Geographic and host species barriers differentially affect generalist and specialist parasite community structure in a tropical sky-island archipelago

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Abstract

Understanding why some parasites emerge in novel host communities while others do not have broad implications for human and wildlife health. In the case of haemosporidian blood parasites, epidemic wild bird mortalities on oceanic islands have been linked to *Plasmodium* spp., but not genera like *Haemoproteus*. Indeed, *Haemoproteus* is absent from many oceanic islands. In contrast, birds on continental islands share long coevolutionary histories with both *Plasmodium* and *Haemoproteus*, and are thus ideal model systems to elucidate eco-evolutionary end-points associated with these parasites in oceanic islands. Here, we examine eco-evolutionary dynamics of avian haemosporidian in the Shola sky-island archipelago of the Western Ghats, India. Our analyses reveal that compared to *Plasmodium*, *Haemoproteus* lineages were highly host-specific and diversified via co-speciation with their hosts. We show that community structure of host-generalist *Plasmodium* was primarily driven by geographic factors (e.g., biogeographic barriers), while that of host-specialist *Haemoproteus* was driven by host-species barriers (e.g., phylogenetic distance). Consequently, a few host species can harbor a high diversity of *Plasmodium* lineages which, in turn, are capable of infecting multiple host species. These two mechanisms can act in concert to increase the risk of introduction, establishment and emergence of novel *Plasmodium* lineages in island systems.

Keywords – avian haemosporidians, *Plasmodium*, *Haemoproteus*, disease emergence, community structure, India

1. Background

Emerging infectious diseases are considered to be one of the greatest challenges of our times from the perspective of human and wildlife health, as well as ecosystem function and stability [1,2]. An important driver for the dramatic increase in disease emergence over the past several decades is the recent and rapid spread of parasites outside their native range due to myriad factors including global climate change and increased human-mediated transport [1]. Such parasite range expansion can lead to serious epidemics in naïve host populations into which these parasites are newly introduced [3–5].

Avian haemosporidians (Apicomplexa: Haemosporida; *Plasmodium* and other related genera such as *Haemoproteus* – hereafter avian malaria) are a globally distributed group of vector-borne blood parasites that infect a wide array of bird taxa [6]. Avian malaria caused by *Plasmodium* spp. is one of the most important emerging infectious diseases of wild bird populations globally [7–9]. Large-scale mortalities in native wild birds have been well documented due to accidental introduction of *Plasmodium* spp. and *Culex quinquefaciatus* into island bird communities which had no coevolutionary history with these parasites (e.g., Hawaii [6,10] and New Zealand [7,8]). However, similar epidemic mortalities by *Haemoproteus* spp. have not been recognized. Indeed, while *Plasmodium* spp. are cosmopolitan [11], *Haemoproteus* spp. only appear to have colonized some oceanic islands systems (e.g. Lesser Antilles [12,13]) and is absent from many others (e.g., Hawaii, New Zealand and French Polynesia [8,11,14]).

The reduced ability to colonize some islands by *Haemoproteus* spp. vs. *Plasmodium* spp., and consequently the lower negative consequences associated with parasite invasions on native bird communities, are likely driven by myriad factors such as parasite specialization and avian host/vector community composition. Previous studies indicate that *Plasmodium* spp. are

relatively generalist, infecting a wide range of host species, whereas *Haemoproteus spp.* generally exhibit specialist associations and are restricted to phylogenetically related host species [11,15–17], but this pattern is not universal [14,19,20]. Such eco-evolutionary differences likely affect the ability of generalist parasites, like *Plasmodium* spp., to readily establish in island communities when introduced by natural or anthropogenic factors [11,21]. However, the taxonomic distinctiveness of host communities on islands may protect them from invasions by specialist parasites, like *Haemoproteus* spp., if island communities consist of species phylogenetically distant to hosts in the parasite's native range. Consequently, the colonization history of avian hosts/vectors, that is specific to each island system, can critically affect the likelihood of colonization by specialist parasites, such as *Haemoproteus* spp., but not generalist ones, such as *Plasmodium* spp.

