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

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Understanding why some parasites emerge in novel host communities while others do not has 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. By contrast, birds on continental islands share long coevolutionary histories with both *Plasmodium* and *Haemoproteus*, and are thus ideal model systems to elucidate eco-evolutionary endpoints 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 geographical 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 harbour 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.

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 owing to a myriad of factors, including global climate change and increased human-mediated transport [1]. Such parasite range expansion can lead to serious epidemics in naive 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 owing to the 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 appears 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. versus *Plasmodium* spp. and, consequently, the lower negative consequences associated with parasite invasions on native bird communities are likely driven by a myriad of 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 [14,18,19]. 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 [11,20]. 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. versus *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 [21]. 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 with 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 endpoints 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 [22]. The Shola sky-islands harbour remarkable species diversity and endemism driven by geographical complexity at multiple spatial scales [22,23]. 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 [24,25]. 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 geographical (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., owing 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 versus 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 versus specialist parasites because geographical range tends to correlate positively with niche breadth [26].

2. Material 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, table S1). Each geographical region corresponded to the sky-island group separated by three biogeographic 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.* [27]. 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 [28] (details in the electronic supplementary material).

(b) Phylogenetic analyses

To assess phylogenetic relationships among the Shola sky-island haemosporidian parasite lineages, we conducted Bayesian phylogenetic analyses in MrBayes [29]. Similarly, we built a host phylogenetic tree based on cytochrome *b* sequence data (1143 bp) for bird species from an earlier study [25]. To examine parasite phylogenetic relationships at the global scale, we obtained cytochrome *b* sequence data from the MalAvi database [30] (accessed February 2018) and built Bayesian parasite phylogenetic trees in MrBayes [29]. We calculated rarefaction curves of expected phylogenetic diversity for host species and parasite lineages to ensure adequate sampling, as implemented in R-package pdcalc [31] (details in the electronic supplementary material). All statistical analyses were carried out in R 3.3.3 [32], 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 (*H*2) [33], a two-dimensional equivalent of the Shannon index [34]. We built null models by randomizing the network interactions (10 000 times) while maintaining the marginal sums (i.e. sum of

