Highlights

1. Parasites must adapt their life cycles to environmental seasonality
2. We show how different transmission strategies evolved in an avian blood-borne parasite
3. Switches from summer transmission to other strategies appeared several independent times
4. Seasonally transmitted parasites have longer evolutionary lives.
5. Switches of transmission strategy could promote parasite spread.

Evolution of seasonal transmission patterns in avian blood-borne parasites

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ABSTRACT

In temperate regions, many vector-borne parasites maximize their transmission prospects by adjusting reproduction to seasonal cycles of host susceptibility and vector availability. Nevertheless, in these regions there are areas where environmental conditions are favourable throughout the year, so that parasites could benefit from a year-round transmission strategy. We analysed how different transmission strategies (strict summer transmission, extended summer transmission - including spring and autumn, and year round transmission) have evolved among the different genetic lineages of *Haemoproteus parabelopolskyi*, an avian blood-borne parasite shared by three sibling species of passerine hosts. Our results indicate that the ancestral state of this clade of parasites had a strict summer transmission with the blackcap (*Sylvia atricapilla*) as the host. Other transmission strategies and switches to the other host species (*Sylvia abyssinica* and *Sylvia borin*) evolved recently, several times, independently. This suggests that, although year-round transmission is ecologically successful at present, seasonal transmission may have become more stable over evolutionary time. Switches from strict summer to an extended or year-round transmission strategy could have ecological consequences, if they promote the spread of parasites into more distant regions, transported by the migrating bird hosts. Therefore, a deeper knowledge of how different parasite transmission strategies are structured among birds in temperate areas is essential for understanding how disease emergence risks may develop in the future.

Keywords: Ancestral state reconstruction; *Haemoproteus parabelopolskyi*; Host switching; Parasite relapse; Parasite transmission seasonality; *Sylvia atricapilla*

1. Introduction

The different life histories of parasitic organisms are shaped by natural selection to overcome hosts' defences without causing excessive harm, at least until transmission to other potential hosts has been completed (Frank, 1996). It is well known that host-parasite dynamics depend on environmental conditions (Blanford et al., 2003; Lafferty and Holt, 2003; Mitchell et al., 2005; Wolinska and King, 2009). If parasites are subjected to changes in transmission success driven by environmental seasonal variations, they also must adapt their life cycles to reduce harm to their hosts during periods of no transmission. In temperate regions, where marked environmental changes among seasons regulate life cycles of most organisms, a way of maximizing parasite exploitation strategies is to adjust parasite replication to the cycles of host susceptibility and/or vector availability (Altizer et al., 2006). Thus, many parasites spend part of the year as dormant forms and then reappear when transmission conditions are favourable. Typically, transmission peaks during or shortly after periods of host reproduction (generally speaking, spring-summer), as this coincides with an abundance of vectors and susceptible hosts that include immunologically naïve juveniles and adults whose immunity is constrained by reproductive physiological investment (Dowell, 2001; Altizer et al., 2006). Accordingly, parasites display seasonal cycles of covert and overt presence where parasite replication corresponds to seasonal constraints (Sorrell et al., 2009).

Given their key role as drivers of parasite replication and spread, understanding how different seasonal patterns of transmission arise among closely-related parasites could help to predict and prevent current and future disease risks (Morgan et al., 2012). The avian blood-borne parasite, *Haemoproteus parabelopolskyi* (Valkiūnas et al., 2007), is a useful model to study the evolution of different seasonal parasite transmission strategies. Within this vector-borne parasite morphospecies, several closely related genetic lineages can be identified by distinct cytochrome *b* (cyt *b*) DNA haplotypes. Lineages behave in turn

as proper biological species (as shown by matching phylogenies of mitochondrial (mt)DNA and nuclear (n)DNA markers showing no trace of recombination among lineages; Bensch et al., 2004; Outlaw and Ricklefs, 2014). The *H. parabelopolskyi* lineages have coevolved with a clade of three sibling passerine species of the genus *Sylvia* (Pérez-Tris et al., 2007): i) the African hill babbler, *Sylvia abyssinica,* which is an Afrotropical resident species, ii) the garden warbler, *Sylvia borin,* which is a Palaearctic long-distance migrant overwintering in the Afrotropics, and iii) the blackcap, *Sylvia atricapilla,* which is a Palaearctic species with both resident and migratory populations whose wintering range expands from the south of the Palaearctic region to the Afrotropics. No *H. parabelopolskyi* lineage has ever been found in other bird species outside this clade, and each lineage typically infects only one of the three host species of the clade, with the exception of H-SYAT01 and H-PABY06 lineages, both of which infect both the blackcap and the African hill babbler (Pérez-Tris et al., 2007; Santiago-Alarcón et al., 2011).

Due to the distribution of their three host species, *H. parabelopolskyi* lineages must survive in regions with dissimilar seasonal variations. Therefore, the different lineages have contrasting strategies of seasonal transmission, which allow us to study the pattern of evolutionary transitions between these strategies among otherwise closely related parasites. Most lineages infecting blackcaps and garden warblers seem to be transmitted only in the Palaearctic breeding grounds of their hosts: during winter parasites disappear from the circulating blood and appear only as dormant forms inside their hosts' internal organs. Then parasites relapse into the bloodstream in spring to start a new transmission episode (Pérez-Tris and Bensch, 2005a; Hellgren et al., 2007). Other lineages instead display a continuous presence in the host's bloodstream, enabling transmission throughout the year. This strategy can be found in two different environmental contexts: first, parasites that infect the tropical African hill babbler are not affected by the constraints that winter imposes on transmission in temperate regions. Second, this strategy is also found in some

of the parasites that infect the blackcaps that spend winter in the Mediterranean region (either resident or short-distance migrant birds), where climatic conditions are mild enough to allow the activity of insect vectors during winter (Pérez-Tris and Bensch, 2005a; Hellgren et al., 2007).