Understanding the underlying eco-evolutionary mechanisms that influence the colonization and maintenance of *Plasmodium* spp. vs. *Haemoproteus* spp. can help elucidate the drivers of disease emergence in natural communities. In this context, continental sky-islands are a fascinating model system because they provide excellent natural laboratories for examining parasite eco-evolutionary dynamics. Sky-islands are isolated montane forests surrounded by a "sea" of low-elevation habitat, limiting dispersal of both bird and parasite lineages, similar to oceanic islands [22]. Thus, sky-island bird communities may face many of the same eco-evolutionary challenges as their oceanic counterparts. However, sky-island bird communities, in contrast to many oceanic counterparts, have generally shared long coevolutionary histories with their parasites. Consequently, the bird communities on continental sky-islands can help elucidate the potential long-term ecological and evolutionary end-points for oceanic island bird communities where avian haemosporidians have recently been introduced.

Here, we examine the eco-evolutionary dynamics of avian haemosporidians in the sky-island archipelago of the Western Ghats, Southern India. These sky-islands (hereafter Shola sky-islands) are high elevation montane ecosystems characterized by unique habitats called *Sholas*, a natural mosaic of wet, tropical evergreen forests and grasslands, isolated by drier lowland habitats [23]. The Shola sky-islands harbor remarkable species diversity and endemism driven by geographic complexity at multiple spatial scales [23,24]. At large spatial scales (i.e., across the Western Ghats), the deep and wide biogeographic barriers (Chaliyar, Palghat and Shencottah gaps; figure 1) have led to avian lineage diversification [25,26]. At small spatial scales (i.e., individual mountains) the steep elevational gradient contributes to colonization of sky-islands by both specialist avian species restricted to montane habitats and generalists with a wide elevational range. Thus, the Shola sky-islands offer an excellent opportunity to better understand the relative importance of geographic (e.g., spatial distance and biogeographic gaps), climatic (e.g., elevational gradients) and host species barriers (e.g., host phylogeny and host ecology) in driving evolution of parasite community structure.

In this study, we test whether *Plasmodium* spp. and *Haemoproteus* spp., due to their varying levels of host specialization differ in terms of: (i) host association patterns, (ii) coevolutionary dynamics, (iii) genetic structure, and (iv) global phylogenetic structure. We predict that: (i) diversity of hosts infected by a single lineage would be greater for generalist vs. specialist parasites; (ii) generalist parasites would likely coevolve with hosts through host-switching, while specialists would likely co-speciate with their hosts; (iii) genetic structure of generalist parasites would primarily be influenced by geography, while specialists would be more affected by host species barriers; and (iv) phylogenetic structure at global scales would be

lower for the generalist vs. specialist parasites because geographical range tends to correlate positively with niche breadth [27].

2. Materials and methods

(a) Field and laboratory methods

Field sampling was conducted at 7-14 sites across four major geographical regions in the southern 600 km mountain range of the Western Ghats (at 100-2500 m.a.s.l.) (figure 1; see electronic supplementary material (ESM), table S1). Each geographical region corresponded to the sky-island group separated by three biogeographical barriers—Chaliyar River valley, Palghat Gap and Shencottah Gap. Adult birds were captured using mist-nets during 2011-2013 and blood samples were collected from bird's ulnar vein in Queen's lysis buffer, following Robin et al. [28]. Genomic DNA was extracted using Qiagen blood and tissue extraction kit (Qiagen, Hilden, Germany) and screened for haemosporidian infection by amplifying 478 bp of mitochondrial cytochrome *b* gene (cytb) of avian haemosporidian parasites [29] (details in ESM).