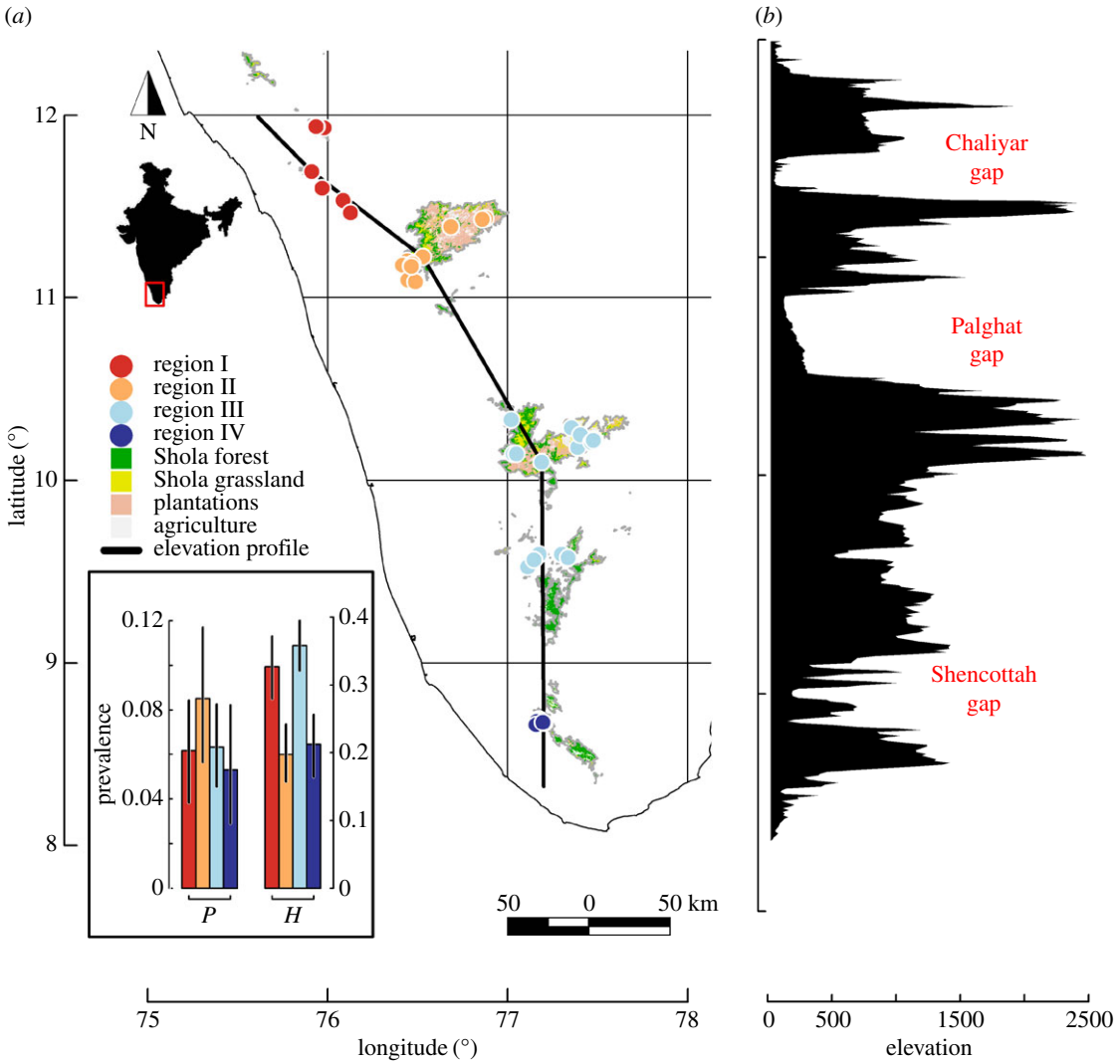

Figure 1. Map of Western Ghats. (*a*) Locations of sampling sites (filled circles) in four geographical regions: I (Bababudan and Banasura hills), II (Nilgiri hills), III (Anamalai-Palni-Highwavies hills), IV (Ashambu hills), corresponding to the major sky-island group separated by three biogeographic barriers—Chaliyar gap, Palghat gap, and Shencottah gap. Underlying natural (i.e. forest and grassland) versus plantation habitats and 1400 m.a.s.l. 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*). (Online version in colour.)

interactions for each species was kept constant) using R-package VEGAN [35]. 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 greater than or equal to two host species by measuring the phylospecificity index—mean phylogenetic distance (MPD) and standardized effect sizes of the MPD values (SES.MPD) [36,37] using R-package PICANTE [38] (details in the electronic supplementary material).

(d) Host–parasite coevolutionary dynamics

We visually assessed phylogenetic congruence between the host and parasite phylogenetic trees by constructing a cophylogenetic tanglegram using TREEMAP [39]. We then statistically tested for host–parasite phylogenetic congruence by conducting a distance-based cophylogenetic analyses in Procrustean Approach to Cophylogeny (PACo) [40], as implemented in R-packages APE and VEGAN [35,41]. We also conducted an event-based cophylogenetic analyses, as implemented in JANE [42] and CORE-PA [43], 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 electronic supplementary material.

(e) Parasite genetic structure

To test whether parasite genetic structure was influenced by host species barriers, biogeographic gaps (figure 1), and geographical structure within each biogeographic region, we used a hierarchical analysis of molecular variance (AMOVA) as implemented in the R-package HIERFSTAT [44]. We assessed the statistical significance of each variance estimate by conducting 1000 randomizations among species (for $F_{\text{Host/Total}}$), regions within each species (for $F_{\text{Region/Host}}$) and sampling sites within regions (for $F_{\text{Site/Region}}$).

Furthermore, we tested the relative effects of geographical, climatic, and host factors on parasite genetic structure using multiple regression on distance matrices (MRM) [45], as implemented in ECODIST [46]. The geographical factors considered were biogeographic gaps (as a Boolean matrix) and geographical distance (i.e. the great circle distance between sampling coordinates); climatic factors included elevational distance (i.e. absolute difference in elevation between sampling sites); host factors

included host phylogenetic and host ecological distance (measured as Gower distance between host ecological traits; [47]). Host ecological data included species traits that could affect haemosporidian infection dynamics and were collected from published sources [48], as well as field observations by V.V.R. and C.K.V. (see electronic supplementary material, table S9).