We set out to analyse how different seasonal patterns of transmission arise among closely related parasites by assessing the pattern of evolution of different seasonal transmission strategies among *H. parabelopolskyi* lineages. To do so, and given that our knowledge of the haemosporidian transmission strategies in temperate regions is still limited to comparisons of parasite prevalences between breeding and mid-winter periods (Pérez-Tris and Bensch, 2005a), our study also reassessed the seasonal transmission patterns of some less well-known lineages by exploring how parasite prevalences progressively change on a monthly basis from the end of one breeding season to the onset of the next. We also looked for seasonal differences in prevalence among parasites, which could offer insight to the relative advantages of one transmission strategy over another. It should be noted that parasite transmission depends on a complex network of factors other than prevalence, such as parasitaemia, vector availability and abundance, and other ecological factors (Cornet et al., 2014); but prevalence can still be considered a good correlate of parasite transmission in haemosporidians (Smith et al., 1993; Beier et al., 1999; Wood et al., 2007; Cornet et al., 2014). All in all, by analysing the evolution of seasonal transmission strategies within phylogenetically restricted clades of parasites and hosts, our study will shed light on the processes promoting such diversity, improving our understanding of putative mechanisms of parasite switching among host species and geographic regions.

2. Materials and methods

2.1. Field sampling of blackcaps during the winter season

To elucidate how the patterns of summer and year-round transmission of the different lineages of *H. parabelopolskyi* infecting blackcaps vary throughout the wintering season, we performed an extensive parasite screening of wintering blackcaps. The blackcaps were captured in the Campo de Gibraltar region (southern Spain; 36º 01' N, 5º 36' W) on a monthly basis, from September 2006 to March 2007, spanning the period during which migratory birds are present in the area in close coexistence with sedentary blackcaps (Cramp, 1992; our personal observations). According to the local climatic conditions, we divided the sampling into three seasons: autumn (September to November), winter (December and January) and spring (February and March).

Shortly after capture, blackcaps were individually identified with a metal ring and sexed and aged by plumage characteristics (Svensson, 1992), distinguishing between juveniles (first-winter birds) and adults (older birds of unknown precise age). The following measures were taken: tarsus length, length of the eighth primary (feather number starting from the body to the wing tip), tail length, and distance between the wing tip and the tip of the first and the ninth primaries. These measures were used to assess the migratory phenotype of blackcaps (migratory or sedentary) using a discriminant function that correctly classifies >97% of birds (Pérez-Tris et al., 1999; De la Hera et al., 2007, 2012). A blood sample (10 - 20 µl) was collected from the jugular vein, preserved in absolute ethanol and refrigerated until analysed. All birds were released after sampling. Bird sampling was performed in compliance with Spanish environmental regulations and with the authorizations issued by the Spanish Ministry of Environment and the Government of the Autonomous Region of Andalucía.

2.2. Molecular analyses

Total DNA was extracted from blood samples following a standard ammonium acetate protocol. DNA was diluted to a working concentration of 25 ng/µl. Parasite presence was

assessed through the nested PCR protocol developed by Waldenström et al. (2004) to detect infections of avian *Plasmodium* and *Haemoproteus,* in which a 479 bp fragment of the parasite's cyt *b* gene was amplified. PCR results were verified in 2% agarose gels, stained with ethidium bromide, and assessed under UV light to look for bands of appropriate size. Negative samples were rerun to look for false negatives. Both a positive (DNA template from an infected bird) and negative (distilled water instead of template DNA) PCR control were included in every PCR batch to test for quality and contamination. No negative control yielded a positive result. All samples were previously tested using a standard bird sexing protocol (Griffiths et al., 1998) to test for sample quality and PCR suitability.

Positive samples were sequenced on an ABI Prism 3730 capillary robot (Applied Biosystems, UK) using the primer HaemF (Waldenström et al., 2004). Sequences were edited manually using BioEdit 7.0.5.3 (Hall, 1999) and unique cyt *b* gene haplotypes were defined by a sequence divergence of at least one base in the amplified fragment. Mixed infections were detected by the presence of double peaks on the electropherograms (Pérez-Tris and Bensch, 2005b) and the identity of the parasites involved was assessed comparing the double peak patterns with the previously known parasite lineages infecting blackcaps. Parasite DNA haplotypes, which were not previously described, were confirmed by repeating the PCR and then sequencing from both ends with primers HaemF and HaemR2. New sequences were given a name according to MalAvi nomenclature (Bensch et al., 2009) and registered in GenBank.

2.3. Analyses of seasonal distribution of blackcaps' parasites

We used log-linear analyses to assess the variation in prevalence of the most frequently found *H. parabelopolskyi* lineages according to sampling season (autumn, winter or spring); as well as its possible interactions with age, sex and migratory behaviour

(sedentary or migratory birds). The log-linear models were obtained by proceeding hierarchically by fitting all interactions of order N to the corresponding null hypotheses that all of them are simultaneously zero. As soon as the reduction in N caused a lack of fit, the terms of that order or lower which significantly contributed to an explanation of the distribution of frequencies were selected; thus generating the final model that best fit the data (StatSoft Inc., 2013. Electronic textbook. URL: http://www.statsoft.com/textbook). All statistical analyses were performed with STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma).

