Host-pathogen-vector continuum in a changing landscape: Drivers of *Bartonella* prevalence and evidence of historic spillover in a multi-host community

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Abstract

Small mammals and their ectoparasites present a unique system to investigate the eco-epidemiology of multi-host vector-borne pathogens and identify specific bacterial spillover determinants. We applied ecological and evolutionary analyses in a rainforest-human-use mosaic to investigate *Bartonella spp.* across small mammal and ectoparasite communities. We observed substantial overlap among small mammal communities in different habitat types, predominantly driven by habitat generalists. Most ectoparasites were generalists, infecting multiple hosts. We observed high *Bartonella* prevalence at both study sites –a forest-plantation mosaic (47.4%) and a protected area (28.8%). Seven of the ten ectoparasite species sampled were also positive for *Bartonella*, following prevalence trends in their hosts. A generalised linear model revealed an independent association between aggregated ectoparasite load in hosts and *Bartonella* prevalence, implicating ectoparasites in transmission. *Bartonella* lineages from small mammals were host-specific, while ectoparasites carried *Bartonella* associated with other small mammal hosts, indicating the potential for cross-species transmission. Phylogenetic ancestral trait reconstruction of *Bartonella* haplotypes suggest historic spillover events in the small mammal community, validating the potential for contemporary spillover events. These results highlight the necessity to disentangle the complex relationship between hosts, ectoparasites, and pathogens to understand the zoonotic implications of undetected spillover events in such multi-host communities.

1. Background

Large-scale modification of natural habitats is associated with loss of biodiversity and ecosystem services [1]. Other unexpected consequences, such as an elevated risk of zoonotic infections, are poorly acknowledged and understood, especially in biodiverse tropical systems. Both theoretical and empirical studies demonstrate that land-use change and associated habitat perturbations can result in modified communities of zoonotic hosts driven by movement of animals across habitat boundaries [2,3].Consequently, there is potential for pathogen spillover between animals that did not previously co-occur, i.e. from reservoir hosts to incidental hosts, directly or indirectly through intermediate hosts and vectors [4,5].

Spillover between wildlife can lead to the emergence of zoonoses [4], and has been implicated in several past epidemics, including SARS-CoV-1 [6]. Pathogen sharing between animal host communities can lead to the recombination and evolution of new variants with altered host ranges, vector specificities, and pathogenic potential [7]. Spillover events, however, are complex and infrequent, influenced by numerous ecological and evolutionary filters that vary in space and time, including the distribution of reservoir hosts, pathogen prevalence, and infection intensity [8]. For example, a high density of reservoir hosts elevates direct interactions, leading to density-dependent transmission among the reservoir population and to other susceptible members of the community [9]. Additionally, in the case of vector-borne pathogens, ectoparasites also respond to host densities and influence pathogen prevalence in reservoirs and incidental hosts [10].

Bats and small mammals (rodents and shrews) are the best-known reservoir taxa for zoonotic pathogens [11]. Small mammals, however, harbour a high number of pathogens [12,13] owing to their disproportionately high diversity within mammalian communities [14], even more so than bats. Small mammals also differ from bats in that they often increase (rather than decrease) in abundance in altered landscapes, resulting in the elevated prevalence and transmission risk of associated zoonoses [15,16]. Small mammal densities are known to increase locally, for example, when large herbivore densities decrease [17], leading to an increase in pathogen prevalence [18]. In addition, small mammals with generalist natural histories (often reservoirs) tend to move across habitat boundaries where they interact with incidental hosts [4,19], thereby moving pathogens across these boundaries. We expect such processes to be especially prevalent in tropical regions, as they harbour some of the richest small mammal communities [20], the highest rates of habitat modification [21,22], and therefore the highest predicted risk of zoonotic infections [23,24].

To understand the zoonotic consequence of small mammals and their ectoparasites in changing landscapes, we must first understand how their communities are structured and how they have responded (and continue to respond) to land-use change and habitat modification. In this study, we characterize differences in host community structure, ectoparasite community structure, and pathogen prevalence in a human-dominated rainforest-plantation landscape in South India, part of the Western Ghats biodiversity hotspot. We used *Bartonella*, a generalist zoonotic bacterial pathogen, as a model system to understand variation in prevalence and transmission dynamics in the landscape. We sampled small mammal communities and their ectoparasites in this landscape to address

the following questions – i) How are small mammal communities structured in a tropical rainforest-plantation landscape? ii) How does *Bartonella* prevalence vary across small mammal species in this human-use landscape? iii) What are the main drivers of *Bartonella* prevalence and transmission in the landscape? (iv) and finally, do we find evidence for spillover, detected as shared *Bartonella* haplotypes between members of these communities? We began this study in a forest-plantation mosaic and expanded to a relatively less disturbed (currently) protected area. However, the protected area had significant human-use in the past [25], similar to the forest-plantation mosaic, allowing us to derive insights that can be broadly applied and generalized. Given the mixed-use nature of the landscape, we expected similarities between small mammals and ectoparasite communities across land-use types [26]. We hypothesized that *Bartonella* prevalence would be high and significantly influenced by the densities of known host species (e.g., *Rattus satarae*) [9,27]. We also expected that *Bartonella* haplotypes would be specific to host species [28] with limited spillover (vector-mediated) among hosts that occupy the same habitats [29].

2. Methods

(a) Field sampling

We carried out this study in two sites in the central Western Ghats: i) Kadamane, a mixed-use, forest-plantation mosaic in the Karnataka state in India, and ii) Kudremukh National Park, a protected area in the same state **(Figure 1)**. We sampled small mammals (rodents and shrews) from different land-use types in Kadamane and Kudremukh between 2016 and 2021. Kadamane was sampled three times during this period (2016, 2017, and 2018) and Kudremukh was sampled once in 2021. In Kadamane, we sampled in forest fragments,

grasslands, tea plantations, rested plantations (unmanaged cardamon and coffee), and built-up (human habitation). In Kudremukh, we sampled forests, grasslands, and built-up (a partially occupied township inside Kudremukh National Park). In both sites, sampling was conducted during dry months between January- April.

