stop-over sites en route (Loiseau et al., 2012). Spatial shifts in the composition of parasite assemblages have previously also been found to be related to prominent geographical barriers (Mata et al., 2015). Alternatively, parasite composition – varying proportions of *Plasmodium* infections and differing lineage composition – could also reflect the different colonisation history of the two populations. While bee-eaters have bred in Portugal since the records began, this species has colonised the German breeding grounds since the 1990s. In this respect, it is interesting to consider the host specificity of the parasites found in these two breeding populations (Fig. 4). Lineages within *Haemoproteus*, the prevailing genus in Portugal, are often more host-specific than *Plasmodium* lineages, which prevailed in Germany. This suggests that colonising new areas might come with the benefit of escaping some vectors of haemosporidian parasites. In this sense, our findings of blood parasite diversity and host population history are very similar to a global study in House sparrows, *Passer domesticus*, where host-specific parasites were also more common in the host within their native range, while generalist parasites prevailed in hosts living in newly occupied parts of the host range (Marzal et al., 2011).

There are no published data describing infection intensities in bee-eaters, except for parasite type specimens, which were chosen due to their particularly high infection intensities (Dimitrov et al., 2016). Based on the distribution of infection intensities, we showed that 97% of infections were of low intensity (less than 1% of the screened erythrocytes were infected), which included mostly chronic infections (according to Valkiūnas, 2005). Additionally, we found six birds with high intensity infections (one in Portugal and five in Germany). It is well known that on average *Haemoproteus* infections develop higher parasitaemias (Valkiūnas, 2005; Hahn et al., 2018; and Fig. 3 of this study), and indeed the maximum infection intensities of both populations (2.5% in Portugal and 9.6% in Germany) were caused by *Haemoproteus* parasites. These cases might encompass acute phase parasitaemias of locally transmitted parasites, but they might also be caused by potential relapses of parasitic infections acquired during the non-breeding period. Hence, we cannot conclusively assess whether transmission takes place at the breeding sites. To further verify or reject the breeding grounds as potential transmission areas would require sampling of first-year birds (EURING age code 3) at the time of autumn migration. Infection intensities were generally lower in Portugal compared with Germany, even when controlling for parasite genus in our statistical analysis. This could be related to a stronger specific defence as a result of a longer co-evolutionary history of the host–parasite system (Clayton et al., 2003) in the native range of a host species compared with populations on the edges of a distribution under a recent range shift.

Long-term datasets are robust for general conclusions as they level out potential between-year variations in prevalence and composition of the parasite fauna. The results of our study across a total of 6 years emphasise that host species undergoing recent range expansions or shifts represent interesting systems to study host–parasite co-evolutionary patterns. To complete the picture of the patterns in haemosporidian parasitism in bee-eaters from western and northern-central (our study) and eastern European populations (Dimitrov et al., 2016), future studies should sample the parasites of the interjacent populations i.e. on the Apennine peninsula, the western Balkans, as well as the large populations in western Asia. We are convinced that there is a great potential in such long-term data sets to study the broad spectrum of potential effects of parasites. Growing datasets with individual infection histories acquired within the framework of bird health monitoring will allow assessment of long-term effects of low pathogenic infections on survival rates and population trends (Dadam et al., 2019), which might be relevant for conservation of vulnerable host species even in well-adapted host–parasite systems.

Genus	Lineage	Found	Known host taxa	Similarity	Most similar	Known host taxa
Haemoproteus	MEAPI01	PT/DE	*Merops apiaster*			
	MEAPI02	PT/DE	*Merops apiaster*			
	MEAPI03 MEAPI04 MEAPI05 MEAPI06	PT/DE DE PT DE	New lineages	98.2% 99.8% 99.6% 99.6%	CYAPIC01	Corvidae, Paridae, Passeridae
	MEAPI07	DE	New lineage	97.5%	MEAPI02	*Merops apiaster*
	MEAPI08	DE	New lineage	98.3%	MEBRE01	*Merops breweri*
Plasmodium	ACCTAC01	DE	6 orders, including Coraciiformes			
	AEMO01	PT	Falconiformes, Passeriformes			
	GRW02	PT/DE	Sylviidae, Hirundinidae, Ploceidae, Fringillidae, Scolopacidae, Estrildidae			
	GRW06	DE	12 families, including Coraciiformes			
	GRW09	DE	19 families of the order Passeriformes			
	MEAPI09	PT/DE	New lineage	99.6%	COLL6	Hirundinidae, Sylviidae
	MEAPI10	DE	New lineage	99.8%	GRW02	Sylviidae, Hirundinidae, Ploceidae, Fringillidae, Scolopacidae, Estrildidae
	MEAPI11	DE	New lineage	99.8%	RTSR1	Columbiformes, Falconiformes, Gruiformes, Passeriformes
	MEAPI12	PT	New lineage	99.8%	SYBOR21	Hirundinidae, Sylviidae
	MILANS06	PT/DE	Falconiformes, Columbiformes, Strigiformes			

Fig. 4. Overview of the known hosts of avian blood parasite lineages found in European bee-eaters (belonging to the family Meropidae in the order Coraciiformes) in our study. For each known parasite lineage, the breeding population(s) where it was found is listed as is the host taxa (to the level of order (-iformes), family (-idae) or species, depending on the host specificity of the lineage) for which it has already been detected (according to the "Hosts and sites table" on the MalAvi database: http://130.235.244.92/Malavi, accessed on the 20 February 2020). For newly described parasite lineages the most similar lineage, the degree of similarity to this lineage (in %) as well as the known host taxa for this most similar lineage are listed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2020.07.005.

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