(b) Phylogenetic analyses

To assess phylogenetic relationships among the Shola sky-island haemosporidian parasite lineages, we conducted Bayesian phylogenetic analyses in MRBAYES [30]. Similarly, we built host phylogenetic tree based on cytochrome *b* sequence data (1143bp) for bird species from an earlier study [26]. To examine parasite phylogenetic relationships at the global scale, we obtained cytochrome *b* sequence data from the MalAvi database [31] (accessed February 2018) and built bayesian parasite phylogenetic trees in MRBAYES [30]. We calculated rarefaction curves of expected phylogenetic diversity for host species and parasite lineages to ensure adequate sampling, as implemented in R-package PDCALC [32] (details in ESM). All statistical analyses were carried out in R 3.3.3 [33], unless specifically mentioned otherwise.

(c) Host-parasite association patterns

We measured the diversity of parasite lineages infecting each host species and diversity of hosts infected by each parasite lineage using the Shannon diversity of interactions index (H2) [34], a two-dimensional equivalent of the Shannon index [35]. We built null models by randomizing the network interactions (10,000 times) while maintaining the marginal sums (i.e. sum of interactions for each species was kept constant) using R-package VEGAN [36]. We performed two-sided tests of the network metric value against the distribution of the null model metric values to assess statistical significance. We quantified host specialization for parasite lineages infecting ≥ 2 host species by measuring the phylospecificity index—mean phylogenetic distance (MPD) and standardized effect sizes of the MPD values (SES.MPD) [37,38] using R-package PICANTE [39] (details in ESM).

(d) Host-parasite coevolutionary dynamics

We visually assessed phylogenetic congruence between the host and parasite phylogenetic trees by constructing a cophylogenetic tanglegram using TREEMAP [40]. We then statistically tested for host-parasite phylogenetic congruence by conducting a distance based co-phylogenetic analyses in Procrustean Approach to Cophylogeny (PACO) [41], as implemented in R-packages APE and VEGAN [36,42]. We also conducted an event-based co-phylogenetic analyses, as implemented in JANE [43] and CORE-PA [44], to determine the type and frequency of different coevolutionary scenarios, e.g., co-speciation, duplication, host switch, sorting or loss of parasite lineages. While JANE assigns an *a priori* cost for each evolutionary event, CORE-PA does not require *a priori* assignment of cost values to compute a cost minimal reconstruction. Run parameters and settings are detailed in the ESM.

147 **(e) Parasite genetic structure**

148 To test whether parasite genetic structure was influenced by host species barriers, biogeographic
149 gaps (see figure 1) and geographical structure within each biogeographical region, we used a
150 hierarchical Analysis of Molecular Variance (AMOVA) as implemented in the R-package
151 HIERFSTAT [45]. We assessed the statistical significance of each variance estimate by conducting
152 1000 randomizations amongst species (for $F_{Host/Total}$), regions within each species (for $F_{Region/Host}$),
153 and sampling sites within regions (for $F_{Site/Region}$).

154 Furthermore, we tested the relative effects of geographic, climatic and host factors on
155 parasite genetic structure using Multiple Regression on Distance Matrices (MRM) [46], as
156 implemented in ECODIST [47]. The geographic factors considered were biogeographic gaps (as a
157 Boolean matrix) and geographical distance (i.e., the great circle distance between sampling
158 coordinates); climatic factors included elevational distance (i.e., absolute difference in elevation
159 between sampling sites); host factors included host phylogenetic and host ecological distance
160 (measured as Gower distance between host ecological traits; [48]**)**. Host ecological data included
161 species traits that could affect haemosporidian infection dynamics and were collected from
162 published sources [49], as well as field observations by VVR and CKV (see ESM, table S9).

163 **(f) Global parasite phylogenetic structure**

164 To test whether specialist vs generalist parasites were phylogenetically more clustered across the
165 global haemosporidian phylogeny, we calculated the nearest neighbor phylogenetic distance
166 (D_{KN}) within *Plasmodium* spp. and *Haemoproteus* spp. lineages. We produced a null distribution
167 of D_{KN} values by randomizing (1000 times) tip labels across the global phylogeny and calculated
168 the probability of obtaining a simulated D_{KN} value $\leq$ observed D_{KN} value. We tested the overall
169 significance (i.e. across lineages within each parasite genus) using the exact binomial test in R.