We used medium-sized Sherman traps in a grid framework to sample small mammals. A trapping grid consisted of 10 trapping lines, each line consisting of 10 traps separated by 10 meters, covering an area of one hectare in total in each habitat type. An additional 20 traps were placed on trees to capture arboreal species in forests and rested plantations. Trapping efforts were proportionately divided based on the area available under each land-use category. Specifically, in Kadamane, this translated to three grids in forests, two grids in grasslands, two grids in rested plantations, one grid in tea plantations, and one grid in built-up areas. Because the rested plantations in Kadamane were often indistinguishable from forests, we grouped them under 'forests' in all analyses. In Kudremukh, three grids were placed in forests, two in grasslands, and one in built-up areas **(Figure 1)**. Each grid was sampled for four consecutive days during each trapping session (assuming a closed population). All traps were baited using peanut butter and locally available seeds. Traps were checked twice a day (morning and evening) for captured individuals. Captured individuals were anesthetised using Isoflurane for collecting measurements and samples. Small mammal species were first identified in field with the help of taxonomic keys and field guides [30–32]. In addition, ear clippings were collected to confirm species identification through molecular methods. We collected dried blood spots from every captured individual in 2018 and 2021 to screen for pathogens. To detect and collect ectoparasites, captured individuals were combed on a white paper for 2-3 minutes. All individuals were marked and

136 released at the site of capture after sample collection (except in 2018; individuals were

137 sacrificed for harvesting organs to screen for various pathogens).

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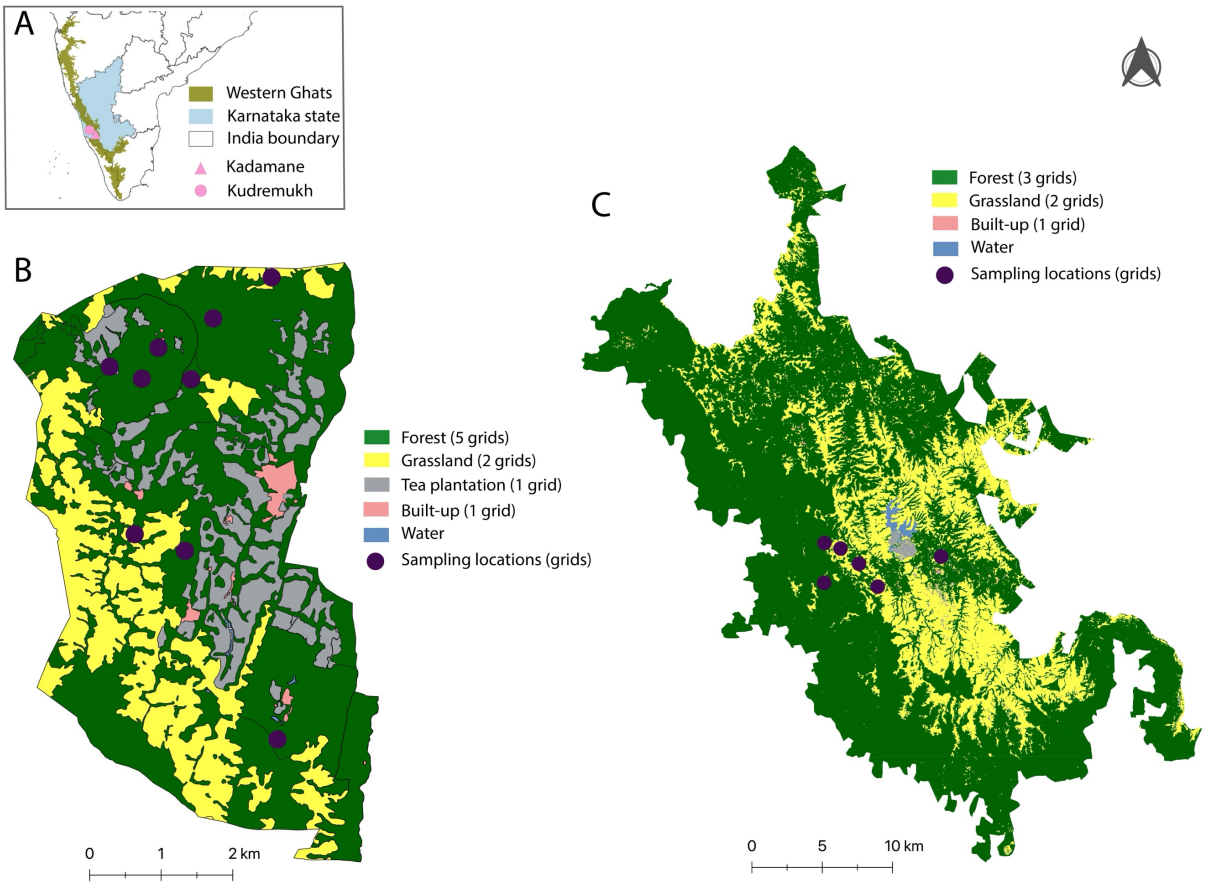

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140 **Figure 1:** Map of the study area. A) Map of India with Karnataka state, Western Ghats and

141 study sites marked. (B) Land-use map of Kadamane forest-plantation mosaic with sampling

142 locations marked. (C) Land-use map of Kudremukh National Park with sampling locations

143 marked. The legends indicate different land-use types and the number of grids sampled per

144 land-use type. Note that rested plantations are grouped under forests.

145

146 **(b) Small mammal community**

147 DNA was extracted from ear clippings using DNeasy Blood & Tissue Kit (Qiagen), following

148 which 1140 bp of Cytochrome b (*Cytb*) gene was amplified and sequenced [33]. Maximum

149 likelihood phylogenetic trees were constructed to identify cryptic *Mus* species and validate

150 morphological species identification. All the other species were identified morphologically
151 and validated using BLAST (blast.ncbi.nlm.nih.gov) similarity scores of *Cytb* sequences.
152
153 Based on species identification, we created a species abundance matrix for each land-use
154 type, followed by a distance matrix using the Morisita-Horn index, a standard beta diversity
155 measure to calculate dissimilarity [34]. The beta diversity index was plotted following
156 hierarchical clustering to visualise the dissimilarity between small mammal communities in
157 different land-use types and sampling grids. We also calculated the relative abundance of
158 each small mammal species in each land-use type to understand species-habitat
159 associations. The community ecology package Vegan [35] in R was used for this analysis.
160
161 We used a Capture-Mark-Recapture approach [36] to estimate densities of the four most
162 common small mammals by creating individual capture histories in forest and grassland. We
163 assumed a closed population with varying capture probability to estimate population size
164 following a customised log-linear model [37]. Density (number of individuals per hectare)
165 for each species was estimated after accounting for the number of grids sampled in each
166 habitat. We used R package *Rcapture* for this analysis [37].
167
168 **(c) Ectoparasite community**
169 Ectoparasites were brought back to the laboratory in 100% ethanol. Mites, ticks, and fleas
170 were segregated using a stereo-zoom microscope. We identified ticks and fleas to the genus
171 level under a high-resolution microscope with the help of taxonomic keys and monographs
172 [38–40]. Mites were mounted on a slide for observation under the microscope and

