Seasonal dynamics in mosquito abundance and temperature do not influence avian malaria prevalence in the Himalayan foothills

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Abstract
We examined seasonal prevalence in avian haemosporidians (Plasmodium and Haemoproteus) in migrant and resident birds in western Himalaya, India. We investigated how infection with haemosporidians in avian hosts is associated with temporal changes in temperature and mosquito abundance along with host abundance and life-history traits (body mass). Using molecular methods for parasite detection and sequencing partial cytochrome b gene, 12 Plasmodium and 27 Haemoproteus lineages were isolated. Our 1-year study from December 2008 to December 2009 in tropical Himalayan foothills revealed a lack of seasonal variation in Plasmodium spp. prevalence in birds despite a strong correlation between mosquito abundance and temperature. The probability of infection with Plasmodium decreased with increase in temperature. Total parasite prevalence and specifically Plasmodium prevalence showed an increase with average avian body mass. In addition, total prevalence exhibited a U-shaped relationship with avian host abundance. There was no difference in prevalence of Plasmodium spp. or Haemoproteus spp. across altitudes; parasite prevalence in high-altitude locations was mainly driven by the seasonal migrants. One Haemoproteus lineage showed cross-species infections between migrant and resident birds. This is the first molecular study in the tropical Himalayan bird community that emphasizes the importance of studying seasonal variation in parasite prevalence. Our study provides a basis for further evolutionary study on the epidemiology of avian malaria and spread of disease across Himalayan bird communities, which may not have been exposed to vectors and parasites throughout the year, with consequential implications to the risk of infection to naïve resident birds in high altitude.

Keywords
Haemoproteus, mosquito abundance, Plasmodium, prevalence, temperature, western Himalaya

1 | INTRODUCTION

Parasite prevalence is a fundamental measure required to understand the temporal and spatial variations and epidemiology of infectious diseases (Ishtiaq, Rao, Huang, & Bensch, 2017; Scheuerlein & Ricklefs, 2004; Wood et al., 2007). The degree to which parasite prevalence varies depends on a suite of underlying mechanisms such as demographic (age, density, sex; Isaksson et al. 2013; Ricklefs et al., 2005),
ecological (season, habitat quality, elevation; Rooyen, Lalubin, Glaziot, & Christie, 2013; Wood et al., 2007), and life-history traits (body mass, fitness; Fecchio et al. 2011. Fecchio et al. 2013; González et al. 2014; Lutz et al. 2015; Matthews et al. 2016; Ricklefs et al., 2005; Svensson-Coelho et al. 2013). The seasonal dynamics and geographic distributions of vectorborne parasites are strongly governed by seasonal changes in vector abundance (Chavasse et al., 1999; Emerson, Bailey, Mahdi, Walraven, & Lindsay, 2000; Lord, Woolhouse, Heesterbeek, & Mellor, 1996; Mabaso, Craig, Vounatsou, & Smith, 2005; Randolph, 2004; Sturrock et al., 2001). For example, seasonal variation in mosquito abundance in response to annual variation in temperature and rainfall can cause strong seasonal patterns of disease incidence in malaria-epidemic regions, such as the Kenyan highlands (Hay et al. 2002). However, the role of temperature and vectors in explaining seasonal patterns in avian haemosporidian prevalence has been relatively understudied (see LaPointe et al. 2005; Medeiros et al. 2015; Okanga, Cumming, & Hockey, 2013).

Avian haemosporidians in the genera Plasmodium and Haemoproteus (Phylum Apicomplexa, Order Haemosporida) are globally distributed vector-mediated parasites found in a broad range of birds (Valkinũas, 2005). Plasmodium and Haemoproteus parasites reproduce sexually in dipteran vectors—culicid mosquitoes and ceratopogonid midges, respectively (Valkinũas, 2005)—and use birds as intermediate hosts in which they undergo asexual reproduction. Parasites in both genera have been shown to be pathogenic to their avian hosts (Atkinson & van Riper, 1991; Bennett et al. 1993; Warner 1968) with deleterious effects on health and reproductive success (e.g., Marzl, De Lope, Navarro, & Müller, 2005) and body condition (Valkinũas, Zickus, Shapoval, & Iezhova, 2006), and possibly lead to extinction in immunologically naïve hosts (Atkinson, Dusek, Woods, & Iko, 2000; Samuel et al., 2011). Thus, these parasites play a significant selective factor in bird populations and exert strong selection pressure on host life-history traits.

The incubation period of malaria parasites within mosquitoes is exquisitely temperature sensitive (Paajimans, Read, & Thomas, 2009); therefore, temperature is a major determinant of malaria transmission and persistence in host populations (LaPointe et al. 2005). Low ambient temperatures prevent sporogonic development of malaria parasites within vectors, influencing their altitudinal distributions (LaPointe, Goff, & Atkinson, 2010). Similarly, change in habitat composition from wet to dry due to climatic shift may alter both the geographic range and local abundance of malaria pathogens because their vectors (mosquitoes, Ceratopogonid midges, and Simuliid flies) require wet habitat to complete their life cycle (Valkinũas, 2005). Furthermore, owing to this vector dependency, both human (Hay et al., 2002) and avian (Cosgrove, Wood, & Sheldon, 2008) Plasmodium show a marked seasonality in transmission which appear to result in skewed prevalence estimates in winters. However, such stark variation in seasonal patterns ought to be more prominent in temperate regions where a peak in malaria prevalence occurs in late summer and autumn when the proportion of juveniles coincides with a rise in vector populations (see Beaudoin, Applegate, David, & McLean, 1971; Cranston, Ramsdale, Snow, & White, 1987; Marshall, 1938). In tropical and subtropical climates, malaria parasites transmission can occur year-round (Valkinũas, 2005). In Hawaii, for example, malaria transmission occurs after the breeding season as chronically infected native birds serve as year-round reservoir of disease (LaPointe, Atkinson, & Samuel, 2012). However, within tropical and subtropical areas, transmission will not occur at very high altitudes or during the cold season. There is a lack of quantitative studies which limits our capacity to understand and predict such changes in other threatened ecosystems and has largely remained unexplored in the tropics and in particular in the Indian subcontinent, despite it being the major staging and wintering ground of the Central Asian Flyway populations.

The Himalayan mountain range is one of the most species-rich areas in the world, harboring about 8% of the world’s bird species (Price et al. 2003). The high species diversity is due to species turnover associated with altitudinal variation in habitat, as well as variation in species composition along the range (Martens & Eck 1995; Price et al. 2003) and the possibility that such regions are buffered from climatic extremes, for example, because habitats and species can shift altitudinally in response to climate change (Fjeldså 1995; Fjeldså & Rahbek 2006). The Indian western Himalaya is species rich but remains a relatively understudied biogeographic region with only a handful of studies on birds (e.g., Price 1991), plants (Oommen and Shankar 2005), and mosquitoes (Devi and Jauhari 2004), with no previous studies on the dynamics of avian diseases and their vectors. Given the effect of climate and host demography which play a crucial role in determining parasite prevalence, as well as the dynamics of parasite transmission and host migration patterns both of which can increase the risk of infection. Many migrants move between altitudes or to the plains, and thereby encounter diverse parasite and vector faunas compared with the resident counterparts which remain at high altitudes throughout. Given that suitable vectors are present to transmit and maintain the infection, migrants can form an effective bridge for parasites between wintering and breeding grounds, hence increasing the risk of infection to naïve resident birds at the higher altitudes.

In this first molecular study on Plasmodium and Haemoproteus prevalence across resident and migrant birds, we aimed to explore the seasonal dynamics in parasite prevalence and its association with seasonal environmental drivers and host life-history traits. Specifically, we examine (1) how mosquito abundance and temperature relate with Plasmodium spp. prevalence in foothills birds; (2) the effect of altitude on haemosporidian prevalence and diversity; and (3) the extent of cross-species infections between resident and migrant birds.