3. Results and Discussion

(a) Parasite prevalence patterns

We sampled 1177 birds belonging to 28 species (including 14 endemics), representing almost the entire Shola sky-island bird community (except two species, see ESM, table S2) and found 24 species (490 birds) infected with haemosporidians (41.6% prevalence; figure 1). *Plasmodium* spp. was found at a prevalence of 13.6% (across 19 bird species), while *Haemoproteus* spp. had a prevalence of 68.9% (across 20 bird species; ESM, table S2). Haemosporidian prevalence varied across species, with *Turdus merula* as a key host species for *Plasmodium* spp. infection (29% prevalence) and *Zosterops palpebrosus* for *Haemoproteus* spp. (77.1% prevalence) infection. Rarefaction analyses revealed that our sampling was adequate to recover the observed parasite phylogenetic diversity (ESM, figure S1). Among the 47 parasite lineages recovered, a majority of *Plasmodium* spp.(10 of 18) and *Haemoproteus* spp. (24 of 29) lineages were novel and unique to the Shola sky-islands (ESM, table S3), indicating that many haemosporidian lineages are generally restricted to a single biogeographic region and characterized by local diversification as suggested by Ellis et al. [50–52].

(b) Host-parasite association patterns

Plasmodium spp. and *Haemoproteus* spp. differed markedly in terms of host-parasite associations, with two *Plasmodium* spp. lineages infected a greater diversity of hosts than expected by chance (P_MSP02: Observed H2 = 1.748; Expected H2 = 0.806; $P = 0.016$; P_MSP03: Observed H2 = 2.246; Expected H2 = 0.795; $P < 0.001$; figure 2; ESM, table S4). However, patterns of generalist host-parasite associations were not statistically significant across all *Plasmodium* spp. lineages (Binomial $P = 0.058$). Additionally, while host individuals were not susceptible to a greater diversity of *Plasmodium* spp. lineages than expected by chance

193 (Binomial $P = 0.340$), it is important to note that a disproportionately high diversity of
194 *Plasmodium* spp. lineages (7 of 18) were recovered from a single host species – *Turdus merula*
195 (Observed H2 = 1.715; Expected H2 = 0.978; $P = 0.046$; figure 2; ESM, table S6). In contrast,
196 for *Haemoproteus* spp., there was a strong positive association between hosts and parasite
197 lineages, with 27 of 29 parasite lineages infecting a lower diversity of hosts (Binomial $P < 0.001$;
198 ESM, table S5) and 23 of 24 host species being infected by a lower diversity of parasites than
199 expected by chance (Binomial $P < 0.001$; figure 2, ESM, table S6).
200 Furthermore, phylogenetic host specificity analyses for parasite lineages infecting
201 multiple host species revealed higher host specialization for *Haemoproteus* spp.
202 (MPD_w mean = 0.132, CI = 0.038, 0.248) compared to *Plasmodium* spp. lineages
203 (MPD_w mean = 0.358, CI = 0.246, 0.443). While four of seven *Haemoproteus* spp. lineages
204 showed higher phylospecificity (based on their significant SES.MPD values), none of the
205 *Plasmodium* spp. lineages had higher host specificity than expected by chance (ESM, table S7).
206 Thus, *Haemoproteus* spp. were highly host specialized, with most lineages infecting one or a
207 very few phylogenetically clustered hosts, compared to *Plasmodium* spp., as observed in other
208 biogeographic regions [11,15–17,53].
209 Interestingly, high prevalence and diversity of *Plasmodium* spp. lineages were recovered
210 from a single host species – *Turdus merula*. Based on existing genetic data and plumage-based
211 taxonomy, *T. merula* is known to harbor cryptic species diversity, with overlapping ranges of
212 resident and migratory races [26,54], which may explain why it was infected by diverse
213 haemosporidian lineages. Additionally, *T. merula* harbored some widespread and pathogenic
214 haemosporidian lineages, which may underscore its role as potential reservoir host in the Shola
215 sky-island bird community. Among the eight *Plasmodium* spp. lineages infecting *T. merula*, one