173 identified through descriptions in a monograph [41]. We serially named and counted
174 individual ectoparasites that belonged to the same morphotypes (potentially species).
175 We constructed a distance matrix based on ectoparasite occurrence per individual small
176 mammal species [42]. We used this matrix for a Principal Coordinate Analysis (PCoA) in R
177 [43], and plotted the clustering using ggplot2 [44] to understand the overlap in ectoparasite
178 communities between small mammal species (the first two PCoA axes). We also estimated
179 ectoparasite density, or the mean number of each morphotype per host species in forests,
180 grasslands, and built-up areas. The infestation rate, i.e., frequency of ectoparasite (mites,
181 ticks, and fleas) occurrence in each host species and habitat, was recorded as aggregated
182 ectoparasite load.
183
184 **(d) *Bartonella* screening and prevalence estimation**
185 To detect *Bartonella* in small mammals, DNA was extracted using QIAamp DNA Mini Kit
186 (Qiagen) from the dried blood spots (DBS) collected in 2018 (Kadamane) and 2021
187 (Kudremukh), and screened for *Bartonella* specific *rpoB* gene (825 bp) using conventional
188 PCR [45]. *Bartonella* prevalence in the Kadamane samples was reported previously [27]. To
189 detect *Bartonella* in ectoparasites, ectoparasite were pooled, mechanically homogenised,
190 and DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen). A pool contained 1-10
191 individual ectoparasites (mean = 3.06) belonging to the same species (previously called
192 morphotypes) collected from the same individual small mammal. We used PCR reagents as
193 follows- 25 µl reaction mixture composed of 6 µl of template DNA, 12.5 µl of 1X HotStarTaq
194 Master Mix (Qiagen), 1 µl each of 5 µM primers and 4.5 µl of nuclease-free water. PCR
195 products were visualized on a 1.5% agarose gel stained with GelRed Nucleic Acid Gel Stain
196 (Biotium Inc) with reference to negative and positive controls set along the test samples.

197 Positive samples were purified using AMPure XP magnetic beads (Beckman Coulter) and
198 sequenced. Sequences were verified for *Bartonella* after comparing with the reference
199 database (NCBI) using BLAST [46]. All *Bartonella* sequences generated in this study have
200 been deposited in GenBank under accession numbers OR574780-OR574826.
201
202 Prevalence was calculated as the proportion of positive individuals against the number of
203 individuals tested. While calculating the overall prevalence, we included two forest grids
204 from Kudremukh twice (randomly selected) to compensate for additional trapping effort in
205 Kadamane (rested plantations) to make the sites comparable. We calculated 95%
206 confidence intervals (Wilson interval) associated with the prevalence using the R package
207 *Hmisc* [47]. Prevalence (positivity) in ectoparasite pools was represented as the proportion
208 of positive pools among the total number of ectoparasite pools tested.
209
210 **(e) Ecological correlates of *Bartonella* prevalence**
211 We used a generalized linear model (GLM) to understand drivers of *Bartonella* prevalence
212 among small mammals in the landscape. We modelled prevalence as the incidence of
213 *Bartonella* (presence/absence) in each individual small mammal as a function of the
214 sampling site, aggregated ectoparasite load (the proportion of all individuals of the species
215 with any ectoparasite presence - mites, ticks, and fleas - within the relevant habitat-site
216 combination), and the density of the small mammal species itself. We assumed a binomial
217 error distribution as our response consisted of 0s and 1s. We restricted this analysis to the
218 10 species-habitat-site combinations that contained data from more than three individuals
219 of the species, together comprising 196 individuals of five species. We also examined host
220 species identity and habitat type as covariates but dropped them because the models did

221 not converge. The covariate 'aggregated ectoparasite load' is the frequency of ectoparasite
222 occurrence in each species and habitat, and is not ectoparasite density per individual small
223 mammal. We considered this a more reliable measure than raw ectoparasite densities since
224 it is possible to occasionally encounter individuals with heavy infestation in the community
225 and this might potentially influence our inference.
226
227 **(f) Phylogenetic analyses**
228 We generated a consensus of forward and reverse sequences for the partial *rpoB* sequence.
229 These sequences were aligned with known *Bartonella* species sequences from the NCBI
230 database and sequences reported in Ansil et al 2021 (accession number MT787671-
231 MT787733) [27]. A 773 bp alignment was created using MUSCLE in MEGA 7.0.26 [48].
232 Sequences generated from the ectoparasites were also included in this alignment.
233 Alignment was codon-optimized and tested for the appropriate nucleotide substitution
234 models using PartitionFinder 2.1.1 [49]. We performed a Bayesian phylogenetic analysis
235 using this alignment in BEAST 1.8.4 [50]. The phylogeny was run for 10^8 Markov Chain
236 Monte Carlo (MCMC) cycles. Effective sample sizes (ESS) and parameter convergence of
237 MCMC sampling were assessed using Tracer 1.6.0 [51]. The resulting trees were summarised
238 into a consensus tree after 25% burnin, and were visualized and annotated using FigTree
239 1.4.4 [52]. The phylogenetic tree was rooted using *Brucella melitensis* (AY562181) as an
240 outgroup. Internal nodes of the tree are marked and coloured based on the Posterior
241 Probability (PP).
242

243 **(g) Host- *Bartonella* association**

244 We also constructed a tanglegram (co-phylogeny) using sequence data from the hosts (Cytb)
245 and *Bartonella* (*rpoB*) in TREEMAP [53] to understand host- *Bartonella* haplotype
246 association. We visually analysed the resulting co-phylogeny for potential host switching
247 (spillover) events.