2.4. Phylogenetic analyses

To determine which *Haemoproteus* spp. lineages belong to the *H. parabelopolskyi* clade, a phylogeny including all *Haemoproteus* spp. sequences registered in the MalAvi database (Bensch et al., 2009; last accession February 2014) was created (Supplementary Data S1). From this phylogeny, the clade that included all lineages that matched, through microscopic examination, the morphospecies *H. parabelopolskyi* (Valkiūnas et al., 2007) was considered the target parasite group (28 lineages in total; Table 1, Supplementary Fig. S1 and S2). As a result of a thorough literature survey (Supplementary Data S2, Supplementary Table S1) and to the outcome of the log-linear analyses (see Results 3.1.), we could distinguish three current states of parasite transmission displayed by the different *H. parabelopolskyi* lineages: (i) lineages with summer transmission (parasites that are not found during non-breeding periods), (ii) lineages with an extended seasonal transmission period (found in autumn and/or early spring in addition to the breeding season, but not in winter) and (iii) lineages transmitted throughout the year (showing little or no change in prevalence among seasons).

To obtain an accurate picture of the branching patterns among the 28 lineages belonging to the *H. parabelopolskyi* clade, their phylogenetic tree topology was re-estimated by

Bayesian inference using BayesPhylogenies1.1 (Pagel and Meade, 2004). A run of four Markov chains (one cold and three heated) was performed for 10^7 generations and sampled every 1,000 generations. The first 25% of generations were discarded as the burn-in period, and the remaining trees were used to elaborate a consensus tree (Fig. 1).

Both blackcaps and garden warblers harbour a great number of *H. parabelopolskyi* lineages. Whereas some of them are widespread and frequent, others have been detected very few times and it is not possible to confidently assess which is their pattern of seasonal transmission (Table 1, Supplementary Data S2). Hence, two different analyses of the ancestral state of parasite transmission seasonality were performed: one assigning the most likely current transmission state to all *H. parabelopolsky* lineages and a conservative one considering only those states known with confidence. A Bayesian estimation of the ancestral host species and the ancestral state of parasite transmission seasonality at each node was done using BayesTraits 2.0 (Pagel et al., 2004) across all of the trees retrieved from the precedent analysis. A Markov chain was allowed to run for 10^7 generations with rate coefficients and ancestral states sampled every 1,000 generations after discarding the first 25% of generations as the burn-in period. Transition rate parameters were adjusted using a gamma-distributed hyperprior. The intervals of the mean and variance of the prior distribution, as well as the deviance rate, were adjusted to obtain an acceptance rate between 20 and 40% (Pagel et al., 2004).

3. Results

3.1. Parasite screening and seasonal distribution

In the course of the field sampling we captured a total of 541 blackcaps, of which 56 were assigned to be sedentary and 485 migratory. Birds that were captured more than once were only included the first time in the dataset. We were able to identify a total of 16 different parasite lineages, with an overall prevalence of 42.1% for *Haemoproteus* spp.

(principally *H. parabelopolskyi*) and 4.6% for *Plasmodium* spp. (Table 2). We found 29 double infections, for all of which we were able to resolve the identity of the parasites. The prevalence of the five most common parasites transmitted outside of the breeding season varied significantly across the study period, and this temporal variation was the strongest effect detected in the log-linear analyses (Table 3, Fig. 2). H-SYAT01 lineage was by far the most frequent parasite in the sample and its prevalence decreased during winter but was still comparatively high. The prevalence of both H-SYAT07 and H-SYAT10 remained relatively stable throughout the study period. H-SYAT02 and H-SYAT13 showed high prevalence in autumn and were almost absent during winter, then reappeared in spring. Thus, in addition to the division between (i) lineages transmitted only during the host breeding season and those transmitted outside this period, this latter group can be further split into (ii) lineages which disappear from the host circulating blood only during winter (i.e. extended transmission) and (iii) lineages present in the host blood throughout the year (i.e. year-round transmission). This classification was based on whether the sampling season did or did not have a significant effect in the log-linear analyses. Besides seasonal variation, prevalences of four of the parasites were significantly different between birds assigned to the two migratory strategies, all showing higher prevalences in sedentary than in migratory birds. No significant effects of sex or age on prevalence were detected, except for a greater prevalence of H-SYAT07 among adult birds.

3.2. Phylogenetic analyses

The reconstruction of the ancestral state of host species suggests that the *H. parabelopolskyi* clade originated in blackcaps with a high posterior probability (0.93), and that host changes were always of recent origin in the evolutionary history of the group. To reconstruct the ancestral state of parasite strategies we first used a data set where all parasite lineages had been assigned a transmission state (Fig. 1A). This analysis

suggested that strict summer transmission was widespread among the different common ancestors of the phylogeny, including the root node (posterior probability of 0.76). However, the conservative analysis did not depict an equally clear pattern (Fig. 1B): the transmission states assigned in the conservative analysis gave greater importance to extended summer and year-round transmission in the deeper nodes of the phylogeny. Thus, parasites showing low prevalences require very large sample sizes to significantly support their absence in a given season (Supplementary Data S2). Regardless of the approach, both year-round and extended summer transmission appear to have arisen several times at the tips of the parasite phylogeny. Interestingly, both reconstructions indicate also that transmission strategies can alternately appear in a clade, as exemplified by the five parasite lineages in the clade including H-SYAT07.