(f) Global parasite phylogenetic structure

To test whether specialist versus generalist parasites were phylogenetically more clustered across the global haemosporidian phylogeny, we calculated the nearest neighbour phylogenetic distance (D_{KN}) within *Plasmodium* spp. and *Haemoproteus* spp. lineages. We produced a null distribution of D_{KN} values by randomizing (1000 times) tip labels across the global phylogeny and calculated the probability of obtaining a simulated D_{KN} value $\leq$ observed D_{KN} value. We tested the overall 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 electronic supplementary material, 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; electronic supplementary material, 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 (electronic supplementary material, 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 (electronic supplementary material, 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*. [49–51].

(b) Host–parasite association patterns

Plasmodium spp. and *Haemoproteus* spp. differed markedly in terms of host–parasite associations, with two *Plasmodium* spp. lineages infecting 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; electronic supplementary material, 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 (binomial $p = 0.340$), it is important to note that a disproportionately high diversity of *Plasmodium* spp. lineages (7 of 18) were recovered from a single host species—*T. merula* (observed $H2 = 1.715$; expected $H2 = 0.978$; $p = 0.046$; figure 2; electronic supplementary material, table S6). By contrast, for *Haemoproteus* spp., there was a strong positive association between hosts and parasite lineages, with 27 of 29 parasite lineages infecting a lower diversity of hosts (binomial $p < 0.001$; electronic supplementary material, table S5) and 23 of 24 host species being infected by a lower diversity of parasites than expected by chance (binomial $p < 0.001$; figure 2; electronic supplementary material, table S6).

Furthermore, phylogenetic host specificity analyses for parasite lineages infecting multiple host species revealed higher host specialization for *Haemoproteus* spp. (MPD_w mean = 0.132, CI = 0.038, 0.248) compared to *Plasmodium* spp. lineages (MPD_w mean = 0.358, CI = 0.246, 0.443). While four of seven *Haemoproteus* spp. lineages showed higher phylospecificity (based on their significant SES.MPD values), none of the *Plasmodium* spp. lineages had higher host specificity than expected by chance (electronic supplementary material, table S7). Thus, *Haemoproteus* spp. were highly host specialized, with most lineages infecting one or a very few phylogenetically clustered hosts, compared to *Plasmodium* spp., as observed in other biogeographic regions [11,15–17,52].

Interestingly, high prevalence and diversity of *Plasmodium* spp. lineages were recovered from a single host species—*T. merula*. Based on existing genetic data and plumage-based taxonomy, *T. merula* is known to harbour cryptic species diversity, with overlapping ranges of resident and migratory races [25,53], which may explain why it was infected by diverse haemosporidian lineages. Additionally, *T. merula* harboured some widespread and pathogenic haemosporidian lineages, which may underscore its role as a potential reservoir host in the Shola 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*) (electronic supplementary material, table S3). While FANTAIL01 is relatively less common, GRW06 is globally widespread and often virulent in naive bird hosts [54]. 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 naive captive parrots in Europe [55,56]. 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 [57], Robinson Crusoe [58], 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 electronic supplementary material, figures S2 and S3). Cophylogenetic analysis with JANE revealed significant topological congruence between host and *Plasmodium* spp. or *Haemoproteus* spp. phylogenies (optimal inferred reconstruction cost lower