248

249 A separate alignment was constructed using unique *Bartonella* haplotypes and considered
250 for a Bayesian phylogenetic ancestral trait reconstruction. We restricted this analysis to
251 unique haplotypes recovered from each host species. We included one additional sequence
252 from *Mus cf. fernandoni* to avoid over-representation and subsequent biases from *Rattus*
253 *satarae* sequences. We used six identified host states as traits to estimate host probabilities
254 for all ancestral nodes in BEAST 1.8.4 [50]. The resultant phylogeny was summarised and
255 annotated as described before.

256

257 ## 3. Results

258 **(a) Small mammal community**

259 We recorded 11 small mammal species, of which nine were rodents, and two were shrews
260 **(Table S1).** The rodent species were *Rattus satarae*, *Rattus rattus*, *Mus cf. fernandoni*, *Mus*
261 *cf. famulus*, *Mus cf. terricolor*, *Golunda ellioti*, *Vandeleuria nilagirica*, *Platacanthomys*
262 *lasiurus*, and *Funambulus tristriatus*. The two shrew species were *Suncus niger* and
263 *Crocidura horsfieldii*. The three *Mus* species were morphologically similar and could only be
264 differentiated using their phylogenetic clustering **(Figure S1)**. The average genetic distances
265 (p distances) for *M. cf. fernandoni*, *M. cf. famulus*, and *M. cf. terricolor* from their respective

266 parent species (for example, *M. cf. fernandoni* vs *M. fernandoni*) were 0.05, 0.07 and 0.09,
267 respectively.
268
269 Among the various habitats sampled, grasslands had the most diverse small mammal
270 communities with five species detected in both Kadamane and Kudremukh. Forest
271 fragments in Kadamane also had five species of small mammals, but forests in Kudremukh
272 only had three species. Built-up areas were less diverse with just three species captured at
273 each site, while tea plantations were the most species-poor land-use type, with only one
274 species (*M. cf. famulus*) captured once in 2016.
275
276 Based on the hierarchical clustering of beta diversity, forests (FR16, FR17, FR18, and FR21)
277 showed similar community assemblages by forming a single cluster with minimal variability
278 across years (<20%); the forest community from Kudremukh (FR21) was also a part of this
279 cluster **(Figure 2A left)**. The grassland and tea plantation (TP16) formed a cluster with 40%
280 dissimilarity with the forest cluster. The grassland communities had minimal variability
281 (<20%) between years (GL16, GL17, GL18) and sites (GL21). Built-up habitats were the most
282 dissimilar (60%) to other land-use types, although they retained two species (*S. niger* and *M.*
283 *cf. famulus*) detected in the other land-use types **(Figure 2A right)**. Overall, Overall, the
284 small mammal community composition within each habitat was similar across years.
285
286 We were able to estimate densities for four (most abundant of the eleven) common small
287 mammal species (*Rattus satarae*, *M. cf. fernandoni*, *M. cf. famulus*, and *M. cf. terricolor*).
288 These densities are reported in Supplementary results and **Figure S2**.