was a generalist while others were restricted to *T. merula* and two lineages matched FANTAIL01 and GRW06 (*Plasmodium elongatum*) (ESM, table S3). While FANTAIL01 is relatively less common, GRW06 is globally widespread and often virulent in naïve bird hosts [55]. Moreover, out of the three *Haemoproteus* spp. lineages detected in *T. merula,* one matched and two were 99% similar to *Haemoproteus minutus*, a widespread European lineage of *Turdus* spp. While *Haemoproteus minutus* is relatively benign for native European birds, lethal outbreaks have been recorded for naïve captive parrots in Europe [56,57]. Previous studies have also shown that Eurasian blackbird and other thrushes (*Turdus* spp.) generally serve as key reservoir hosts for *Plasmodium* spp. infections with high prevalence and diversity in continental communities; and contribute to high spillover risk to naive host communities when introduced to islands (such as in Azores [58], Robinson Crusoe [59] and New Zealand [8]). Thus, *T. merula* could be a potential key reservoir host in the Western Ghats with several virulent lineages.

(c) Host-parasite coevolutionary dynamics

We found no evidence of significant cophylogenetic congruence between hosts and *Plasmodium* spp. phylogenies (PACo, $m^2 = 5.297$, $P = 0.640$) but there was significant cophylogenetic congruence between host and *Haemoproteus* spp. phylogenies (PACo, $m^2 = 7.39$, $P = 0.047$; see also ESM figures S2, S3). Co-phylogenetic analysis with JANE revealed significant topological congruence between host and *Plasmodium* spp. or *Haemoproteus* spp. phylogenies (optimal inferred reconstruction cost lower than expected by chance; $P < 0.001$; ESM, figures S4, S5). However, CORE-PA revealed co-speciation for *Haemoproteus* spp., with inferred co-speciation events significantly greater than expected by chance ($P = 0.05$) while other host-switching, sorting or duplication events did not differ significantly from random

238 expectations. For *Plasmodium* spp., none of the events occurred significantly more than expected
239 by chance (ESM, table S8).
240 Overall, as expected, our cophylogenetic analyses revealed a signal of host-parasite
241 congruence mediated by co-speciation for specialist *Haemoproteus* spp., but lack of congruence
242 for the generalist *Plasmodium* spp. The significant role of co-speciation vs. host-switching in the
243 evolutionary history of *Haemoproteus* spp. in the Shola sky-islands is in contrast to previous
244 studies that recognize host switching as the dominant coevolutionary mechanism [60–62]. Our
245 study suggests that coevolutionary mechanisms underlying diversification of avian
246 haemosporidians are likely more complex than has been anticipated earlier. Employing a
247 probabilistic approach such as approximate Bayesian computation (ABC) represent useful future
248 directions for an improved understanding of avian haemosporidian diversification as has been
249 proposed recently [63].
250 The specialist strategy of *Haemoproteus* spp. and history of co-speciation may have
251 facilitated its diversification in the Shola sky-island bird community. For example, three
252 specialist lineages – MONCAC03, MONFAI02, MONMER02 showed signal of co-speciation
253 and have co-diversified with their endemic hosts *Montecincla cachinnans*, *Montecincla fairbanki*
254 and *Montecincla meridionalis*, respectively (see ESM, figure S5). Our results further strengthen
255 the patterns of local diversification of avian haemosporidians observed in other tropical bird
256 communities [50,52]. Broadly, empirical data from other host-parasite systems suggests that
257 parasites tend to be host-specialists in species rich communities [64]. Similarly, in the highly
258 diverse Shola sky-island bird communities with old host evolutionary histories and many
259 endemic host radiations, parasites likely benefit by establishing host-specialized associations and

diversify by co-speciation rather than adapting a generalist strategy and having more opportunities for host-switching, as suggested earlier [20,62].