2 | METHODS

2.1 | Study site and bird blood sampling

From December 2008 to December 2009, we conducted bird blood sampling in Dehradun [DUN: 30.174099N, 77.582535E; 640 m above sea level (a.s.l.) inside the campus of Wildlife Institute of India, n = 413]. The Wildlife Institute of India (WII) campus is nearly 200 acres of mixed habitat in the southern valley of Dehradun (Uttarakhand). To compare the patterns in parasite prevalence with high-altitude
breeding birds with foothills in April-May 2009, in addition to DUN (n = 38), we conducted sampling across three additional sites in forested habitat ranging between 1,800 and 3,200 m altitudes within Uttarakhand state, 2) Magra [MAG: N30.45587°E78.16158°; 1,800 m a.s.l.; n = 24], 3) Chakrata [CHAK: N30.702°E77.869°; 2,200 m a.s.l.; n = 12], and 4) Shokharakh (SHOK: N30.47860°E79.21798°; 3,100 m a.s.l.; n = 45; Figure 1).

At each location, we set up 10–12 mist nets in high bird activity locations, often along forest edges, footpaths, or off-road nature trails. Mist nets were 38-mm gauge, 2.6 m tall, and 6, 9, or 12 m long. Birds were sampled between 0540 and 1230, and nets were checked every 5–10 min. Birds were identified to species according to Rasmussen and Anderton (2005) and ringed. Wing length was measured (maximum wing-cord) with a steel rule (±1 mm) and body mass recorded with an electronic balance (±0.1 g). All birds were measured by F.I. Captured individuals were released at the site immediately after processing. We sampled 20–40 μl of bird blood from the sub-brachial wing vein (never exceeding 1% of the individual’s body weight). All samples were stored in SET Buffer (20–40 μl in 500 μl buffer 0.15 mol/L NaCl, 0.05 mol/L Tris, 0.001 mol/L EDTA, pH 8.0) at room temperature and subsequently transferred to −20°C.

2.2 | Mosquito sampling and temperature data across seasons in foothills

Mosquito sampling was conducted during January–December 2009 four times in each month using standard Centers for Disease Control and Prevention (CDC) miniature black light (UV) traps without dry ice baits. For all sampling, the traps were suspended within the forest canopy, no more than 2 m from the ground on trees (DiMenna et al., 2006; Okanga et al., 2013) around avian blood sampling (mist-netting) sites. Traps were operated overnight starting at 1.800 hr and picked up next morning at 0700 hr. Mosquitoes were collected from traps in the morning and stored at −20°C before identification. The species and sex of all mosquito samples were determined using a morphological mosquito-identification key (Barraud, 1934; Christophers, 1933), and sorted by species and date followed by DNA barcoding technique (Kumar, Rajavel, Natarajan, & Jambulingam, 2007). As only female mosquitoes are responsible for parasite transmission, we provide estimates of abundance for female mosquitoes. Daily temperature records were obtained from the on-site WII weather station in Dehradun.

2.3 | Molecular methods

DNA extractions were performed using phenol chloroform extraction method (Sambrook, Fritsch, & Maniatis, 1987) or ammonium acetate protocol (Nicholls, Double, Rowell, & Magrath, 2000). We screened all bird samples for the combined presence of parasites of the genera Plasmodium and Haemoproteus using a polymerase chain reaction (PCR) protocol designed to amplify a 160-bp fragment of mitochondrial ribosomal RNA gene (rRNA) of avian haemosporidians (213F/372R; Beadell & Fleischer, 2005) followed by a restriction enzyme-based assay.

For samples that screened positive for the 16S rRNA gene fragment of the parasites, we amplified the cytochrome b (cyt-b) gene fragments ranging from 533, 477, or 351 bp following Beadell et al. (2004), Hellgren, Waldenström, and Bensch (2004), and Ishtiaq et al. (2006), respectively. Each plate accompanied a parasite positive control and also a negative control to examine for any potential contamination. We screened all parasite-negative samples for bird DNA (for cyt-b gene) following Dumbacher, Pratt, and Fleischer (2003). The resulting PCR products were then sequenced in both directions. Sequences were assembled, aligned, and edited using SEQUENCHER version 5.2. We then identified sequences to genus using their closest sequence matches in GenBank or MalAvi database (Bensch, Hellgren, & Pérez-Tris, 2009). Novel lineages were defined as lineages that
differed by one or more nucleotides from any lineage deposited in GenBank prior to this study. All sequences are deposited in GenBank accession numbers: MF565807-MF565836 (Table S1).

2.4 | Phylogenetic analysis

To explore ecological and evolutionary relationships, a model-based approach was used following phylogenetic reconstruction using the maximum-likelihood analysis on 27 Haemoproteus sequences isolated from western Himalayan birds as well as 46 reference sequences downloaded from MalAvi database (Bensch et al., 2009). The Plasmodium phylogeny included 12 sequences isolated from western Himalayan birds and 27 reference sequences downloaded from MalAvi database (Bensch et al., 2009). Leucocytozoon majoris (GenBank accession number: AY393804) served as an outgroup in both phylogenies. The maximum-likelihood tree was constructed using Bayesian phylogenetics as implemented in BEAST version 1.4.3 (Drummond & Rambaut, 2007) using the most appropriate substitution model (GTR + G) according to the Akaike Information Criterion implemented MEGA version 5.2 (Tamura et al., 2011). We present a maximum clade credibility tree using a relaxed molecular clock approach (Drummond & Rambaut, 2007). Rates of substitution were drawn from a lognormal distribution, and Yule prior was used for branching rates. We conducted two runs of 20 million generations, each with sampling conducted every 1,000 generations. Tracer (Rambaut & Drummond, 2003) was used to assess convergence, and whether two chains were mixing and whether the estimated sample size (ESS) for each parameter was sufficient (ESS > 200) to obtain robust parameter estimates. Four million generations were discarded as burn-in from each run, leaving a posterior distribution of 32,000 trees.

2.5 | Statistical analysis

We used contingency table analyses using G tests for the heterogeneity in parasite prevalence across host species and families followed by partitioned analyses (Sokal & Rohlf, 1995).

2.6 | Individual-level traits

We used generalized linear mixed models (GLMMs, function glmer in lme4; Bates, Maechler, Bolker, & Walker, 2015) to assess whether individual infection was influenced by individual-level trait such as body condition, as fixed effects and bird taxonomy (species nested in genus and genus nested in family) as a random effect. Body condition (or size-corrected body mass) is measured as the residuals of a species-specific log (mass) by log (wing length) regression, which is often used as a proxy for overall condition (Schulte-Hostedde, Zinner, Millar, & Hickling, 2005).

2.7 | Species-level traits

We also used GLMMs to assess whether infection prevalence in each host species was influenced by species-specific traits, including avian abundance, mean species body mass (g), and migratory status as fixed effect and avian families with ≥5 individuals with bird taxonomy (species nested in genus and genus nested in family). We derived abundance estimates for each species by a single experienced observer (CB) recording the maximum number of birds seen on a given fixed circuit on dates within each week. We ensured that for any given week, at least one circuit of the nature trail within the WII campus was completed, but in some weeks, it may have been as many as 3–4 times. For any species known to be migratory or simply scarce, the exact date was noted, but for the remainder, simply the maximum figure seen during the week was noted. This may sound obvious, but by doing this, on a weekly (or even monthly) basis, it not only flags up when migrants are arriving, but in some cases, it identifies species that are not generally known to be migrants, but clearly do undergo at least localized movements. This presence/absence information can be a first step to detecting such localized seasonal movements. The averaged weekly maximum count for two visits per month was used as an index of abundance of each species. Abundance estimates (which are effectively an index for many of the more cryptic species) represent the predicted number of individuals of a given species detected in ~2.5 hr of surveying.

2.8 | Seasonal effects on parasite prevalence, mosquito abundance, and temperature

Samples were ordered by the season collected (spring, summer, monsoon, autumn, and winter). We used GLMMs to assess whether individual infection was influenced by season, mosquito abundance, or average monthly temperature as fixed effects, and bird taxonomy (species nested in genus and genus nested in family) as a random effect. Mosquito abundance was the mean number of mosquitoes caught, and this was calculated to provide comparability with months where mosquitoes were trapped less than four nights in a month. Pearson’s product–moment correlations (r) were run between mosquito abundance and temperature. To explore variation in parasite prevalence, we plotted correlation between prevalence in winter against prevalence in spring-summer, including the bird species for which at least four individuals were sampled in both seasons.