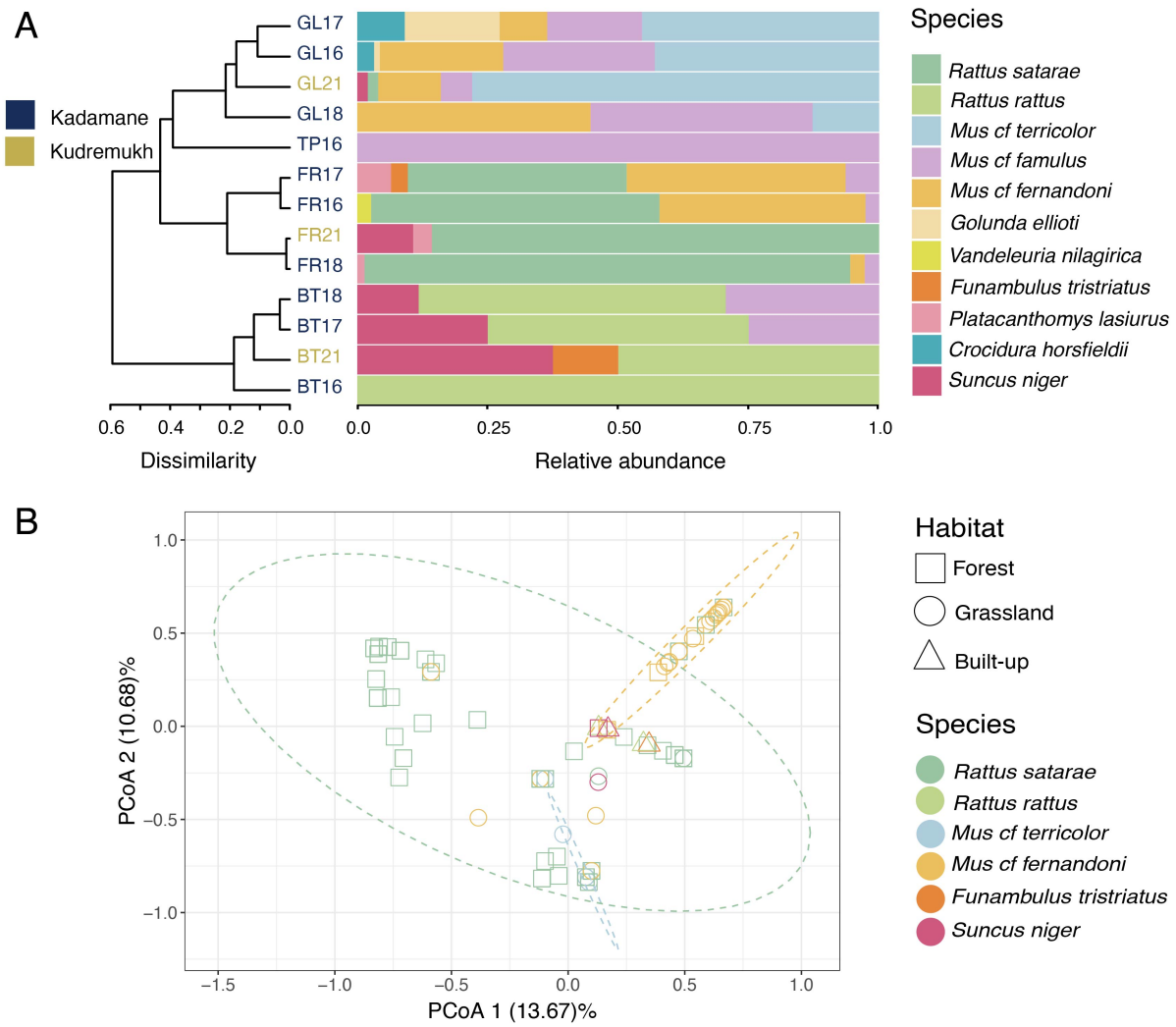

Figure 2: Community structure of small mammals and their ectoparasites. A) Left: a dendrogram showing dissimilarity between small mammal communities inhabiting different habitats sampled and sampling years (2016, 2017, 2018, 2021). Abbreviations: FR- Forest, GL-Grassland, TP-Tea plantations, and BT-Built-up areas. Right: Relative abundance of different small mammal species captured in different habitats. B) Principal Coordinate (PCoA) plot showing ectoparasite community structure across different small mammals and habitats. The ellipses represent the 95% confidence level of the respective sample group centroid.

300 **(c) Ectoparasite community**

301 Ten ectoparasite species were recorded from the small mammals; two species of mites
302 belonging to the genus *Laelaps*, three species of ticks belonging to the genus *Rhipicephalus*,
303 *Ixodes* and *Haemaphysalis* and five species of fleas belonging to the genus *Xenopsylla* **(Table**
304 **S2)**. Since more than one species was detected under *Laelaps* (mites) and *Xenopsylla* (fleas),
305 these morphotypes were named serially *(e.g. Xenopsylla sp 1, Xenopsylla sp 2* and so on).
306 Only one morphotype each was identified from three tick genera. Interestingly, six
307 ectoparasite species (60%) were detected in multiple hosts (generalists), and six of eight
308 small mammals sampled (75%) were polyparasitic (infested with two or more ectoparasite
309 species).

310

311 Our PCoA revealed substantial overlap and variation in ectoparasite communities hosted by
312 small mammals (**Figure 2B**). *R. satarae* showed significant variation in ectoparasite
313 community along the PCoA axis 1 and 2 (13.67 % and 10.68 % explained, respectively), while
314 *M. cf.* fernandoni predominantly displayed limited variation (similar ectoparasite
315 community dominated by *Laelaps* 1). However, individuals from *R. rattus*, *F. tristriatus*, *M.*
316 *cf. fernandoni*, *M. cf. terricolor*, and *S. niger* showed ectoparasite community similarity with
317 *R. satarae*. We also estimated the mean density of each ectoparasite species recovered
318 from each small mammal species per habitat presented in Supplementary results and **Figure**
319 **S2**.

320

321 **(d) *Bartonella* prevalence in small mammals**

322 We detected *Bartonella* in five species of small mammals from both sites; *R. satarae*, *R.*
323 *rattus*, *M. cf. fernandoni*, *M. cf. famulus,* and *S. niger* (**Table 1**) with varying prevalence (0-

75.8%). In both sites, *R. satarae* showed the highest prevalence of 75.8% (n= 66) in Kadamane and 72% (n= 25) in Kudremukh, followed by *M. cf. fernandoni* that showed a low prevalence of 54.5% (n=22) in Kadamane and 40% (n=5) in Kudremukh. *M. cf. famulus*, on the other hand showed a low prevalence of 3.8% in Kadamane (n=26, only one individual positive) and zero prevalence in Kudremukh (n=3), while *M. cf. terricolor* showed zero prevalence at both sites despite good sample size (n =6 and 37 in Kadamane and Kudremukh, respectively). Other species, such as *R. rattus* and *S. niger* had low prevalence, but also had low sample sizes, hence we have minimal confidence in their prevalence estimates.

We observed similar patterns of *Bartonella* prevalence at both sites (*R. satarae*, *M. cf. fernandoni*, *M. cf. famulus*, and *M. cf. terricolor*) and overall community at both sites **(Figure 3A)**. Since samples were collected in different years (2018 in Kadamane and 2021 in Kudremukh), we did not attempt any comparison between sites, but treated them separately. Pooled ectoparasite positivity was largely correlated with prevalence in small mammals, albeit slightly lower in every case. Positivity in specific ectoparasite species is provided in **Table S3.**

343 **Table 1:** *Bartonella* prevalence in small mammal blood and ectoparasites. Prevalence
344 reported for ectoparasites is not true prevalence since ectoparasites were pooled for
345 screening (see methods).

	***Bartonella* prevalence; number of positives / number of samples tested (%)**			
	Dried blood spot (DBS)		Pooled ectoparasites	
	Kadamane	Kudremukh	Kadamane	Kudremukh
Rattus satarae	50/66 (75.8)	18/25 (72)	24/71 (33.8)	6/24 (25)
Rattus rattus	0/10 (0)	1/4 (25)	-	0/3 (0)
Mus cf. fernandoni	12/22 (54.5)	2/5 (40)	9/29 (31)	0/6 (0)
Mus cf. famulus	1/26 (3.8)	0/3 (0)	0/1 (0)	-
Mus cf. terricolor	0/6 (0)	0/37 (0)	-	0/7 (0)
Platacanthomys lasiurus	-	0/1 (0)	-	0/1 (0)
Funambulus tristriatus	-	0/1 (0)	0/2 (0)	0/2 (0)
Suncus niger	0/2 (0)	2/4 (50)	0/1 (0)	1/2 (50)

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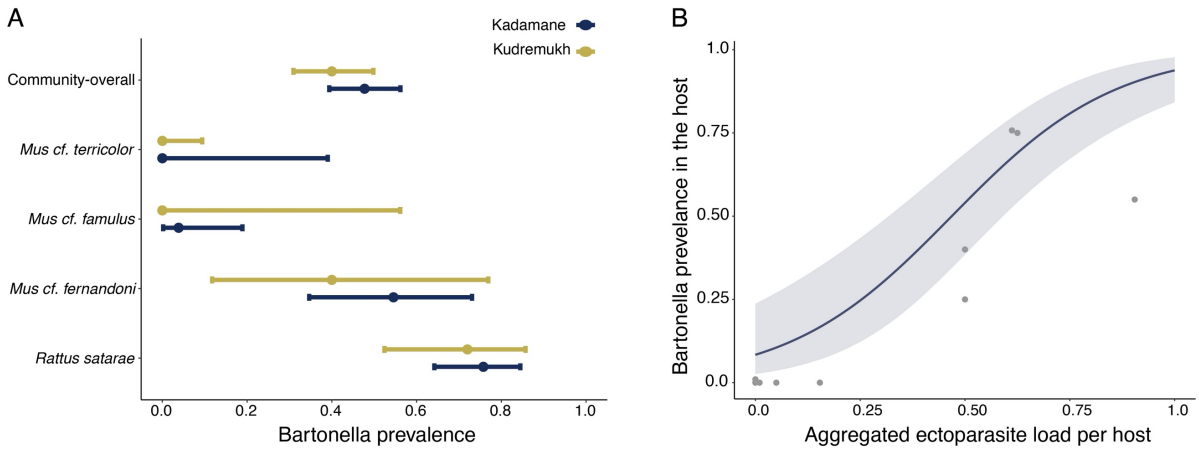