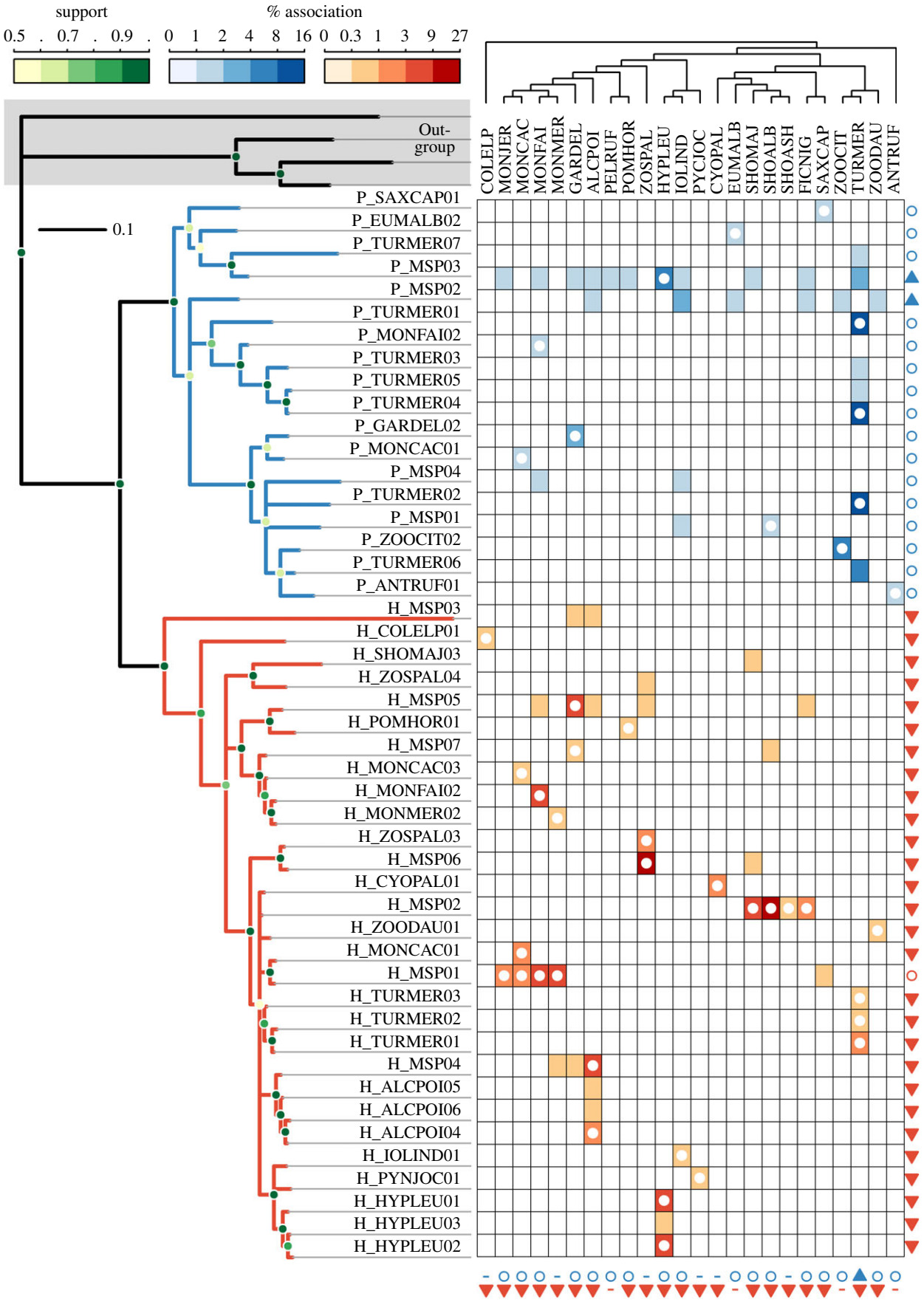

Figure 2. Host association matrix for avian haemosporidians in the Shola sky-islands. (Left) Bayesian phylogenetic tree of *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red) lineages based on cytochrome *b* gene sequence data, with *Leucocytozoon* spp. as outgroups. Bayesian posterior probability support values are colour coded. (Top) Bayesian phylogenetic tree of Shola sky-island bird species. See electronic supplementary material, tables S2 and 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 coloured cells indicate significance 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. (Online version in colour.)