2.9 | Altitudinal effects on parasite prevalence and diversity

We used GLMMs to assess whether individual infection was influenced by altitude and migratory status (resident, seasonal migrant, and long-distance migrant) as fixed effects, and bird taxonomy (species nested in genus and genus nested in family) as a random effect.

All models were specified with a binomial error distribution and logit link function. The significance of fixed effects was evaluated with Wald’s chi-square tests (Bolker et al., 2009). Analyses were conducted in R v. 3.0.1 (R Development Core Team, 2010; R: A language and environment for statistical computing).
3 | RESULTS

3.1 | Year-round sampling in Himalayan foothills (Dehradun: 600 m)

Among 413 bird blood samples representing 49 species from 19 families that were screened for infection with *Plasmodium* spp. or *Haemoproteus* spp., we detected an overall prevalence of 153 birds (37.68%) in which 24 species (49%) were infected. Of these, 37 were infected with *Plasmodium*, 95 were infected with *Haemoproteus*, and 21 were mixed infections. *Haemoproteus* spp. infections were more frequent than *Plasmodium* spp. infections (binomial test, $p < .01$; Figure 2). Overall haemosporidian prevalence was significantly heterogeneous among host species sampled with ten or more individuals ($G_{adj} = 115.92, df = 13, p < 10^{-5}$) due to the high proportion of infected individuals in two species, *Zosterops palpebrosus* showed particularly high *Haemoproteus* prevalence (62.35%) followed by migrant *Acrocephalus dumetorum* (45%; Table 1). For host families sampled with five or more individuals, the total haemosporidian prevalence varied significantly ($G_{adj} = 115.92, df = 11, p < 10^{-5}$).

3.2 | Influence of individual- and species-level traits on parasite prevalence

For individual-level trait, infection status did not vary with body condition for *Plasmodium* spp. (Wald’s $\chi^2 = 1.14$, degrees of freedom ($df$) = 1, $p = .28$) nor for *Haemoproteus* spp. (Wald’s $\chi^2 = 0.58$, $df = 1, p = .46$). For species-level traits, the log-transformed avian abundance did not show significant effect on prevalence of *Plasmodium* spp. (Wald’s $\chi^2 = 2.80, df = 1, p = .09$; Table 2) and *Haemoproteus* spp. (Wald’s $\chi^2 = 0.37, df = 1, p = .37$; Table 2). However, total haemosporidian prevalence was marginally significant, increasing with host abundance (Wald’s $\chi^2 = 4.17, df = 1, p < .04$; Table 2; Figure 3).

Both total haemoparasite prevalence (Wald’s $\chi^2 = 7.44, df = 1, p < .01$) and *Plasmodium* spp. prevalence (Wald’s $\chi^2 = 4.96, df = 1, p < .02$; Figure 4) increased with average species body mass, but *Haemoproteus* spp. (Wald’s $\chi^2 = 2.65, df = 1, p = .10$) prevalence showed no effect with body mass. There was no significant difference in prevalence of *Plasmodium* spp. (Wald’s $\chi^2 = 3.49, df = 1, p = .75$), *Haemoproteus* spp. (Wald’s $\chi^2 = 0.17, df = 1, p = .89$), or total prevalence (Wald’s $\chi^2 = 2.29, df = 1, p = .12$) between migrant and resident birds.

3.3 | Seasonal effects on parasite prevalence and mosquito abundance

The total haemosporidian prevalence (Wald’s $\chi^2 = 1.20, df = 4, p = .87$), *Plasmodium* spp. prevalence (Wald’s $\chi^2 = 7.07, df = 4, p = .13$), and *Haemoproteus* spp. prevalence (Wald’s $\chi^2 = 3.56, df = 4, p < .46$; Figure 2) did not vary by season.

Fifteen species representing six genera were identified from 588 specimens of mosquitoes. The *Culex* genus comprising of five species was the dominant group with *Culex quinquefasciatus* as the prevalent species which is a known vector of avian *Plasmodium*. There was significant correlation between the log mosquito abundance and temperature ($p < .02$; Figure 5), with change in mosquito species composition (Fig. S1). The abundance of *Culex* mosquito species dominated summer period and *Uranotenia* species in winter. The prevalence of *Plasmodium* spp. decreased (Wald’s $\chi^2 = 7.30, df = 1, p < .01$) with increase in temperature and mosquito abundance (Wald’s $\chi^2 = 3.94, df = 1, p < .04$; Table 2; Figure 6). Prevalence of *Haemoproteus* spp., however, showed no
| Scientific name | Host family | Spring | Summer | Monsoon | Autumn | Winter |
|-----------------|-------------|--------|--------|---------|--------|--------|
| *Prinia socialis* | Cisticolidae | 2/5 P_DELURB5 | 0/6 0 | 0/1 0 | 0/2 0 | 2/7 P_GRW04; P_APR15 |
| *Prinia hodgsoni* | Cisticolidae | 0 0 | 0/1 0 | 0 0 | 0 0 | 2/4 P_UPUPA01 |
| *Orthotomus sutorius* | Cisticolidae | 1/3 P_UPUPA01 | 0/2 | 0 | 0/1 | 2/8 P |
| *Prinia philippinus* | Ploecidae | 0 0 | 0/2 0 | 0 0 | 0 0 | 1/3 H_PLOPHIL01 |
| *Stachyris pyrrocephs* | Timaliidae | 2/7 P_GRW06 | 1/3 P | 0/3 0 | 0/1 0 | 0/9 H |
| *Turdoides striata* | Timaliidae | 0 0 | 1/2 H_TURSTR03 | 3/3 H_TURSTR02 | 0 0 | 6/6 H_TURSTR02; H_TURSTR04 |
| *Pellorneum ruficeps* | Timaliidae | 0 0 | 0/5 0 | 0/1 0 | 0 0 | 0 |
| *Pomatorhynius erythrogenys* | Timaliidae | 0 0 | 2/3 H_ZOSPAL01 | 1/2 P_POMERY01 | 0 0 | 1/1 P_POMERY01 |
| *Pnoepyga albivenor* | Timaliidae | 0 0 | 0 0 | 0 0 | 0 0 | 0/1 |
| *Chrysomme sinense* | Timaliidae | 0/1 0 | 0 0 | 0 0 | 0 0 | 2/5 P_DELURB5; P_GRW04 |
| *Acrocephalus dumetorum* | Acrocephalidae | 7/16 H_ACDUM1; H_ACDUM2; H_ACDUM3; H_MW1 | 11/14 H_ACDUM1; H_ACDUM2; H_ACDUM3 | 0/2 0 | 2/6 H_ACDUM1; H_MW1 | 0 0 |
| *Cettia flavolivacea* | Sylviidae | 1/1 P | 0 0 | 0 0 | 0/1 0 | 0/2 0 |
| *Sylvia curruca* | Sylviidae | 1/1 H_WTH177 | 0 0 | 0 0 | 0 0 | 0 0 |
| *Luscinia s. svecica* | Sylviidae | 0 0 | 0 0 | 0 0 | 0 0 | 0/1 0 |
| *Cettia brunifrons* | Sylviidae | 0 0 | 0 0 | 0 0 | 0 0 | 0/2 0 |
| *Locustella major* | Sylviidae | 0/2 0 | 0 0 | 0 0 | 0 0 | 1/1 0 |
| *Phylloscopus coronatus* | Phylloscopidae | 0/1 0 | 0 0 | 0 0 | 0 0 | 0 0 |
| *Selercus burkii* | Phylloscopidae | 0/3 0 | 0 0 | 0 0 | 0 0 | 0 0 |