4. Discussion

Our results show that closely related parasites may differ in their life cycles, showing different patterns of seasonal presence in blood that have changed many times during their evolution. This pattern of phenotypic variation at narrow phylogenetic scales provides evidence of adaptive evolution of parasite lifestyles. For *H. parabelopolskyi*, the data strongly support the existence of the different seasonal transmission strategies described by Pérez-Tris and Bensch (2005a), as each parasite lineage detected in our study scored the same transmission dynamics as previously reported (references provided in Supplementary Data S2). Our study allowed us to detect further differences in the transmission strategy displayed by parasites that are present in the blood of blackcaps during non-breeding periods. We could distinguish between strict year-round transmission (high winter prevalence and/or lack of significant variation in prevalence throughout the year, as for H-SYAT01, H-SYAT07 and H-SYAT10) and extended summer transmission (high prevalence in autumn that drops in winter and quickly recovers in early spring, as for

H-SYAT02 and H-SYAT13). In addition, parasites such as H-SYAT14 or H-SYAT16, which are common in blackcaps during summer (Pérez-Tris and Bensch, 2005a; Pérez-Rodríguez et al., 2013), were not detected in the present study among the birds sampled in the non-breeding season. This is surprising from a transmission point of view, because the climatic conditions of early autumn or spring at our Mediterranean study site should be highly favourable for parasite transmission. Since parasites disappear from the blood of migratory and sedentary blackcaps alike, it does not seem to be governed by weather changes or host physiological adjustments related to migration activity, pointing instead towards a suppression of gametocyte production mediated by day length, as for the spring relapse (Valkiūnas et al., 2004). This diversity of transmission strategies has evolved within a closely related clade of host-restricted parasites and demonstrates the great potential of avian haemosporidians to change their dynamics of seasonal transmission.

Transmission outside of the host breeding season is particularly relevant for parasites in the Mediterranean region, given that it confers on them a dispersal advantage over parasites with a strict summer transmission strategy. Parasites transmitted only during the breeding season could find themselves limited to isolated host populations if transmission is restricted within the range of vector movements. In contrast, a seasonally transmitted parasite that manages to switch to a non-seasonal transmission strategy would potentially get access to hosts from different locations that coexist during the winter season. Consequently, this process could result in increased dispersal capacity of the parasite through the host species' range, a beneficial effect of year-round transmission for which evidence has already been found in our study system (Pérez-Tris and Bensch, 2005a). It is worth noting that the same advantageous result (in this case, the ability to be present in the host's blood for longer periods) is achieved by the parasites through different transmission strategies (extended summer transmission or year-round transmission).

Despite the aforementioned advantages of year-round transmission, most haemosporidians in temperate areas seem to exhibit seasonal transmission (Hellgren et al., 2007). Thus, an overriding question is: if an extended transmission season is advantageous, why are most of the switching events from summer transmission to other strategies recent evolutionary phenomena? Changes in transmission strategy are by no means rare events in the phylogeny of *H. parabelopolskyi*, indicating that they may not be regulated very tightly (a rigid genetic control of gametocyte seasonal production, for instance). Thus, the consequence of natural selection acting in the long-term against year round transmission may be a more probable cause. In principle, parasites which affect their host for a longer period are at higher risk of being targeted by the host immune system, which may be particularly efficient against parasites that have a longer history of interaction with that host species (Altizer et al., 2003; Woodworth et al., 2005). Then, in the context of competition between highly diverse and closely related parasite lineages (such as in *H. parabelopolskyi* parasites of blackcaps), any selective disadvantage for parasites that are very conspicuous to host immunity may drive them to extinction or promote reversion to a summer transmission strategy (as shown in the H-SYAT07 clade). In fact, true year-round transmission may not be as beneficial as previously thought. This is because parasite lineages with an extended summer transmission strategy exhibit both greater geographic range distributions and higher local prevalences than those with year-round transmission (Pérez-Tris and Bensch, 2005a; Pérez-Rodríguez et al., 2013). An extended summer transmission would combine the best of both strategies, thereby promoting parasite survival. Parasites that remain in the host blood during periods of coexistence with different host populations (autumn and spring) increase dispersal prospects but also keep host exploitation at a low level during the most restrictive periods for vectors, when host physiology might also be more compromised.

Relative differences in the prevalence of each *H. parabelopolskyi* lineage among migratory and sedentary blackcaps remained unchanged during the study period (no significant season x migration interactions were detected). This may indicate that parasite exchange between sedentary and migratory birds during the non-breeding season is rare, despite parasite gametocytes circulating in birds' blood throughout the year. Nevertheless, a few events of transmission of parasites during periods of coexistence between migratory and sedentary blackcaps may be enough for consequences to be detected over time, since a small number of immigrants per generation may allow parasites to reach the level of gene flow necessary to obtain fitness benefits associated with increased local infectivity (Pérez-Tris and Bensch, 2005).

Our results broaden knowledge about the processes regulating parasite transmission throughout the year and raise new questions for future research. It would be interesting to know the precise mechanisms regulating parasite presence in peripheral host blood and if they are the same for the different transmission strategies uncovered in this or other studies (Mendes et al., 2013). Future research including data from more than one complete winter season should also be undertaken to look for relationships between specific environmental conditions and seasonal variations in prevalence. Thus, if annual meteorological features turn out to have a detectable effect on the average winter prevalence of a given parasite, then climate change could modify parasite abundances by affecting parasite transmission on the wintering grounds and not only on the breeding grounds, where studies addressing this issue are usually focussed (Møller, 2010; Garamszegi, 2011; Loiseau et al., 2013; Pérez-Rodríguez et al., 2013). It would also be pertinent to examine how, under natural conditions, the actual presence of vectors might determine the persistence or relapse of parasites into the host's bloodstream, as shown under laboratory conditions for *Plasmodium relictum* (Cornet et al., 2014). To conclude, our analysis of the emergence of the different transmission strategies within a phylogenetic

framework indicates that, although the changes between transmission strategies do not show much stability over evolutionary time, they can arise frequently and temporarily achieve great ecological success (Pérez-Tris and Bensch, 2005a). The unpredictability of these switches requires monitoring parasites of several host species across both seasons and years (Fuller et al., 2012) in order to determine how widespread different transmission strategies are and to detect any possible hazard that, in a context of fast and unpredictable global change, might promote parasite spread among previously unaffected host species.