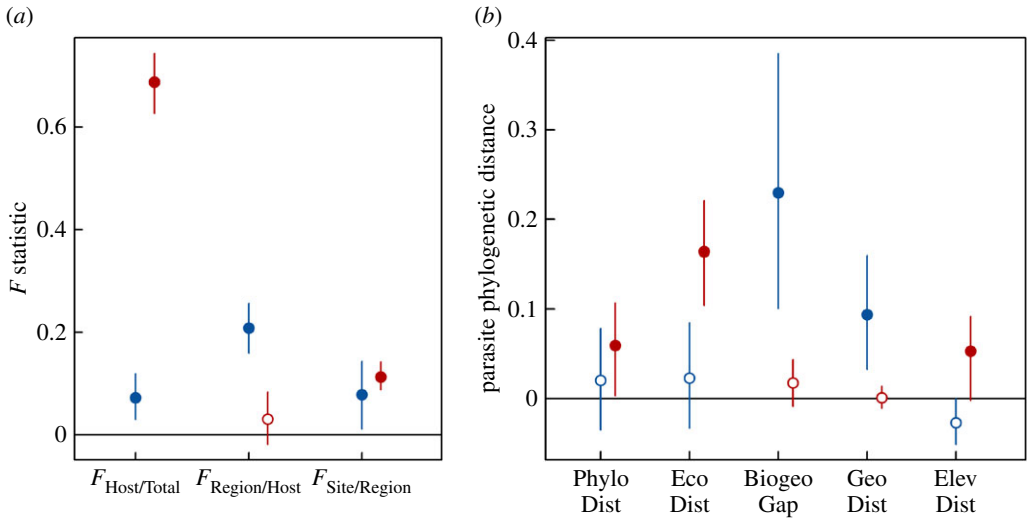

Figure 3. Biogeographic structuring of Shola sky-island haemosporidian lineages. (*a*) AMOVA representing the effects of host species barriers ($F_{Host/Total}$), biogeographic regions within host species ($F_{Region/Host}$), and geographical site within each biogeographic region ($F_{Site/Region}$) on parasite genetic structure for *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red). (*b*) MRM analysis representing the effects of host phylogenetic distance (Phylo Dist), host ecological distance (Eco Dist), biogeographic gaps (Biogeo Gap), geographical 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. (Online version in colour.)

than expected by chance; $p < 0.001$; electronic supplementary material, figures S4 and 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 expectations. For *Plasmodium* spp., none of the events occurred significantly more than expected by chance (electronic supplementary material, table S8).

Overall, as expected, our cophylogenetic analyses revealed a signal of host–parasite congruence mediated by co-speciation for specialist *Haemoproteus* spp., but lack of congruence for the generalist *Plasmodium* spp. The significant role of co-speciation versus host-switching in the evolutionary history of *Haemoproteus* spp. in the Shola sky-islands is in contrast with previous studies that recognize host-switching as the dominant coevolutionary mechanism [59–61]. Our study suggests that coevolutionary mechanisms underlying diversification of avian haemosporidians are likely more complex than has been anticipated earlier. Employing a probabilistic approach such as approximate Bayesian computation represents a useful future direction for an improved understanding of avian haemosporidian diversification as has been proposed recently [62].

The specialist strategy of *Haemoproteus* spp. and history of co-speciation may have facilitated its diversification in the Shola sky-island bird community. For example, three specialist lineages—MONCAC03, MONFAI02, and MONMER02—showed signals of co-speciation and have co-diversified with their endemic hosts *Montecincla cachinnans*, *Montecincla fairbanki*, and *Montecincla meridionalis*, respectively (see electronic supplementary material, figure S5). Our results further strengthen the patterns of local diversification of avian haemosporidians observed in other tropical bird communities [49,51]. Broadly, empirical data from other host–parasite systems suggest that parasites tend to be host-specialists in species-rich communities [63]. Similarly, in the highly diverse Shola sky-island bird communities with old host evolutionary histories and many 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 [19,61].

(d) Parasite genetic structure

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; electronic supplementary material, 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 biogeographic 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; electronic supplementary material, 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 geographical 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; electronic supplementary material, 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 geographical distance ($B = 0.001$, $t = 0.389$, $p = 0.909$; figure 3; electronic supplementary material, table S11).