(Continues)
| Scientific name | Host family | Spring No. infected | Spring Lineage | Summer No. infected | Summer Lineage | Monsoon No. infected | Monsoon Lineage | Autumn No. infected | Autumn Lineage | Winter No. infected | Winter Lineage |
|----------------|-------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|------------------|----------------|
| Phylloscopus trochiloides | Phylloscopidae | 0 | 0 | 0/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phylloscopus xanthoschistos | Phylloscopidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/3 | 0 |
| Phylloscopus humei | Phylloscopidae | 0/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 |
| Phylloscopus chloronotus | Phylloscopidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phylloscopus tristis | Phylloscopidae | 0/2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phylloscopus fulgiventer | Phylloscopidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phylloscopus griseolus | Phylloscopidae | 0/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Parus major | Paridae | 0/3 | 0 | 0/1 | 0 | 0 | 0 | 0 | 0/2 | 0 | 0/3 | 0 |
| Saxicola ferreus | Muscicapidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/4 | 0 |
| Luscinia pectoralis | Muscicapidae | 0/2 | 0 | 0 | 0 | 0 | 0 | 0 | 0/4 | 0 | 0 | 0 |
| Ficedula strophiata | Muscicapidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/2 | 0 |
| Ficedula parva | Muscicapidae | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 | 0 | 0 | 0 |
| Ficedula tricolor | Muscicapidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 |
| Saxicola torquatus | Muscicapidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 |
| Rhipidua albicollis | Muscicapidae | 0/3 | 0 | 0/5 | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 |
| Pycnonotus leucogenys | Pycnonotidae | 2/2 | H | 0/2 | 0 | 0/2 | 0 | 4/8 | P_PYCAFF01 | 0/4 | 0 |
| Pycnonotus cafer | Pycnonotidae | 1/1 | H_PYCAFF02 | 1/3 | P_PYCAFF01 | 0/1 | 0 | 0 | 0 | 7/17 | P_PYCAFF01 |
| Pycnonotus jocosus | Pycnonotidae | 0 | 0 | 0/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lanius schach | Laniidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/2 | 0 |

(Continues)
significant variation with temperature (Wald’s $\chi^2 = 1.21, df = 1, p = .27$). Temporal variation across six bird species suggested time-independent species-specific prevalence levels ($r = 0.76, p < .01$; Table 1).

### 3.4 | Seasonal sampling across altitudinal gradient

Of 119 bird samples representing 50 species from 17 families that were screened for *Plasmodium* spp. and *Haemoproteus* spp. infections, an additive model of altitude + status showed significant variation (Wald’s $\chi^2 = 9.87, df = 2, p < .01$). Total prevalence did not show variation across altitude (Wald’s $\chi^2 = 5.35, df = 3, p = .14$; Table 3). However, resident birds were marginally less infected (Wald’s $\chi^2 = 6.01, df = 2, p < .49$; Table 2) than seasonal migrants. There was no relationship detected between prevalence of *Plasmodium* spp. and *Haemoproteus* spp. when accounting for host taxonomy.

### 3.5 | Cross-species infections between migrant and resident birds

Using PCR-based detection methods, 37 cyt-b sequences of parasite lineages were isolated, with a high proportion of infections of *Haemoproteus* spp., representing 27 lineages whereas *Plasmodium* spp. had just 10 lineages (GenBank accession numbers are listed in Table S1). *Plasmodium* and *Haemoproteus* mitochondrial lineage relationships are presented independently in Figures 7 and 8, respectively. Most of the lineages found in the resident foothill birds were locally transmitted based on their presence in young birds hatched on-site as well as the presence of lineages in the birds throughout the year. *Plasmodium* lineages found in the resident birds have been recorded earlier in host species with varying taxonomic affiliations whereas most of the *Haemoproteus* lineages recorded showed no overlap with parasite communities sampled elsewhere and shared within a host family. In particular, one *Haemoproteus* lineage (ACDUM1) sampled in Palearctic migrant warbler (*Acrocephalus dumetorum*) showed cross-species infection with resident Himalayan species sampled at a high-altitude site.

### 4 | DISCUSSION

Our study is the first seasonal study in the tropics which captures the absence of seasonal pattern in prevalence of avian *Plasmodium* spp. These findings are in contrast with the Beaudoin et al. (1971) model for temperate areas that predicts a within-year bimodal pattern of spring and autumn peaks with absence of infection in winter. Our 1-year molecular study in tropical Himalayan foothills revealed a strong correlation between mosquito abundance and temperature; however, there was a lack of seasonal variation in *Plasmodium* spp. prevalence. There was no effect of body condition on parasite prevalence; however, log avian abundance contributed to variation in total haemosporidian prevalence. *Haemoproteus* spp. prevalence showed no variation across seasons and life-history traits. Migrant birds were slightly more infected than resident species across altitudes.

| Scientific name | Host family | No. infected | Lineage |
|-----------------|-------------|-------------|---------|
| Copyscytus saularis | Turdidae | 0 | 0/1 |
| Turdus unicolor | Turdidae | 1/1 | P_AFTRU5 |
| Zosterops palpebrosus | Zosteropidae | 7/8 | H_ZOSPAL01; H_ZOSPAL02; H_ZOSPAL03; H_ZOSPAL04 |
| Lonchura punctulata | Estrildidae | 0/1 | 0/1 |
| Zosterops pectoralis | Zosteropidae | 7/8 | H_ZOSPAL01; H_ZOSPAL02; H_ZOSPAL03; H_ZOSPAL04 |

**Table 1** (Continued)
TABLE 2  GLMM (generalized linear mixed model with binomial error distribution and logit link function) to test the influence of individual and species-level traits along with environmental variables as fixed effects on Plasmodium and Haemoproteus infection rates after controlling for host taxonomy as random effect in birds sampled across seasons (n = 412) and altitudes (n = 119) in western Himalaya. Significant variables are in bold.

| Variable | β   | SE  | z value | p   |
|----------|-----|-----|---------|-----|
| A) Individual-level trait | | | | |
| Body Condition Index | | | | |
| Total infection | 4.09 | 2.69 | 1.51 | .12 |
| Plasmodium | 0.73 | 3.06 | 0.24 | .80 |
| Haemoproteus | 2.52 | 3.53 | 0.71 | .47 |
| B) Species-level traits | | | | |
| Avian abundance (log) | | | | |
| Total infection | 1.05 | 0.51 | 2.04 | .04* |
| Plasmodium | 1.10 | 0.68 | 1.61 | .10 |
| Haemoproteus | 0.72 | 0.51 | 1.40 | .15 |
| Mean body weight (log) | | | | |
| Total infection | 3.76 | 1.18 | 3.17 | .001* |
| Plasmodium | 0.81 | 0.34 | 2.34 | .01* |
| Haemoproteus | 1.95 | 1.18 | 1.62 | .10 |
| Status (migrant versus resident) | | | | |
| Total infection | 0.61 | 0.79 | 0.77 | .44 |
| Plasmodium | 1.78 | 1.09 | 1.63 | .10 |
| Haemoproteus | 0.50 | 1.59 | 0.31 | .74 |
| C) Temperature | | | | |
| Plasmodium | −0.09 | 0.037 | −2.55 | .01* |
| Haemoproteus | 0.03 | 0.027 | 1.09 | .27 |
| D) Mosquito abundance (log) | | | | |
| Plasmodium | −0.67 | 0.33 | −1.98 | .04* |
| E) Season | | | | |
| Total infection | | | | |
| Spring (Intercept) | −1.21 | 0.51 | −2.36 | .01 |
| Summer | −0.08 | 0.39 | −0.21 | .82 |
| Monsoon | −0.36 | 0.50 | −0.72 | .47 |
| Autumn | −0.39 | 0.45 | −0.88 | .37 |
| Winter | −0.15 | 0.39 | −0.39 | .69 |
| Plasmodium | | | | |
| Spring (Intercept) | −2.79 | 0.62 | −4.46 | .01 |
| Summer | −0.84 | 0.71 | −1.17 | .24 |
| Monsoon | −1.62 | 1.16 | −1.39 | .16 |
| Autumn | 0.40 | 0.65 | 0.61 | .53 |
| Winter | 0.17 | 0.54 | 0.32 | .74 |
| Haemoproteus | | | | |
| Spring (Intercept) | −5.15 | 2.19 | −2.35 | .01 |
| Summer | 0.60 | 0.49 | 1.21 | .22 |
| Monsoon | 0.10 | 0.59 | 0.18 | .85 |
| Autumn | −0.13 | 0.56 | −0.24 | .80 |

TABLE 2  (Continued)

| Variable | β   | SE  | z value | p   |
|----------|-----|-----|---------|-----|
| F) Altitude + status | | | | |
| Total infection* | 3.28 | 1.82 | 1.79 | .07 |
| Altitude_600 m (Intercept) | | | | |
| Status_resident | −4.47 | 1.82 | −2.45 | .01 |
| Status_seasonal migr | −2.49 | 1.48 | −1.6 | .09 |
| Altitude_1,800 m | −1.73 | 1.14 | −1.51 | .12 |
| Altitude_2,200 m | −0.75 | 1.29 | −0.58 | .56 |
| Altitude_3,000 m | −3.21 | 1.60 | −1.99 | .04 |

*Plasmodium and Haemoproteus did not show significant results.