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Legends to figures

Fig. 1. Reconstruction of ancestral states of seasonal transmission strategy and host species of the different *Haemoproteus parabelopolskyi* lineages, either assigning a given transmission strategy to all of the parasite lineages (A), or considering only those transmission strategies known with confidence (B). The 50% majority rule consensus tree is shown; Bayesian posterior probabilities are depicted at each node. Current transmission states and estimated posterior probabilities for the transmission state of the Most Recent Common Ancestor (MRCA) are identified by colours (black, year round transmission; grey, extended summer transmission; white, strict summer transmission; the shaded percentage of each circle indicates the probability of the MRCA showing that trait). Parasites host shifts from blackcaps (*Sylvia atricapilla*) to other species are indicated by arrowheads. *Haemoproteus majoris* lineage H-WW2 was used as outgroup.

Fig. 2. Seasonal variation in prevalence of *Haemoproteus parabelopolskyi* lineages. Variation in prevalence of the five most frequently found *H. parabelopolskyi* lineages over the course of the wintering period (September 2006 to March 2007). The number of sampled birds per month is stated in parentheses.

Supplementary Figure legends

Supplementary Fig. S1. Phylogenetic tree of *Haemoproteus* spp. The 50% majority rule consensus Bayesian tree including all *Haemoproteus* spp. sequences registered in the MalAvi database. Blue, outgroup *Leucocytozoon mathisi* L-ACNI04; red, *Haemoproteus parabelopolskyi* clade.

Supplementary Fig. S2. Enlarged picture of the subtree of the phylogenetic tree of *Haemoproteus* spp. (Supplementary Figure S1) including all *H. parabelopolskyi* lineages. Numbers above branches indicate Maximum Likelihood (ML) bootstrap values; numbers below branches indicate Bayesian posterior probabilities. Parasite lineages which have been positively assigned to *H. parabelopolskyi* through microscopic examination are marked with an asterisk (*).

Table 1. Parasite lineages belonging to the *Haemoproteus parabelopolskyi* clade. Parasite host species (*Sylvia abyssinica*/ *Sylvia atricapilla*/ *Sylvia borin*) and current transmission states were retrieved from the literature (see Supplementary Data S2).

Parasite lineage	GenBank accession number	Transmission state	Host species
H-PABY04	**DQ368355**	Year-round	*S. abyssinica*
H-PABY06	**DQ368356**	Year-round	*S. atricapilla* *S. abyssinica*
H-SYAT01	**AY831750**	Year-round	*S. atricapilla* *S. abyssinica*
H-SYAT02	**AY831751**	Extended	*S. atricapilla*
H-SYAT04	**AY831753**	Summer [a]	*S. atricapilla*
H-SYAT07	**AY831754**	Year-round	*S. atricapilla*
H-SYAT09	**AY831756**	Summer [a]	*S. atricapilla*
H-SYAT10	**AY831757**	Year-round	*S. atricapilla*
H-SYAT11	**AY831758**	Summer [a]	*S. atricapilla*
H-SYAT12	**AY831759**	Summer [a]	*S. atricapilla*
H-SYAT13	**AY831760**	Extended	*S. atricapilla*
H-SYAT14	**AY831761**	Summer	*S. atricapilla*
H-SYAT16	**AY831762**	Summer	*S. atricapilla*
H-SYAT17	**AY831763**	Summer	*S. atricapilla*
H-SYAT19	**AY831765**	Summer [a]	*S. atricapilla*
H-SYAT21	**AY831766**	Summer [a]	*S. atricapilla*
H-SYAT26	**AY831767**	Year-round	*S. atricapilla*

H-SYAT28	**AY831768**	Summer [a]	*S. atricapilla*
H-SYAT29	**AY831769**	Summer [a]	*S. atricapilla*
H-SYAT30	**GU784849**	Summer [a]	*S. atricapilla*
H-SYAT33	**GU784852**	Summer [a]	*S. atricapilla*
H-SYAT34	**GU784853**	Summer [a]	*S. atricapilla*
H-SYAT36	**JN164702**	Year-round	*S. atricapilla*
H-SYAT37	**JN164703**	Year-round	*S. atricapilla*
H-SYAT41	**JQ670873**	Summer [a]	*S. atricapilla*
H-SYBOR1	**AF495575**	Year-round	*S. borin*
H-SYBOR3	**DQ368365**	Summer	*S. borin*
H-SYBOR4	**DQ368366**	Summer [a]	*S. borin*

[a]Transmission states considered as unknown in the conservative ancestral state reconstruction analysis.

Table 2. Winter season parasite screening results for September 2006 to March 2007; parasite lineages detected and prevalence per month.

Parasite morphospecies	Lineage	GenBank accession number	Number of infections	Prevalence per month (number of sampled birds)						
				Sep (74)	Oct (71)	Nov (85)	Dec (81)	Jan (129)	Feb (69)	Mar (32)
Haemoproteus parabelopolskyi	H-SYAT01	**AY831750**	145	31.1	39.4	28.2	18.5	20.2	20.3	46.9
Haemoproteus parabelopolskyi	H-SYAT02	**AY831751**	51	37.8	19.7	4.7	1.2	0.8	1.4	6.2
Haemoproteus parabelopolskyi	H-SYAT07	**AY831754**	55	5.4	18.3	10.9	16.1	9.3	4.3	3.1
Haemoproteus parabelopolskyi	H-SYAT13	**AY831760**	24	10.8	7.1	5.9	0	0.8	0	15.6
Haemoproteus parabelopolskyi	H-SYAT10	**AY831757**	9	4.1	0	3.5	1.2	0.8	0	3.1
Haemoproteus parabelopolskyi	H-SYAT16	**AY831762**	2	1.4	0	0	0	0	1.4	0