(d) Parasite genetic structure

Analysis of Molecular Variance (AMOVA) revealed that parasite genetic differentiation between host species was low for *Plasmodium* spp. ($F_{Host/Total}$ = 0.073, *P* = 0.045) and high for *Haemoproteus* spp. ($F_{Host/Total}$ = 0.688, *P* = 0.001; figure 3; ESM, table S10). We found a significant effect of biogeographic gaps, within host species on the genetic structure of *Plasmodium* spp. ($F_{Region/Host}$ = 0.208, *P* = 0.004) but not *Haemoproteus* spp. ($F_{Region/Host}$ = 0.031, *P* = 0.464). However, there was significant parasite genetic structure between sampling sites within biogeographical regions for both *Plasmodium* spp. ($F_{Site/Region}$ = 0.079, *P* = 0.007) and *Haemoproteus* spp. ($F_{Site/Region}$ = 0.113, *P* = 0.018; figure 3; ESM, table S10).

Furthermore, Multiple Regressions on Distance Matrices (MRM) analyses showed that *Plasmodium* spp. parasite genetic distance was significantly associated with biogeographic gaps (B = 0.229, t = 4.686, P = 0.003) and geographic distance (B = 0.094, t = 4.425, *P* = 0.002) but not with host phylogenetic (B = 0.020, t = 0.809, *P* = 0.592), ecological (B = 0.023, t = 0.656, *P* = 0.609) or elevational distance (B = -0.027, t = -1.101, *P* = 0.360; figure 3; ESM, table S11). Alternatively, *Haemoproteus* spp. parasite genetic distance was significantly associated with host phylogenetic (B = 0.059, t = 18.157, *P* = 0.014), ecological (B = 0.164, t = 44.794, *P* = 0.001) and elevational distance (B = 0.053, t = 16.614, *P* = 0.037), but was not affected by biogeographic gaps (B = 0.017, t = 3.114, *P* = 0.558) or geographic distance (B = 0.001, t = 0.389, *P* = 0.909; figure 3; ESM, table S11).

From an eco-evolutionary perspective, parasites are intrinsically tied to their hosts and may be affected by host phylogeography. Thus, given the effect of biogeographic gaps in the

283 Western Ghats on host phylogeographic structure, we expected to find similar phylogeographic
284 structure among the parasite lineages. Indeed, at large spatial scales, *Plasmodium* spp. lineages
285 revealed phylogeographic structure across the biogeographic gaps. Surprisingly, *Haemoproteus*
286 spp. structure was not affected by biogeographic gaps, suggesting that these parasites tend to
287 track their hosts closely and have likely colonized their hosts before hosts genetic divergence. It
288 was especially surprising that even host species (e.g., *Sholicola* spp. and *Montecincla* spp.) that
289 showed deep genetic divergence (~4-5 Ma; [25]) across the biogeographic gaps were infected by
290 similar *Haemoproteus* spp. lineages across their range. This could likely occur due to differences
291 in mutation rates of parasites compared to their hosts. Additionally, an open and interesting
292 question remains regarding the role of the dipteran vectors in facilitating dispersal of
293 *Haemoproteus* spp. lineages across the biogeographic gaps.
294 Within a biogeographical region, we found that *Plasmodium* spp. lineages were shared
295 more among geographically closer hosts and did not show any host phylogenetic or ecological
296 constraints, coherent with their generalist strategy and a characteristic that likely contributes to
297 its role as an emerging parasite in novel bird communities. In contrast, specialist *Haemoproteus*
298 spp. lineages were shared more among closely related hosts (phylogenetically and ecologically),
299 despite their geographical isolation, a finding consistent with earlier studies [16,53].
300 Interestingly, *Haemoproteus* spp. populations were structured by elevation compared to
301 *Plasmodium* spp., indicating a higher probability of elevational spread by *Plasmodium* spp.,
302 which has critical implications from the perspective of disease emergence in novel climatic
303 niches.
304 Broadly, our results provide interesting insights into how hosts may be analogous to
305 islands from the perspective of parasite's colonization [65]. For instance, in the case of

306 *Plasmodium* spp., biogeographic gaps influenced parasite genetic structure, indicating that host
307 communities in each sky-island group served as islands. In contrast, host phylogenetic and
308 ecological differences constrained the dispersal of *Haemoproteus* parasites, thus characterizing
309 each host species as islands.