4.1 Seasonal variation in parasite prevalence

Our year-round study shows the lack of seasonal variation in parasite prevalence and its association with tropical climate which allows for the presence of mosquitoes and other hematophagous arthropod vectors and therefore facilitates avian malaria transmission throughout the year. Human malaria, however, shows a seasonal pattern in the Himalayan foothills with peak transmission from July–October (Dhiman, Chavan, Pant, & Pahwa, 2011). We did not find any peak in prevalence in spring due to a relapse of the previous year’s infections (Beaudoin et al., 1971), which coincides with a peak in vector abundance (Atkinson & van Riper, 1991). In temperate regions, most Haemoproteus spp. show peak prevalence in the spring months when vectors (e.g., Culicoides spp.) become abundant or in the summer with acute infections in newly recruited immunologically naïve juveniles (Cranston et al., 1987; Klei & DeGiusti, 1975; Marshall, 1938). It is very difficult to differentiate recrudescence and relapse in parasitemia in the tropics as the optimal conditions without any thermal constraints facilitate parasite transmission throughout the year. Furthermore, our use of sensitive PCR-based screening methods provides a better estimate of parasite prevalence (Ishtiaq et al., 2017) and suggests that Plasmodium spp. and Haemoproteus spp. were indeed present in the blood throughout the year without undergoing a latent (dormant) phase where parasitemia survive in the tissues of avian hosts (Atkinson & van Riper, 1991). In temperate regions, dormancy is thought to minimize clearance from the host and to seasonally reduce virulence, thereby enhancing overwinter survival of the parasite (Worms, 1972). Re-emergence of the parasite coincides with a peak in vector abundance. As our study is PCR-based, we appreciate that parasite intensity data can shed more light on the dynamics of haemosporidian emergence with season providing insights into relapse of parasitemia by the presence of infective stages (gametocytes). The lack of temporal variation in parasite lineages across bird species suggests time-independent species-specific prevalence levels despite the change in temperature and mosquito species composition and abundance around the year.
4.2 | Effect of host life-history traits and environmental drivers on parasite prevalence

Prevalence of parasites varied significantly across host species and families. Of the three species-level traits, we found no significant relationship between host body condition and infection status. However, both host abundance and mean body mass influenced the parasite prevalence in the foothills Himalayan bird community. These findings are similar to patterns in prevalence observed in temperate regions where species with the large body mass and at greater abundance are known to facilitate transmission and increase parasite prevalence (Matthews, Ellis, Roberts, Ricklefs, & Collins, 2015; Scheuerlein & Ricklefs, 2004). Large birds' surface area provides more biting opportunities (Atkinson & van Riper, 1991) and emits more carbon dioxide which attracts host-seeking vectors (Scheuerlein & Ricklefs, 2004). Epidemiological theory predicts that host density plays a central role in transmission of directly transmitted parasites (Anderson and May 1978). The positive relationship between host abundance and parasite prevalence is consistent with results from Dobson (2004) and Brown et al. (2001). In particular, the U-shaped relationship between host abundance and parasite prevalence was similar to the temperate study by Ricklefs et al. (2005).
where the most abundant host species had the highest infection prevalence followed by the least abundant host species. The high prevalence in our study was primarily driven by *Haemoproteus* spp. in highly abundant hosts: Zosteropidae, Acrocephalidae, Timaliidae, Cisticolidae families, whereas the least common host were migrant *Sylvid* warblers. High prevalence of *Haemoproteus* in hosts with high abundance can be explained by two underlying mechanisms: (1) high host-to-vector transmission rates in a densely populated community (Brown et al., 2001); (2) large numbers of susceptible hosts within a community will increase parasite transmission (Keesing...
| Scientific name               | Host family | Status | DUN (600 m) | MAG (1,800 m) | CHAK (2,200 m) | SHOK (3,200 m) |
|------------------------------|-------------|--------|-------------|--------------|---------------|---------------|
|                             |             |        | No. infected | Lineage      | No. infected   | Lineage       |
|                             |             |        |             |              | No. infected   | Lineage       |
|                             |             |        |             |              | No. infected   | Lineage       |
|                             |             |        |             |              | No. infected   | Lineage       |
| Prinia socialis             | Cisticolidae| R      | 0/3         |              |               |               |
| Prinia hodgsoni             | Cisticolidae| R      | 0/1         |              |               |               |
| Orthotomus sutorius         | Cisticolidae| R      | 0/2         |              |               |               |
| Stachyris pyrrhops          | Timaliidae  | R      | 2/4         | P_GRW06      |               |               |
| Turdoides striata           | Timaliidae  | R      | 1/2         | H_TURSTR03   |               |               |
| Pellorneum ruficeps         | Timaliidae  | R      | 1/4         | P            |               |               |
| Garrulax lanceolatus        | Timaliidae  | SM     | 1/1         | H_GARLAN01   |               |               |
| Garrulax albovularis        | Leiothrichidae| SM   | 1/1         | P_NILSUN01   |               |               |
| Garrulax striata            | Leiothrichidae| SM   | 0/1         |              |               |               |
| Minla strigula              | Leiothrichidae| SM   | 0/2         |              |               |               |
| Trochalapteron erythrocephalum | Leiothrichidae| SM | 1/1         | H_TROERY01   |               |               |
| Trochalapteron lineatum     | Leiothrichidae| SM   | 3/5         | H_TROLIN01;  |               |               |
| Trochalapteron variegatum   | Leiothrichidae| SM   | 0/1         |               |               |               |
| Acrocephalus dumetorum      | Acrocephalidae| M   | 18/30       | H_ACDUM1;    |               |               |
|                             |             |        |             | H_ACDUM2;    |               |               |
|                             |             |        |             | H_ACDUM3;    |               |               |
|                             |             |        |             | H_MW1        |               |               |
| Aegithalos concinnus        | Aegithalidae| R      | 0/6         |              | 2/2           | H_AEGCON01    |
| Procarduelis nipalensis     | Fringillidae| SM     | 0/5         |              |               |               |
| Carpodacus rodochroa        | Fringillidae| R      | 1/6         | H_ACDUM1     |               |               |
| Aethopygea nipalensis       | Nectariniidae| SM  | 0/1         |              |               |               |
| Phylloscopus occipitalis    | Phylloscopidae| SM | 0/1         | 0            | 0             | 0             |
| Seicercus burkii            | Phylloscopidae| SM | 0/1         | 0            | 0             | 0             |
| Phylloscopus trochiloides   | Phylloscopidae| M   | 0           | 3/4          | H_GW1;        |               |
|                             |             |        |             |              | H_GW7;        |               |
|                             |             |        |             |              | H_GW8         |               |
| Phylloscopus xanthochlilosis| Phylloscopidae| SM | 0/1         | 0/2          |               |               |
| Phylloscopus humei           | Phylloscopidae| SM | 1/1         | P            |               |               |