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349 **Figure 3:** (A) *Bartonella* prevalence in the overall community and the four most abundant
350 small mammals. (B) *Bartonella* prevalence positively correlated with aggregated
351 ectoparasite densities within a small mammal species at each habitat site. The trend line
352 indicates predicted *Bartonella* prevalence as a function of aggregated ectoparasite load. The
353 shaded area represents the 95% confidence interval of the predicted prevalence estimate.

354 **(e) Factors affecting *Bartonella* prevalence**

355 *Bartonella* prevalence increased with aggregated ectoparasite load (mites, ticks, and fleas)
356 within a species at each habitat site (GLM, $p<0.05$**, Figure 3B**). We did not find evidence that
357 *Bartonella* prevalence was influenced by host densities and sampling locations (**Table 2**). A
358 second GLM with host species identity and habitat type as additional covariates did not
359 converge; hence we tested these variables separately and found them not significant.

360
361
362 **Table 2:** Results of generalised linear model (GLM) examining drivers of *Bartonella*
363 prevalence. Significant variables are marked with (*)

	Estimate	Std. Error
Intercept	-1.633595	1.05266
Host density	-0.07574	0.06711
Site	-0.47880	0.72288
Aggregated ectoparasite load	5.10933*	0.96562

364

365

366 **(f) Phylogenetic relationship**

367 Phylogenetic analysis (*rpoB*) revealed eight distinct *Bartonella* lineages (BL1-BL8) from small
368 mammals and ectoparasites. We found that five lineages among them (BL1-BL5) were
369 phylogenetically related to known zoonotic *Bartonella* species (**Figure 4**). We observed
370 three lineages in *R. satarae* (BL1, BL6, and BL7) and two lineages in *M. cf. fernandoni* (BL4
371 and BL8). BL1 included *Bartonella* sequences from *R. satarae* and ectoparasites (*Laelaps sp*
372 *1, Rhipicephalus sp, Ixodes sp, Xenopsylla sp 1, and Xenopsylla sp 5*). Interestingly,
373 sequences from *Laelaps sp 1* and *Xenopsylla sp 5* in this lineage were not collected from *R.*
374 *satarae* but *M. cf. fernandoni* and *S. niger*, respectively. BL2 consisted of a single sequence
375 collected from *R. rattus*, and closely related to BL1. These two lineages were

phylogenetically related to *B. queenslandensis*. BL3 consisted of two sequences, one from *S. niger* and the other from *Laelaps sp 1* collected from *M. cf. fernandoni*. This lineage was related to *B. tribocorum*. BL4 consisted of *Bartonella* sequences from *M. cf. fernandoni*, *M. cf. famulus,* and ectoparasites (*Laelaps sp 1, Rhipicephalus sp, Ixodes sp, Haemaphysalis sp, and Xenopsylla sp 3*). In this lineage, the *Haemaphysalis sp* was recovered from *R. satarae* and not from any of the *Mus* species. BL5 was a single sequence from *F. tristriatus* (reported in Ansil et al. 2021 [27], accession number- MT787671), forming a sister lineage to BL4. We included this sequence in our analysis since it was collected during our study in Kadamane. The remaining lineages (BL6-BL8) were related to non-pathogenic *Bartonella* species. BL6 comprised sequences from *R. satarae* (second lineage from *R. satarae*) and *Ixodes sp* recovered from them. This lineage was related to *B. phoceensis*. BL7 and BL8 were two distinct lineages from *R. satarae* (third lineage from *R. satarae*) and *M. cf. fernandoni* (second lineage from *M. cf. fernandoni*), respectively. Both these lineages were phylogenetically related to *B. thailandensis*.