310 **(e) Global parasite phylogenetic structure**

311 We found that phylogenetic clustering in *Plasmodium* spp. lineages from the Shola sky-
312 islands did not differ from a random sample of lineages from the global parasite pool at the
313 community or lineage level (Mean D_{KN} = 0.533, P = 0.272; 2 of 18 lineages had D_{KN} lower than
314 expected; figure 4; ESM, table S12). In contrast, *Haemoproteus* spp. lineages showed strong
315 phylogenetic clustering at both community and lineage level (Mean D_{KN} = 0.281, P = 0.002; 14
316 of 29 lineages had D_{KN} less than expected; figure 4; ESM, table S13). Overall, *Haemoproteus*
317 spp. lineages had a significantly higher chance of being clustered compared to *Plasmodium* spp.
318 lineages ($\beta \pm SE = 1.958 \pm 0.86$, Odds ratio = 7.086; $z = 2.277$, P = 0.023).

319 In line with our expectations, the generalist *Plasmodium* spp. lineages were widely
320 interspersed across their global phylogeny whereas specialist *Haemoproteus* spp. lineages were
321 phylogenetically more clustered. This suggests that *Haemoproteus* spp. have likely diversified in
322 the Western Ghats, owing to the relatively old origin [66] and the deep evolutionary history of
323 Western Ghats endemic avian hosts [26] such as *Sholicola* spp. and *Montecincla* spp., which
324 diverged from their most recent ancestor about 11-12 Ma and later diversified on the Shola sky-
325 islands about 4-5 Ma [25]. Lack of phylogenetic clustering among the *Plasmodium* spp. lineages
326 suggests that these parasites are a random sample of their global phylogenetic pool and remain
327 unconstrained by host phylogeny, further highlighting their potential as emerging parasites in
328 novel host communities.

4. Conclusion

We present one of the first comprehensive investigation of avian haemosporidian dynamics in the Indian subcontinent by sampling almost the entire bird community in an important biodiversity hotspot(see also [18]). Here, we addressed the differential effects of geographic, climatic and host species barriers in shaping generalist and specialist haemosporidian parasite community structure. Our results reveal that, in a continental island system with long host-parasite coevolutionary history, there were several novel haemosporidian parasite lineages, endemic to the Shola sky-islands. *Plasmodium* spp. and *Haemoproteus* spp. clearly differed in terms of their host diversity, with higher host specialization in the case of the latter but not in the former. Consequently, there was a strong signal of co-speciation in the coevolutionary history of *Haemoproteus* spp., but not in *Plasmodium* spp. These parasites also differed dramatically in terms of their emerging infectious disease risk, with sharing of generalist *Plasmodium* spp. lineages among multiple host species primarily constrained by geographic factors such as geographic proximity, whereas specialist *Haemoproteus* spp. lineages were more influenced by host species factors such as host phylogeny, host ecology and climatic factors driven by elevation. Critically, our analyses revealed that *Plasmodium* spp. were less affected by climatic gradients (i.e., elevation), indicating that these parasites had a higher likelihood of elevational range expansion and were more likely to emerge when introduced to novel environments. In the Shola sky-islands, this is an especially troubling finding as high elevation habitats harbor higher number of endemic host species, which are also more likely to have evolved with avian haemosporidian parasites (for example, see [8]).