(Continues)
| Scientific name | Host family | Status | DUN (600 m) | MAG (1,800 m) | CHAK (2,200 m) | SHOK (3,200 m) |
|-----------------|-------------|--------|------------|--------------|----------------|---------------|
| Phylloscopus maculipennis | Phylloscopidae | R | | | | 0/5 |
| Phylloscopus chloronotus | Phylloscopidae | SM | | | | 0/4 |
| Motacilla cinerea | Motacillidae | M | | 1/2 | H_YWT7 | |
| Anthus hodgsonii | Motacillidae | | | | | 0/1 |
| Parus major | Paridae | R | 0/2 | 0 | | |
| Parus monticolus | Paridae | R | 0/2 | 0/2 | 0/1 | |
| Sylviparus modestus | Paridae | SM | | | | 0/6 |
| Myophonus caeruleus | Muscicapidae | SM | 0 | 0 | 1/1 | H_MYOCA01 |
| Rhapidua albicollis | Muscicapidae | R | 0/4 | 0 | | |
| Niltava macgrigoriae | Muscicapidae | SM | | | | 0/1 |
| Enicurus maculatus | Muscicapidae | | | | | 0/1 |
| Tarsiger cyanurus | Muscicapidae | SM | | | | 0/1 |
| Ficedula superciliaris | Muscicapidae | SM | | 0/1 | | 0/1 |
| Ficedula strophiata | Muscicapidae | SM | | 0/1 | | 0/2 |
| Chaimarrornis leucocephalus | Muscicapidae | R | | | | 0/2 |
| Pycnonotus leucogenys | Pycnonotidae | R | 1/2 | H | | 0 |
| Pycnonotus jocosus | Pycnonotidae | R | 0/1 | 0 | | 0 |
| Picus squamatus | Picidae | R | | | | 0/1 |
| Dicrurus leucopheus | Dicrurus | SM | | | | 0/1 |
| Turdus unicolor | Turdidae | SM | 1/1 | P_AFTRU5 | 0 | 0 |
| Zoothera citrina | Turdidae | SM | 0/1 | 0 | | 0 |
| Amandava amandava | Estrildidae | R | 0/2 | 0 | | 0 |
| Lonchura punctulata | Estrildidae | R | 0/2 | 0 | | |
| Glaucidium brodiei | Strigidae | R | 0 | 0 | 0/1 | |
| Zosterops palpebrosus | Zosteropidae | R | 4/7 | H_ZOSPAP01; H_ZOSPAP03; H_ZOSPAP04 | | |
| Yuhina flavicollis | Zosteropidae | R | | | | 0/2 |

M, migrant; R, resident; SM, seasonal migrant.
et al. 2006). In our study, the high infection prevalence was a result of host-specific Haemoproteus lineages which were shared within phylogenetically closely related species (see Ishtiaq, 2017). In contrast, Medeiros et al. (2015) found abundant hosts were more often bitten by mosquito vectors and were also more likely to be infected by Plasmodium parasites compared with less abundant hosts. In the Himalayan bird community, the vector–host encounter rates contribute to the positive relationships between host abundance and parasite prevalence; however, the temperature and mosquito abundance showed a negative effect on the Plasmodium spp. prevalence. The seasonal dynamics in parasite prevalence are a function of vector–host interactions leading to the transmission of parasites and vector abundance, host specificity, and ecological requirements of the vectors (van Riper et al. 1986). Temperature drives the transmission probability of parasites, which might be optimal in temperate regions only in summer, facilitating an increase in Plasmodium prevalence (e.g., Medeirovs et al. 2015). However, even in our tropical site, suboptimal conditions including the temperature threshold above which parasite cannot develop to infective stage within mosquito for a long period (e.g., 30°C for P. relictum; LaPointe et al., 2010), could result in a decrease in parasite prevalence. Temperature is an important determinant in malaria transmission, influencing both mosquito and parasite biology not only through variation in average temperature, but also by the extent of fluctuation in the daily temperature throughout the day (Blanford et al., 2013; Paaijmans et al., 2010) and noting that warmer temperatures reduces vectorial capacity of mosquitoes (Paaijmans et al., 2009). Given that incubation period of Plasmodium spp. within the mosquito is highly sensitive to a temperature threshold with development completely blocked under 15°C and over 30°C, thus potential of Plasmodium spp. transmission declines with increased temperature, despite a strong correlation between mosquito abundance and temperature.

4.3 | Effect of altitude and seasonal migration on parasite prevalence

In contrast to studies in wet tropics and temperate regions (Atkinson et al., 2014; Rooyen et al., 2013; Zamora-Vilchis, Williams, & Johnson, 2012) on altitudinal variation in haemosporidian distribution in bird community to single species model, we found no change in prevalence of Plasmodium spp. and Haemoproteus spp. across altitudes. The parasite prevalence in high-altitude Himalayan sites is mainly driven by migrant hosts which move between altitudes or to the plains, and thereby encounter more diverse parasite and vector faunas compared with the resident counterparts occurring at high altitude which do not migrate to low altitudes. Avian haemosporidian parasites represent a complex, spatially heterogeneous host–parasite system, and their occurrence is strongly influenced and limited by environmental factors that affect vector prevalence and distribution. Seasonal movement of birds between altitudes or to the plains certainly adds to the complexity of the host–parasite associations, which warrants further investigation by longitudinal study across Himalayan sites.
4.4 | Cross-species infections

Many lineages observed in the Haemoproteus tree were host specific at a family level, which appears to be a trait in Haemoproteus species where the host ranges are often restricted to a limited number of closely related host species (Atkinson & van Riper, 1991; Bennett & Peirce, 1988; Perez-Tris et al., 2007; Savage & Greiner, 2004). Among resident species sampled, Zosterops palpebrosus (Oriental white-eye) was the most infected species with an assemblage of Haemoproteus lineages which appears to have diversified within the Zosteropidae family. Among migrants, Acrocephalus dumetorum was the most infected species with a diverse assemblage of Haemoproteus belopolskyi, which have a relatively low genetic distance from prevalent and widespread parasites of migrant Acrocephalids and Sylvids in Europe and African (Ishtiaq, 2017). This appears to be polymorphism within H. belopolskyi clade. The Haemoproteus lineage ACDUM1 was shared with a pink-browed rosefinch Carpodacus rodochroa, a high-altitude species which points toward potential cross-species transmission; however, as our study utilized only PCR, and not microscopy technique, there are several caveats associated with this methodology that could bias our lineage sharing results. First, the development of haemosporidians can be abortive in resistant or partly resistant hosts, resulting in no development of gametocytes (Cannell et al., 2013; Valkinûnas, Ashford, Bensch, Killick-Kendrick, & Perkins, 2011). Second, the low-frequency infection could be spillover infections in resistant hosts leading to a dead end for parasite transmission, and indeed, this was confirmed with microscopy by the absence of gametocytes. Nonetheless, there was no sharing of parasite lineages between foothills and high-altitude sites, which suggests demarcation of parasite transmission zones with a varying vector and thermal gradient. In Plasmodium tree, many lineages were generalists that infected multiple host species across multiple families.

Overall, this is the first molecular study of a tropical Himalayan bird community that reveals a high diversity of Haemoproteus lineages. While there was no seasonal variation in the prevalence of either Haemoproteus or Plasmodium, thus the lack of thermal constraints appears to facilitate year-round transmission of these parasites. Many Haemoproteus lineages were host specific and have not been reported previously. These new data contribute to our understanding of seasonal dynamics of temperature, mosquito abundance, and thermal constraints in parasite transmission in the tropics. They also provide a basis for furthering our understanding on the ecology and epidemiology of avian malaria and the spread of disease across Himalayan bird communities, which may not generally be exposed to vector and
parasites throughout the year. This can have important potential implications for the risk and susceptibility to infection for naïve resident birds at higher altitudes.

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ETHICAL APPROVAL

The field experiments comply with the current laws of the India where study was performed.

We thank Uttarakhand Forest Department for ethical approval and permission for collection of avian blood samples.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

FI conceived the idea. FI, CB, and YVJ designed the experiment; FI conducted the field and laboratory experiments; CB helped with fieldwork; FI wrote the manuscript, and FI, CB, and YVJ reviewed the manuscript. All authors approved the final version of the manuscript.

REFERENCES

Anderson, R. M., & May, R. M. (1978). Regulation and stability of host-parasite population interactions. I. Regulatory processes. Journal of Animal Ecology, 47, 219–247.