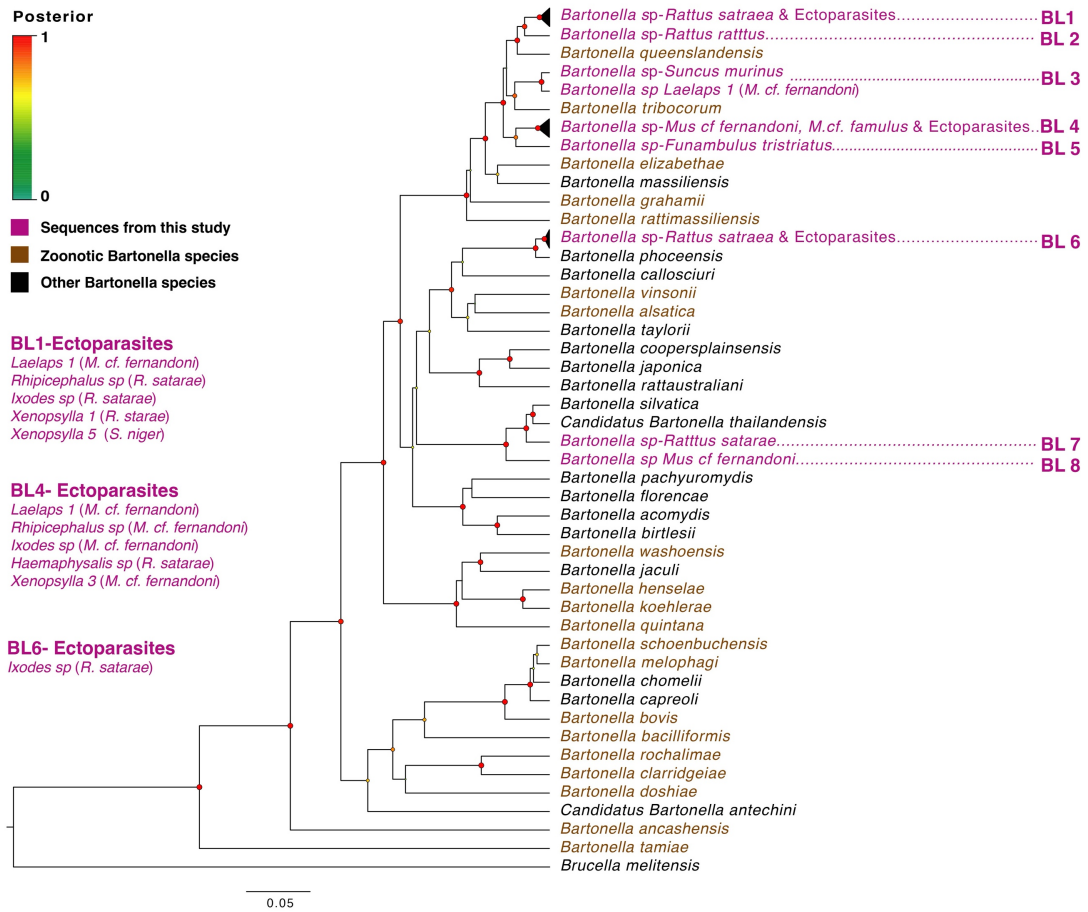

Figure 4: Bayesian phylogenetic inference of *Bartonella* based on 773 bp of *rpoB* sequences. Nodes are coloured based on posterior probability, and monophyletic clades are collapsed for easy visualization. Ectoparasites part of collapsed lineages and their hosts are expanded on the left side.

(g) Host-*Bartonella* association and historic spillover

The co-phylogeny of small mammals and *Bartonella* revealed host-specific lineages **(Figure 5A)**. However, one *Bartonella* lineage from *M. cf. fernandoni* (BL8) was observed to be phylogenetically related to *Bartonella* sequences from *R. satarae* (BL7). Our phylogenetic ancestral trait reconstruction revealed a high probability (0.87) of *R. satarae* being the host state of the common ancestor of these lineages **(Figure 5B)**, suggesting a potential past spillover event. Similarly, we also observed a moderately high probability (0.73) of *R.*

404 *satarae* being the host state for the *Bartonella* sequence from *R. rattus* and the remote

405 common ancestor (0.8) of the *Bartonella* community level phylogeny. We found no

406 evidence for contemporary *Bartonella* spillover or sharing between small mammal hosts.

407

408

409 **Figure 5:** A) Co-phylogeny of small mammals and *Bartonella* association. One lineage of *M.*
410 *cf. fernandoni* (BL8) was observed to have phylogenetic similarity with one of the *R. satarae*
411 lineage (BL7). B) Bayesian phylogenetic inference showing ancestral host state of different
412 *Bartonella* haplotypes. Each colour represents a specific host, and the number at the
413 common ancestor nodes indicates the probability for the corresponding host state.
414

415 ## 4. Discussion

416 We characterized small mammal communities, their ectoparasites, and associations with
417 *Bartonella* in a human-dominated rainforest-plantation landscape in the Western Ghats. We
418 demonstrated that certain small mammal host species occur across habitats, bringing them
419 in contact with specialist hosts with specific *Bartonella* lineages. We also demonstrate that
420 ectoparasites can serve as potential vectors, because *Bartonella* prevalence in the
421 community is influenced by the cumulative ectoparasite load in small mammals.
422 Additionally, we present evidence of a historic spillover event between two major hosts in
423 the community and substantiate the potential for future *Bartonella* sharing between hosts,
424 including other domestic animals and humans, possibly through ectoparasites.
425

426 **(a) Some small mammals have the potential to carry *Bartonella* across habitat boundaries**
427 We observed strong species-habitat associations in the focal small mammal community,
428 characterised by exclusive occurrences and high densities, mirroring several ecological
429 communities [54,55]. While species like *R. satarae* (a major *Bartonella* host with highest
430 prevalence) showed high abundance and nearly exclusive occurrence in forested habitats, *R.*
431 *rattus*, a known synanthropic species, was only detected in built-up areas, reaffirming its
432 commensal nature [56]. Similarly, *M. cf. terricolor* was exclusively recorded in grasslands in
433 both sites, albeit with higher density in Kudremukh. Other *Mus* species , *M. cf. fernandoni*

434 and *M. cf. famulus* were also abundant in grasslands, indicating a generalizable association
435 of *Mus* species with grassland ecosystems. Significantly from a disease ecology perspective,
436 few species like *M. cf. famulus* and *S. niger*, however, were recorded in multiple habitats,
437 including forests, grasslands, and built-up areas. Species like *M. cf. famulus* can also be
438 incidental hosts (as may be the case with the single positive), connecting potential reservoir
439 hosts like *R. satarae* and *M. cf. fernandoni*, with *R. rattus* (another seemingly incidental host
440 with a single positive) and humans. Similarly, *M. cf. fernandoni* was found to occur at low
441 densities in forests in addition to grasslands, bringing them in contact with *R. satarae*,
442 potentially paving the way for the historic spillover (discussed later) from *R. satarae* to *M.*
443 *cf. fernandoni*.
444
445 We did not find any notable difference between small mammal communities within
446 Kudremukh (protected area) and Kadamane (forest-plantation mosaic). One reason for this
447 might be that both landscapes (and much of the Western Ghats) have a long history of
448 human use [25,57], dampening differences that might exist between 'natural' and human
449 dominated areas. However, inadequate temporal replicates and delay in sampling
450 Kudremukh diminishes the generality of the pattern observed. Interestingly, all *Mus* species
451 in Kadamane showed variation in annual densities, indicating annual population oscillations
452 [58,59]. We could not fully capture this pattern due to the relatively short duration of this
453 study. However, understanding such variation in host density is crucial as it can be a
454 determinant of zoonotic hazard and spillover [8,60]. This reiterates the importance of
455 detailed ecological studies on host communities to understand spillover dynamics.
456