From an eco-evolutionary perspective, parasites are intrinsically tied to their hosts and may be affected by host

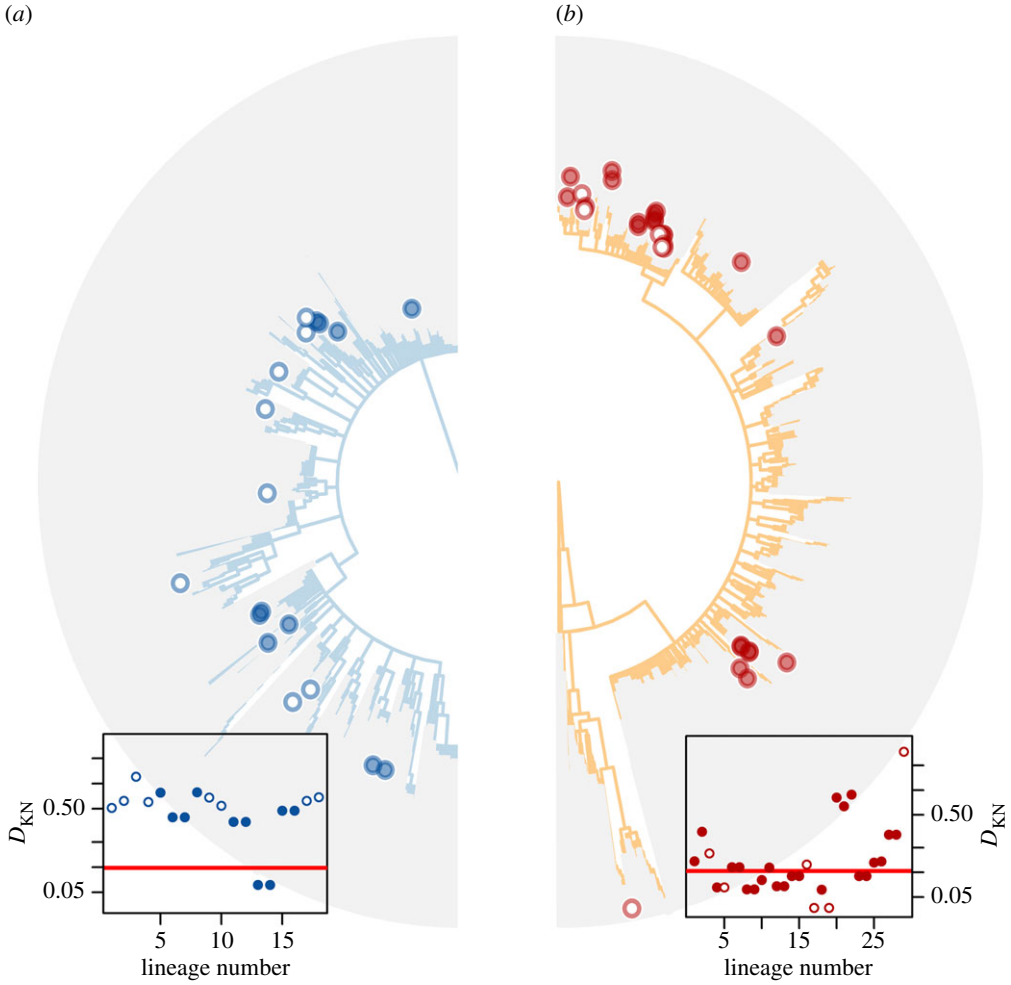

Figure 4. Global phylogenetic structure based on nearest neighbour phylogenetic distance (D_{KN}). Bayesian phylogenetic trees for (*a*) *Plasmodium* spp. and (*b*) *Haemoproteus* spp. lineages based on cytochrome *b* gene sequence data obtained from the MalAvi database and endemic (closed circles) and 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 neighbour phylogenetic distance (D_{KN}) for each Shola sky-island haemosporidian lineage. (Online version in colour.)

phylogeography. Thus, given the effect of biogeographic gaps in the Western Ghats on host phylogeographic structure, we expected to find a similar phylogeographic structure among the parasite lineages. Indeed, at large spatial scales, *Plasmodium* spp. lineages revealed a phylogeographic structure across the biogeographic gaps. Surprisingly, *Haemoproteus* spp. structure was not affected by biogeographic gaps, suggesting that these parasites tend to track their hosts closely and have likely colonized their hosts before genetic divergence of the hosts. It was especially surprising that even host species (e.g. *Sholicola* spp. and *Montecincla* spp.) that showed deep genetic divergence (approx. 4–5 Ma; [24]) across the biogeographic gaps were infected by similar *Haemoproteus* spp. lineages across their range. This could likely occur owing to differences in mutation rates of parasites compared to their hosts. Additionally, an open and interesting question remains regarding the role of the dipteran vectors in facilitating dispersal of *Haemoproteus* spp. lineages across the biogeographic gaps.