Atkinson, C. T., Dusek, R. J., Woods, K. L., & Iko, W. M. (2000). Pathogenicity of avian malaria in experimentally infected Hawaii Amakihis. Journal of Wildlife Diseases, 36, 197–204.

Atkinson, C. T., Utzurrum, R. B., LaPointe, D. A., Camp, R. J., Crampton, L. H., Foster, J. T., & Giambelluca, T. W. (2014). Changing climate and the altitudinal range of avian malaria in the Hawaiian Islands—An ongoing conservation crisis on the island of Kaua‘i. Global Change Biology, 20, 2426–2436.

Atkinson, C. T., & van Riper III, C. (1991). Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoon, and Haemoproteus. In J. E. Love & M. Zuk (Eds.), Bird–parasite interactions: Ecology, evolution and behaviour (pp. 19–48). New York: Oxford University Press.

Barraud, P. J. (1934). The fauna of British India including Ceylon and Burma. Diptera vol. V. Family Culicidae Tribes Megarhinini and Culicini. London: Taylor & Francis.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67, 1–48.

Beadell, J. S., & Fleischer, R. C. (2005). A restriction enzyme-based assay to distinguish between avian hemoparasitids. Journal of Parasitology, 91, 683–685.

Beadell, J. S., Gering, E., Austin, J., Dumbacher, J. P., Peirce, M. A., Pratt, T. K., ... Fleischer, R. C. (2004). Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Molecular Ecology, 13, 3829–3844.

Beaudoin, R. L., Applegate, J. E., David, D. E., & McLean, R. G. (1971). A model for the ecology of avian malaria. Journal of Wildlife Diseases, 7, 5–13.

Bennett, G. F., Pierce, M. A., & Ashford, R. W. (1993). Avian haematozoa: Mortality and pathogenicity. Journal of Natural History, 27, 993–1001.

Bennett, G. F., & Peirce, M. A. (1988). Morphological form in the avian Haemoproteidae and an annotated checklist of the genus Haemoproteus Kruse, 1890. Journal of Natural History, 22, 1683–1686.

Bensch, S., Hellgren, O., & Pérez-Tris, J. (2009). MalAvi: A public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Molecular Ecology Resources, 9, 1353–1358. http://mBio-serv4.mbioekol.lu.se/avianmalaria/index.html

Blanford, J. I., Blanford, S., Crane, R. G., Mann, M. E., Paaijmans, K. P., Schreiber, K. V., & Thomas, M. B. (2013). Implications of temperature variations for malaria parasite development across Africa. Scientific Reports, 3, 1300.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H., & White, J. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. Trends in Ecology Evolution, 24, 127–135.

Brown, C. R., Komar, N., Quick, S. B., Sethi, R. A., Panella, N. A., Brown, M. B., & Pfeffer, M. (2001). Arbovirus infection increases with group size. Proceedings of Royal Society London B Biological Sciences, 268, 1833–1840.

Cannell, B. L., Krasnec, K. V., Campbell, K., Jones, H. I., Miller, R. D., & Stephens, N. (2013). The pathology and pathogenicity of a novel Haemoproteus sp. infection in wild little penguins (Eudyptula minor). Veterinary Parasitology, 197, 74–84.

Chavasse, D. C., Shier, R. P., Murphy, O. A., Hotfly, S. R. A., Cousens, S. N., & Akhtar, T. (1999). Impact of fly control on childhood diarrhoea in Pakistan: Community-randomised trial. Lancet, 353, 22–25.

Christophers, S. R. (1933). The fauna of British India including Ceylon and Burma. Diptera vol. IV. Family Culicidae Tribe Anophelini. London: Taylor & Francis.

Cosgrove, C. L., Wood, M. J., & Sheldon, B. C. (2008). Seasonal variation in Plasmodium prevalence in a population of blue tits Cyanistes caeruleus. Journal of Animal Ecology, 77, 540–548.

Cranston, P. S., Ramsdale, C. D., Snow, K. P., & White, G. B. (1987). Adults, larvae and pupae of British mosquitoes (Culicidae). Ambleside: Freshwater Biological Association.

Devi, P. N., & Jauhari, R. K. (2004). Altitudinal distribution of mosquitoes in mountainous areas of Garhwal region, Part I. Journal of Vector Borne Diseases, 41, 17–26.

Dhiman, R. C., Chavan, L., Pant, M., & Pahwa, S. (2011). National and regional impacts of climate change on malaria by 2030. Current Science, 101, 372–382.

DiMenna, M. A., Bueno, R., Parmenter, R. R., Norris, D. E., Sheyka, J., Molina, J., ... Glass, G. E. (2006). Comparison of mosquito trapping method efficacy for West Nile virus surveillance in New Mexico. Journal of American Mosquito Control Association, 22(2), 246–253.

Dobson, A. (2004). Population dynamics of pathogens with multiple host species. The American Naturalist, 164, S64–S78.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology, 7, 214.

Dumbacher, J. P., Pratt, T. K., & Fleischer, R. C. (2003). Phylogeny of the owlet-nightjars (Aves: Aegothelidae) based on mitochondrial DNA sequence. Molecular Phylogenetics Evolution, 29, 540–549.

Emerson, P. M., Bailey, R. L., Mahdi, O. S., Walraven, G. E. L., & Lindsay, S. W. (2000). Transmission ecology of the fly Musca sorbens, a putative vector of trachoma. Transactions of Royal Society of Tropical Medical Hygiene, 94, 28–32.
Fecchio, A., Lima, M. R., Silveira, P., Braga, E. M., & Marini, M. A. (2011). High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu – Australian Ornithology, 111, 132–138.

Fecchio, A., Lima, M. R., Svensson-Coelho, M., Marini, M. A., & Ricklefs, R. E. (2013). Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. Parasitology, 1–12.

Fjeldså, J. (1995). Geographical patterns of neoeendemic and older relic species of Andean birds: the significance of ecological stability areas. In S. P. Churchill, H. Balise, E. Forero & J. L. Luteyn (Eds.), Biodiversity and conservation of Neotropical montane forests (pp. 89–102). The New York Botanical Garden.

Fjeldså, J., & Rahbek, C. (2006). Diversification of tanagers, a species rich bird group, from lowlands to montane regions of South America. Integrative and Comparative Biology, 46, 72–81.

González, A. D., Matta, N. E., Ellis, V. A., Miller, E. T., Ricklefs, R. E., & Gutiérrez, H. R. (2014). Mixed species flock, nest height, and elevation partially explain avian haemoparasite prevalence in Colombia. PLoS ONE, 9 (6), e100695. https://doi.org/10.1371/journal.pone

Hay, S. L., Cox, J., Rogers, D. J., Randolph, S. E., Stern, D. I., Shanks, G. D., ... Snow, R. W. (2002). Climate change and the resurgence of malaria in the East African highlands. Nature, 415, 905–909.

Hellgren, O., Waldenström, J., & Bensch, S. (2004). A new PCR assay for barcodes can distinguish species of Indian Mosquitoes (Diptera: Culicidae). Journal of Medical Entomology, 1249, 543–559.

Ishtiaq, F., Rao, M., Huang, X., & Bensch, S. (2017). Estimating prevalence of avian haemosporidians in natural populations—A comparative study on screening protocols. Parasites and Vectors, https://doi.org/10.1186/s13071-017-2066-z

Keeling, F., Holt, R. D., & Ostfeld, R. S. (2006). Effects of species diversity on disease risk. Ecology Letters, 9, 485–498.

Kle, T. R., & DeGiusti, D. L. (1975). Seasonal occurrence of Haemoproteus columbae Kruse and its vector Pseudolynchia canariensis Bequaert. Journal of Wildlife Diseases, 11, 130–135.

Kumar, P. N., Rajavel, A. R., Natarajan, R., & Jambulingam, P. (2007). DNA barcodes can distinguish species of Indian Mosquitoes (Diptera: Culicidae). Journal of Medical Entomology, 44, 1–7.