Overall, our results reveal that the higher likelihood of emergence in novel host communities by *Plasmodium* spp. vs. *Haemoproteus* spp. was likely driven by two interrelated

mechanisms. First, there are a few *Plasmodium* spp. lineages that can infect a diverse array of host species without being constrained by host phylogenetic/ecological similarity, and thus these lineages could emerge rapidly when introduced into a novel host community. Second, a few host species harbor a high diversity of *Plasmodium* spp. lineages, and thus invasion of such hosts into a novel bird community will be associated with the introduction of multiple parasite lineages, increasing the likelihood of spill-over to native hosts. Consequently, *Plasmodium* spp. lineages were globally widespread, reiterating their increased potential for colonization and emergence in novel host communities. Elucidating the underlying ecological and evolutionary factors that contribute to the rapid emergence of some parasites (e.g. *Plasmodium* spp.) but not others (e.g. *Haemoproteus* spp.) has critical implications for an improved understanding of emerging infectious diseases.

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Figures

Figure 1. Map of Western Ghats. (a) Locations of sampling sites (filled circles) in four geographical regions: I (Bababudan & Banasura hills), II (Nilgiri hills), III (Anamalai-Palni-Highwavies Hills), IV (Ashambu hills), corresponding to the major sky-island group separated by three biogeographical barriers—Chaliyar Gap, Palghat Gap and Shencottah Gap. Underlying natural (i.e. forest and grassland) vs. plantation habitats and 1400 msl isoclines are also depicted. Inset shows the proportion of individuals infected with *Plasmodium* spp. and *Haemoproteus* spp. in each geographical region with their 95% bootstrap confidence intervals; **(b)** Elevation profile of the Western Ghats along a linear transect connecting the highest elevation points in each geographical region (black transect line in A).

Figure 2. Host-association matrix for avian haemosporidians in the Shola sky-islands. Left: Bayesian phylogenetic tree of *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red) lineages based on cytochrome b gene sequence data, with *Leucocytozoon* spp. as outgroups. Bayesian posterior probability support values are color coded. Top: Bayesian phylogenetic tree of Shola sky-island bird species. See electronic supplementary material, tables S2, S3 for details on tree tip labels. The network matrix represents the heat map of abundance and distribution of each *Plasmodium* spp. and *Haemoproteus* spp. lineage, ranging from cool blues/reds (low abundance) to warm blues/reds (high abundance), respectively. White circles in the colored cells indicate significance of the of the network metric value against null expectations. Triangles depicted on the edges of the matrix indicate significant values of Shannon diversity of interactions (two-tailed test), circles show non-significance and dashes indicate an absence of infection.

Figure 3. Biogeographic structuring of Shola sky-island haemosporidian lineages. **(a)** Analysis of Molecular Variance (AMOVA) representing the effects of host species barriers ($F_{Host/Total}$), biogeographic regions within host species ($F_{Region/Host}$), and geographic site within each biogeographic region ($F_{Site/Region}$) on parasite genetic structure for *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red). **(b)** Multiple Regression on distance Matrices (MRM) analysis representing the effects of host phylogenetic distance (Phylo Dist), host ecological distance (Eco Dist), biogeographic gaps (Biogeo Gap), geographic distance (Geo Dist) and elevational distance (Elev Dist) on parasite phylogenetic structure for *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red). Filled symbols indicate *F* values significantly different from random expectation with their 95% bootstrap confidence intervals.

Figure 4. **Global phylogenetic structure based on nearest neighbor phylogenetic distance (D_{KN}).** Bayesian phylogenetic trees for **(a)** *Plasmodium* spp. and **(b)** *Haemoproteus* spp. lineages based on cytochrome *b* gene sequence data obtained from MalAvi database and endemic (closed circles) and non-endemic (open circles) lineages recovered from the Shola sky-islands. *Leucocytozoon* spp. were used as an outgroup. Inset shows the observed (circles) and expected (line) nearest neighbor phylogenetic distance (D_{KN}) for each Shola sky-island haemosporidian lineage.