Haemoproteus parabelopolskyi	H-SYAT36	**JN164702**	1	0	1.4	0	0	0	0	0
Haemoproteus parabelopolskyi	H-SYAT37	**JN164703**	1	0	0	0	0	0.8	0	0
Haemoproteus pallidulus	H-SYAT03	**AY831752**	3	1.4	0	0	0	0	0	6.2
Plasmodium relictum	P-SGS1	**AF495571**	26	2.7	4.2	7.1	4.9	3.9	5.8	6.2
Plasmodium relictum	P-GRW11	**AY831748**	1	0	0	0	0	0.8	0	0
Plasmodium elongatum	P-GRW06	**DQ368381**	1	0	0	0	0	0	0	3.1
Plasmodium sp.	P-COLL1	**AY831747**	1	0	0	1.2	0	0	0	0
Plasmodium sp.	P-SYAT24	**AY831749**	1	1.4	0	0	0	0	0	0
Plasmodium sp.	P-SYAT38	**JN164704**	1	0	0	0	1.2	0	0	0
Plasmodium circumflexum	P-TURDUS1	**AF495576**	1	0	0	0	0	0	1.4	0

Table 3. Relationships between the five most frequently found *Haemoproteus parabelopolskyi* lineages and sampling season or host age, sex and migratory behaviour. Each log-linear model was built from the five-way contingency table including infection status with the corresponding parasite, season, age, sex and migratory behaviour. Only significant interactions (all of those two-way) involving infection status are reported for each model. All models had a good fit to the data (maximum likelihood chi-square tests with $P > 0.13$).

		Partial association		**Marginal association**	
Interactions in the model	**d.f.**	χ^2	***P***	χ^2	***P***
H-SYAT01 x Season	2	8.05	0.018	9.57	0.008
H-SYAT01 x Migration	1	6.99	0.008	7.91	0.005
H-SYAT02 x Season	2	38.94	< 0.001	43.48	< 0.001
H-SYAT02 x Migration	1	5.75	0.016	7.75	0.005
H-SYAT07 x Age	1	12.41	< 0.001	11.82	< 0.001
H-SYAT10 x Migration	1	9.32	0.002	9.83	0.002
H-SYAT13 x Season	2	10.08	0.006	11.45	0.003
H-SYAT13 x Migration	1	4.52	0.033	7.06	0.008

d.f., degrees of freedom.

Supplementary Data S1.

1. *Haemoproteus* phylogeny based on the cytochrome *b* (cyt *b*) gene

A phylogeny including all *Haemoproteus* spp. sequences registered in the MalAvi database was created with Maximum Likelihood (ML) (10^3 bootstrap replications using RAxML v7.2.6 (Stamatakis, 2006), as implemented on the CIPRES Science Gateway; URL: http://www.phylo.org/) and Bayesian inference (10^6 generations with the first 20% discarded as burn-in using MrBayes 3.2.2; Ronquist et al., 2012). Both analyses were run according to the HKY85+G+I model of molecular evolution, as selected with jModelTest 0.1.1 (Posada, 2008). *Leucocytozoon mathisi* lineage L-ACNI04 (in blue, Supplementary Fig. S1) was used as an outgroup. The 50% consensus tree was largely congruent between analyses (Bayesian tree is shown; Supplementary Fig. S1). The clade (in red, Supplementary Fig. S1) that included all lineages previously matched with the *Haemoproteus parabelopolskyi* morphospecies (Valkiūnas et al., 2007) was considered as the target parasite group (Supplementary Fig. S2).

To further assess the support of the *H. parabelopolskyi* clade, we performed a stepping-stone evaluation of the reliability of the clade, as implemented in MrBayes 3.2.2. In short, the stepping-stone provides a reliable comparison of the marginal likelihoods of two phylogenies: in this particular case, one with a defined constraint (the presence of the *H. parabelopolskyi* clade) and the other without the constraint. After running the analyses for 10^6 generations and discarding the first 20% as burn-in, the marginal likelihood of the phylogeny in which the *H. parabelopolskyi* clade was forced to appear was

noticeably higher than the likelihood of the unconstrained phylogeny (-13,570.65 versus -13,657.42, respectively), corroborating the actual existence of the clade as a natural entity.

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Supplementary Data S2.

1. Assignment of transmission strategy to the *Haemoproteus parabelopolskyi* lineages

The average parasite prevalence per lineage and season retrieved from literature (Pérez-Tris and Bensch, 2005; Križanauskienė et al., 2006; Hellgren et al., 2007, 2013; Santiago-Alarcón et al., 2011; Pérez-Rodríguez et al., 2013) and from this study showed a large amount of variation (Supplementary Table S1). Hence, to determine to what extent these differences could affect winter parasite detection in both host species living in temperate areas (blackcaps, *Sylvia atricapilla* and garden warblers, *Sylvia borin*), we compared the parasite prevalence observed outside of the breeding season with its expected prevalence, under the assumption that the occurrence of a given parasite in a sample of non-breeding hosts equals its probability of occurrence during the breeding season regardless of variation in overall parasite prevalence. To generate a null distribution we simulated a population of 100,000 infected hosts (separate analyses for blackcaps and garden warblers), in which each parasite haplotype occurred with the same frequency as the observed average prevalence during the breeding season. From the pool of hosts, we randomly selected 10,000 groups of 490 blackcaps and 298 garden warblers (equalling the number of birds sampled outside of the breeding season; Supplementary Table S1), and measured the prevalence of each parasite in each random trial.