LaPointe, D. A., & M.L. Goff, C. T. Atkinson. (2005). Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawaii to avian malaria, Plasmodium relictum. Journal of Parasitology, 91, 843–849.

LaPointe, D. A., Atkinson, C. T., & Samuel, S. D. (2012). Ecology and conservation biology of avian malaria. Annals of the New York Academy of Sciences, 1249, 211–226.

LaPointe, D. A., Goff, M. L., & Atkinson, C. T. (2010). Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria Plasmodium relictum in Hawaii. Journal of Parasitology, 96, 318–324.

Lord, C. C., Woolhouse, M. E. J., Heesterbeek, J. A. P., & Mellor, P. S. (1996). Vector-borne diseases and the basic reproduction number: A case study of African horse sickness. Medical Veterinary Entomology, 10, 19–28.

Lutz, H. L., Hochachka, W. M., Engel, J. I., Bell, J. A., Tkach, V. V., Bates, J. M., et al. (2015). Parasite prevalence corresponds to host life history in a diverse assemblage of afrotropical birds and haemosporidian parasites. PLoS ONE, 10(4), e0121254. https://doi.org/10.1371/journal.pone.0121254

Mabaso, M. L. H., Craig, M., Vounatsou, P., & Smith, T. (2005). Towards empirical description of malaria seasonality in southern Africa: The example of Zimbabwe. Tropical Medical International Health, 10, 909–918.

Martens, J., & Eck, S. (1995). Towards an ornithology of the Himalayas: Systematics, ecology and vocalizations of Nepal birds. Bonner Zoologische Monographien, 38, 1–445.

Marshall, J. F. (1938). The British mosquitoes. London: British Museum.

Marzal, A., De Lope, F., Navarro, C., & Mellor, A. P. (2005). Malarial parasites decrease reproductive success: An experimental study in a passerine bird. Oecologia, 142, 541–545.

Matthews, A. E., Ellis, V. A., Hanson, A. A., Roberts, J. R., Ricklefs, R. E., & Collins, M. D. (2016). Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. Journal of Ornithology, 157, 533–548.

Medeiros, M. C., Ricklefs, R. E., Brawn, J. D., & Hamer, G. L. (2015). Plasmodium prevalence across avian host species is positively associated with exposure to mosquito vectors. Parasitology, 142(13), 1612–1620.

Nicholls, J. A., Double, M. C., Rowell, D. M., & Magrath, R. D. (2000). The evolution of cooperative and pair breeding in thornbills Acanthiza (Pardalotidae). Journal of Avian Biology, 31, 165–176.

Oomen, M. A., & Shankar, K. (2005). Elevational species richness patterns emerge from multiple local mechanisms in Himalayan woody plants. Ecology, 85, 3039–3047.

Okanga, S., Cumming, G. S., & Hockley, P. A. R. (2013). Avian malaria prevalence and mosquito abundance in the western Cape, South Africa. Malaria Journal, 12, 370.

Paaamijans, K. P., Blanford, S., Bell, A. S., Blanford, J. I., Read, A. F., & Thomas, M. B. (2010). Influence of climate on malaria transmission depends on daily temperature variation. Proceedings of Natural Academy of Sciences USA, 107, 15135–15139.

Paaamijans, K. P., Read, A. F., & Thomas, M. B. (2009). Understanding the link between malaria and climate. Proceedings of Natural Academy of Sciences USA, 106, 13844–13849.

Perez-Tris, J., Hellgren, O., Krizanauskiena, A., Waldenström, J., Secondi, J., Bonneauad, C., ... Bensch, S. (2007). Within-host speciation of malaria parasites. PLoS One, 2, 1–7.

Price, T. (1991). Morphology and ecology of breeding warblers along an altitudinal gradient in Kashmir, India. Journal of Animal Ecology, 60, 643–664.

Price, T., Zee, J., Jamdar, K., & Jamdar, N. (2003). Bird species diversity along the Himalayas: A comparison of Himachal Pradesh and Kashmir. Journal of the Bombay Natural History Society, 100, 394–409.

R Development Core Team (2010). R: A language and environment for statisti- cal computing. Vienna, Austria: R Foundation for Statistical Computing.

Rambaut, A., & Drummond, A. J. (2003). Tracer v1.3. Retrieved from http:// evolve.zoo.ox.ac.uk/

Randolph, S. E. (2004). Tick ecology: Processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology, 129, 537–565.

Rasmussen, P. C., & Anderton, J. C. (2005). Birds of South Asia. The Ripley guide. Washington, DC and Barcelona: Smithsonian Institution and Lynx Edicions.

Ricklefs, R. E., Swanson, B. L., Fallon, S. M., Martinez-Abrain, A., Scheuerlein, A., Gray, J., & Latta, S. (2005). Community relationships of avian malaria parasites in southern Missouri. Ecological Monograph, 75, 543–559.

Rooyen, J., Lalubin, F., Glaziot, O., & Christe, P. (2013). Altitudinal varia- tion in haemosporidian parasite distribution in great tit populations. Parasites and Vectors, 6, 139.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1987). Molecular cloning, a labora- tory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Samuel, M. D., Hobbelen, P. H. F., DeCastro, F., Ahumada, J. A., LaPointe, D. A., Atkinson, C. T., ... Duffy, D. C. (2011). The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: A modeling approach. Ecological Applications, 21(8), 2960–2973.
Savage, A. F., & Greiner, E. C. (2004). Hematozoa of the avian family Brachypteraciidae (the groundrollers). *Journal of Parasitology, 90*, 1468–1472.

Scheuerlein, A., & Ricklefs, R. E. (2004). Prevalence of blood parasites in European passeriform birds. *Proceedings of Royal Society London B Biological Sciences, 271*, 1363–1370.

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S., & Hickling, G. J. (2005). Restitution of mass-size residuals: Validating body condition indices. *Ecology, 86*, 155–163.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practice of statistics in biological research*, 3rd ed.. New York, W.H: Freeman.

Sturrock, R. F., Diaw, O. T., Talla, I., Niang, M., Piau, J. P., & Capron, A. (2001). Seasonality in the transmission of schistosomiasis and in populations of its snail intermediate hosts in and around a sugar irrigation scheme at Richard Toll, Senegal. *Parasitology, 123*, S77–S89.

Svensson-Coelho, M., Blake, J. G., Loiselle, B. A., Penrose, A. S., Parker, P. G., & Ricklefs, R. E. (2013). Diversity, prevalence, and host specificity of avian Plasmodium and Haemoproteus in a Western Amazon assemblage. *Omnithology Monographs, 76*, 1–47.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology Evolution, 28*, 2731–2739.

van Riper, C., van Riper III, S. G., Goff, M. L., & Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian (USA) land birds. *Ecological Monographs, 56*, 327–344.

Valkinūnas, G., Ashford, R. W., Bensch, S., Killick-Kendrick, R., & Perkins, S. (2011). A cautionary note concerning *Plasmodium* in apes. *Trends in Parasitology, 27*(6), 231–232.

Valkinūnas, G. (2005). *Avian malaria parasites and other haemosporidia*. Boca Raton, FL: CRC Press.

Valkinūnas, G., Zickus, T., Shapoval, A. P., & Iezhova, T. A. (2006). Effect of Haemoproteus belopolskyi (Haemosporida: Haemoproteidae) on body mass of the blackcap Sylvia atricapilla. *Journal of Parasitology, 92*, 1123–1125.

Warner, R. E. (1968). The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor, 70*, 101–120.

Wood, M. J., Cosgrove, C. L., Wilkin, T. A., Knowles, S. C., Day, K. P., & Sheldon, B. C. (2007). Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, Cyanistes caeruleus. *Molecular Ecology, 16*, 3263–3273.

Worms, M. (1972). Circadian and seasonal rhythms in blood parasites. In E. Canning, & C. Wright (Eds.), *Behavioural aspects of parasite transmission* (pp. 53–67). London: Academic Press.

Zamora-Vilchis, I., Williams, S. E., & Johnson, C. N. (2012). Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: Implications for disease in a warming climate. *PLoS One, 7*, e39208–e39210.

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Additional Supporting Information may be found online in the supporting information tab for this article.