Within a biogeographic region, we found that *Plasmodium* spp. lineages were shared more among geographically closer hosts and did not show any host phylogenetic or ecological constraints, coherent with their generalist strategy and a characteristic that likely contributes to its role as an emerging parasite in novel bird communities. By contrast, specialist *Haemoproteus* spp. lineages were shared more among closely related hosts (phylogenetically and ecologically), despite their geographical isolation, a finding consistent with earlier studies [16,52]. Interestingly, *Haemoproteus* spp. populations were structured by elevation compared to *Plasmodium* spp., indicating a higher probability of elevational spread by *Plasmodium* spp., which has critical implications from the perspective of disease emergence in novel climatic niches.

Broadly, our results provide interesting insights into how hosts may be analogous to islands from the perspective of parasite colonization [64]. For instance, in the case of *Plasmodium* spp., biogeographic gaps influenced parasite genetic structure, indicating that host communities in each sky-island group served as islands. By contrast, host phylogenetic and ecological differences constrained the dispersal of *Haemoproteus* parasites, thus characterizing each host species as islands.

(e) Global parasite phylogenetic structure

We found that phylogenetic clustering in *Plasmodium* spp. lineages from the Shola sky-islands did not differ from a random sample of lineages from the global parasite pool at the community or lineage level (mean $D_{KN} = 0.533$, $p = 0.272$; two of 18 lineages had D_{KN} lower than expected; figure 4; electronic supplementary material, table S12). By contrast, *Haemoproteus* spp. lineages showed strong phylogenetic clustering at both community and lineage level (mean $D_{KN} = 0.281$, $p = 0.002$; 14 of 29 lineages had D_{KN} less than expected; figure 4; electronic

supplementary material, table S13). Overall, *Haemoproteus* spp. lineages had a significantly higher chance of being clustered compared to *Plasmodium* spp. lineages ($\beta \pm$ s.e. $= 1.958 \pm 0.86$, odds ratio $= 7.086$; $z = 2.277$, $p = 0.023$).

In line with our expectations, the generalist *Plasmodium* spp. lineages were widely interspersed across their global phylogeny, whereas specialist *Haemoproteus* spp. lineages were phylogenetically more clustered. This suggests that *Haemoproteus* spp. have likely diversified in the Western Ghats, owing to the relatively old origin [65] and the deep evolutionary history of Western Ghats endemic avian hosts [25] such as *Sholicola* spp. and *Montecincla* spp., which diverged from their most recent ancestor about 11–12 Ma and later diversified on the Shola sky-islands about 4–5 Ma [24]. The lack of phylogenetic clustering among the *Plasmodium* spp. lineages suggests that these parasites are a random sample of their global phylogenetic pool and remain unconstrained by host phylogeny, further highlighting their potential as emerging parasites in novel host communities.

4. Conclusion

We present one of the first comprehensive investigations of avian haemosporidian dynamics in the Indian subcontinent (see also [66]) by sampling almost the entire bird community in an important biodiversity hotspot. Here, we addressed the differential effects of geographical, 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 geographical factors such as geographical 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 harbour a 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. versus *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 harbour 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 spillover 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.

Data accessibility. The parasite genetic data generated in the current study have been deposited in the National Center for Biotechnology Information (NCBI) under the following GenBank Accession Numbers: MK493368–MK493401. Other datasets supporting this article have been provided as part of the electronic supplementary material.

Authors' contributions. P.G., V.V.R., and G.D.: conceived and designed the study; C.K.V. and V.V.R.: coordinated and collected field data; P.G.: molecular laboratory work and sequence alignments; P.G. and G.D.: data analyses, drafted the manuscript; P.G., C.K.V., U.R., V.V.R., and G.D.: revised and edited the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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