We used these simulations to estimate the probability of finding particular parasite lineages during the non-breeding season, if present at the same prevalence as observed during the breeding season, and computed the 95% confidence interval of this expected prevalence. Parasites infecting the tropical host, the African hill babbler, *Sylvia abyssinica,* and those appearing outside of the breeding season were considered as having year-round transmission or extended summer transmission (see Section 3.1 in the main text). Parasites that were not found outside of the host breeding season but had an expected prevalence in that period significantly greater than zero (the confidence interval of expected prevalence excluded zero), were considered to have strict summer transmission. Finally, those lineages that were found only in summer but could not be proven as absent outside of the host breeding season (the confidence interval of expected prevalence included zero), were considered of unknown transmission state in the conservative analysis and of summer transmission in the general analysis, since summer transmission is the pattern common to most *Haemoproteus* lineages infecting European birds (Hellgren et al., 2007).

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Supplementary Table S1. Breeding and non-breeding prevalence of each *Haemoproteus parabelopolskyi* lineage. Number of samples per host species and season (Br., breeding; NB., non-breeding) and prevalence of each *H. parabelopolskyi* lineage as obtained from bibliographic sources and this study. The expected non-breeding prevalence according to simulations is shown. Lineages infecting only *Sylvia abyssinica* (H-PABY04 and H-PABY06) are considered to have a year round constant prevalence (2.33%; one bird infected by each parasite lineage, out of 43 sampled birds).

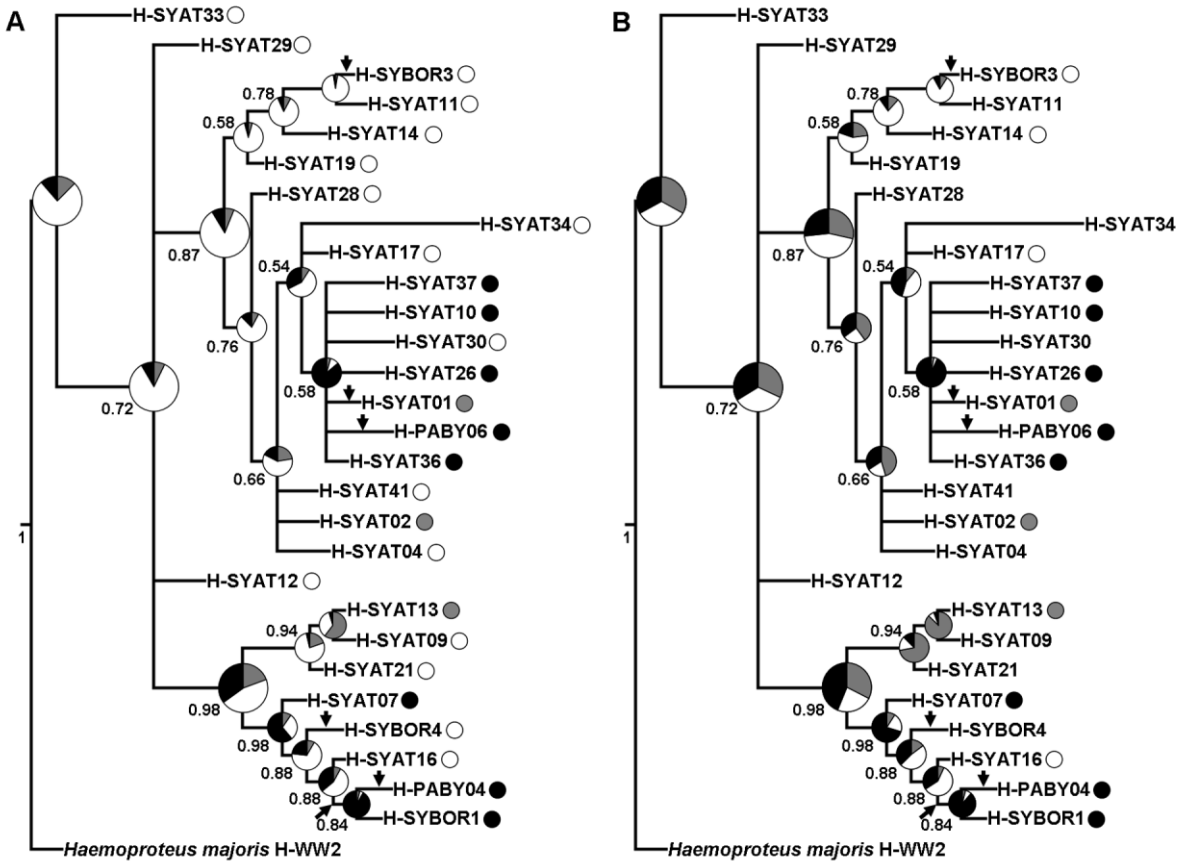
Host species	**Br. sample**	**NB. sample**
Sylvia atricapilla	2340	490
Sylvia borin	892	298

Parasite lineage	**Br. prevalence**	**NB. prevalence**	**Exp. NB. prevalence (95% CI)**
H-SYAT01	22.39	18.57	22.37 (18.77 - 25.97)
H-SYAT02	20.47	4.08	20.45 (16.96 - 23.94)
H-SYAT04 [a]	0.47	0.00	0.48 (0 - 1.07)
H-SYAT07	3.76	8.37	3.76 (2.13 - 5.39)
H-SYAT09 [a]	0.73	0.00	0.74 (0 - 1.47)
H-SYAT10	0.98	1.43	0.99 (0.14 - 1.84)
H-SYAT11 [a]	0.68	0.00	0.69 (0 - 1.4)
H-SYAT12 [a]	0.26	0.00	0.26 (0 - 0.7)
H-SYAT13	4.10	0.82	4.1 (2.4 - 5.79)
H-SYAT14	4.10	0.00	4.1 (2.4 - 5.79)
H-SYAT16	1.11	0.00	1.12 (0.22 - 2.02)

H-SYAT17	0.77	0.00	0.78 (0.02 - 1.53)
H-SYAT19 [a]	0.21	0.00	0.21 (0 - 0.6)
H-SYAT21 [a]	0.34	0.00	0.34 (0 - 0.85)
H-SYAT26 [a]	0.04	0.61	0.04 (0 - 0.22)
H-SYAT28 [a]	0.34	0.00	0.34 (0 - 0.85)
H-SYAT29 [a]	0.26	0.00	0.26 (0 - 0.7)
H-SYAT30 [a]	0.04	0.00	0.04 (0 - 0.22)
H-SYAT33 [a]	0.04	0.00	0.04 (0 - 0.22)
H-SYAT34 [a]	0.04	0.00	0.04 (0 - 0.22)
H-SYAT36 [a]	0.00	0.20	0.04 (0 - 0.22)
H-SYAT37 [a]	0.00	0.20	0.04 (0 - 0.22)
H-SYAT41 [a]	0.04	0.00	0.04 (0 - 0.22)
H-SYBOR1	11.43	19.46	11.43 (7.89 - 14.97)
H-SYBOR3	1.68	0.00	1.67 (0.25 - 3.1)
H-SYBOR4 [a]	0.45	0.00	0.45 (0 - 1.19)

[a] Lineages having an expected prevalence with a 95% confidence interval (CI) including zero. Even in the cases where these parasites have not been recorded during the non-breeding period (NB. prevalence = 0), we cannot exclude that they would have been detected if sample sizes had been larger.

Figure 1


A
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H-PABY06
H-SYAT36
H-SYAT41
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H-SYAT09
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Haemoproteus majoris H-WW2
0.78
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B
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Figure 2

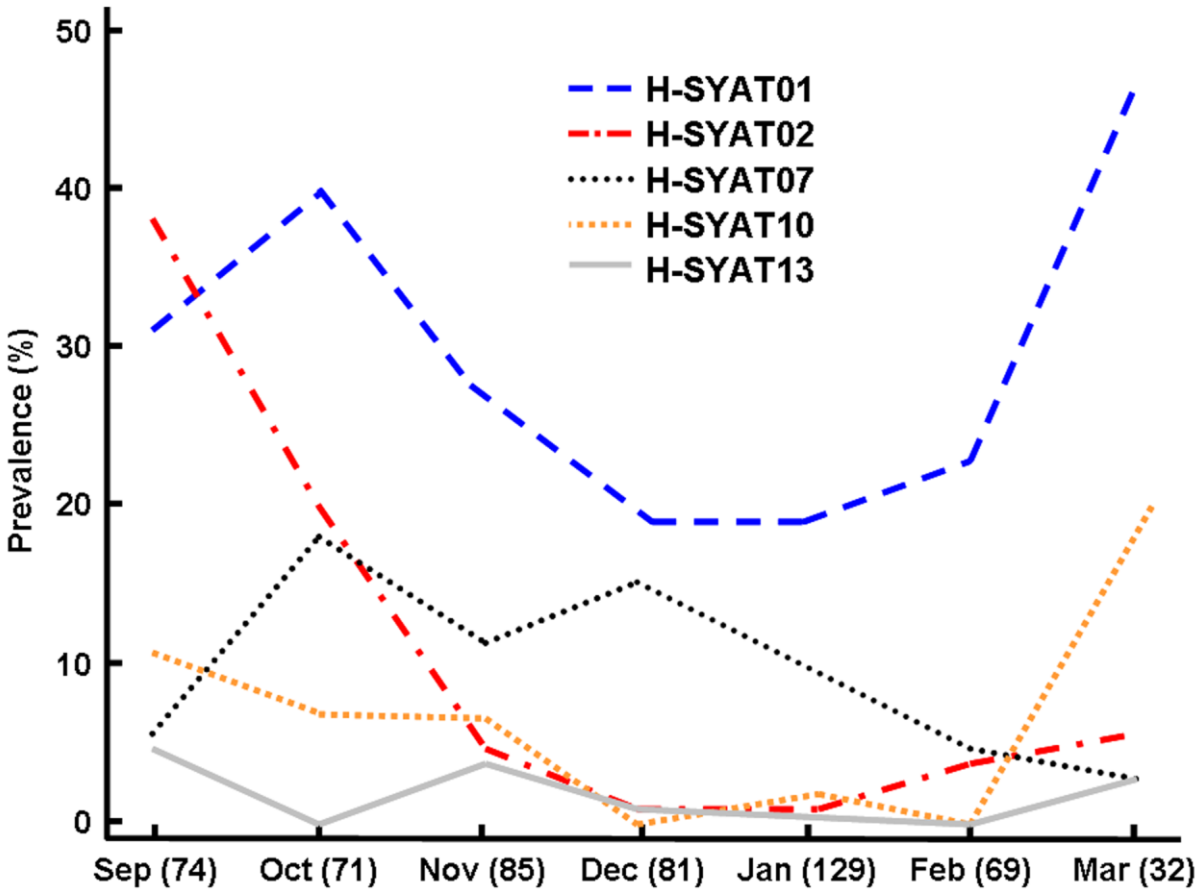
50
40
30
20
10
0
Prevalence (%)
H-SYAT01
H-SYAT02
H-SYAT07
H-SYAT10
H-SYAT13
Sep (74)
Oct (71)
Nov (85)
Dec (81)
Jan (129)
Feb (69)
Mar (32)

Figure S1