457 Our study suggests that small mammals in this part of the Western Ghats are in urgent need
458 of taxonomic revision. Previous studies from the landscape morphologically identified *M. cf.*
459 *fernandoni* as *M. musculus* and *M. cf. famulus* and *M. cf terricolor* as *M. booduga* [27].
460 Phylogenetic analysis and genetic distances suggest that these are distinct lineages related
461 to *M. fernandoni*, *M. famulus*, and *M. terricolor*, respectively. We urge researchers to
462 sample rodents in this landscape and resolve their taxonomy on priority, because pathogen
463 and habitat associations are often linked with species identity as we show in this study.
464
465 **(b) *Bartonella* prevalence and spillover may be mediated by ectoparasites**
466 Our results have revealed some broad ectoparasite-host associations that may have
467 implications for *Bartonella* maintenance and transmission. The observed patterns include
468 instances of polyparasitism, host sharing, and the influence of ectoparasitism on *Bartonella*
469 prevalence. In our dataset, ectoparasite diversity and density was centered around medium
470 to large-bodied, and synanthropic species. Specifically, *R. satarae* and *M. cf. fernandoni*, two
471 relatively large-bodied and abundant species with high *Bartonella* prevalence, displayed
472 polyparasitism, often with high densities of specific ectoparasites. This pattern aligns with
473 the positive correlation between body size and ectoparasitism observed in other studies
474 [61]. Potentially, habitat sharing of these species with other small mammals facilitates novel
475 ectoparasite acquisitions and driving overall ectoparasite richness on them [62,63].
476
477 Host sharing was another predominant pattern in the ectoparasite community. Several
478 ectoparasites (e.g., *Rhipicephalus sp*, *Xenopsylla sp 1*) showed a wider host range,
479 presumably due to their ability to infect multiple locally available hosts [64]. These results
480 are consistent with previous studies and indicate that host sharing among vectors is more

481 common than previously believed [65]. Such non-specific host-ectoparasite associations can
482 facilitate transmission of several pathogens, making their ecology a crucial part of vector-
483 borne pathogen dynamics [66].
484
485 Contrary to our expectations based on host density, our results revealed ectoparasitism
486 (aggregated ectoparasite load per species) was the most important predictor for
487 prevalence. While we did not find the patterns we expected for *Bartonella* prevalence, the
488 correlation between ectoparasitism and *Bartonella* prevalence holds ecological and
489 epidemiological significance. *Bartonella* is a vector-borne pathogen known for its
490 transmission through blood-feeding arthropods [67]. Hence, elevated densities of infected
491 ectoparasites in the environment (and on a specific host) can increase transmission (density-
492 dependant transmission) and impact prevalence and risk of infection [68]. Polyparasitism
493 and lack of host specialization suggests high chances of *Bartonella* transmission between
494 hosts through ectoparasites. In addition, known synanthropic species such as *R. rattus* with
495 multiple generalist fleas (occur in many hosts as demonstrated in this study) pose risks of
496 *Bartonella* transmission to humans and domestic animals [67].
497
498 Unfortunately, ecological drivers of aggregated ectoparasite load remain poorly understood.
499 As discussed earlier, longitudinal sampling targeting larger sample sizes from each
500 representative species in combination with ecological models incorporating additional
501 appropriate variables (e.g., host traits, season, etc.) might help explain variance in
502 ectoparasitism. We caution that other variables previously shown to influence pathogen
503 prevalence, like seasonality [9] and environmental stressors [69] were unavailable in our
504 study.

505 **(c) Non-specific ectoparasite-*Bartonella* associations and evidence of historic spillover**
506 **suggest future spillover risk**
507 Characterizing *Bartonella* diversity and its association with small mammals and
508 ectoparasites is crucial from a public health perspective, especially since many of the
509 *Bartonella* species are zoonotic and known to associate with small mammals and their
510 ectoparasites [70]. For instance, endemic *Bartonella* variants such as *B. bacilliformis*,
511 responsible for Carrion's disease, can be fatal and cause serious mortality [71]. Five (of
512 eight) *Bartonella* lineages identified in this study share a close evolutionary relationship with
513 known zoonotic species such as *B. queenslandensis* and *B. tribocorum*. Previous studies have
514 documented the widespread presence and global circulation of these *Bartonella* species,
515 primarily facilitated by small mammals and domestic animals [72]. However, it is important
516 to note that phylogenetic similarity alone does not confirm their ability to cause disease in
517 humans. Additional validation through molecular and *in vitro* experiments is warranted to
518 establish their pathogenicity in humans [73,74].
519
520 We observed contrasting patterns in *Bartonella* association with small mammals and
521 ectoparasites. In hosts, *Bartonella* displayed species-specific associations, as we expected
522 based on previous studies [27,75]. Remarkably, *Bartonella* showed non-specific association
523 with ectoparasites (e.g.*, Xenopsylla sp* 5 collected from *S. niger* carried *Bartonella* associated
524 with *R. satarae*), leading to a higher overall diversity of haplotypes in specific ectoparasites
525 compared to their corresponding hosts. These complex patterns are not entirely
526 unprecedented, as they have been documented elsewhere [76]. Nevertheless, these non-
527 specific associations between *Bartonella* and ectoparasites have significance for cross-
528 species transmission, given vector competency.

529 Our phylogenetic ancestral trait reconstruction also highlighted the possibility for cross-
530 species transmission in the community. We observed *R. satarae* as the ancestral host for
531 several *Bartonella* lineages with a medium to high probability, indicating past spillover
532 events involving this species. This was evident between the BL7 and BL8 lineages, recovered
533 from *R. satarae* and *M. cf. fernandoni*, respectively. Interestingly, these two species stood
534 out as the primary *Bartonella* hosts in our dataset, displaying high prevalence, high
535 haplotype diversity, and co-occurrence in forested habitats. Such pathogen movement
536 between host species could provide an opportunity to evolve new virulent variants through
537 recombination [4], similar to those observed in other pathogen systems [6]. Although we
538 did not find instances of contemporary spillover, our results suggest past spillover events,
539 potentially through ectoparasites, emphasizing the likelihood of similar events. The
540 communities we studied include many small mammals and their ectoparasites that overlap
541 between habitats, increasing the likelihood of such inter-species transmissions [77]. In
542 addition, the movement of generalist hosts across multiple habitats carrying generalist
543 ectoparasites with non-specific *Bartonella* associations is likely to further drive pathogen
544 spillover in the landscape [78].
545

546 **5. Conclusion**

547 This study has uncovered several significant insights and nuances about the intricate
548 relationship between small mammal hosts, their ectoparasites, and bacterial agents within a
549 tropical mixed-use landscape. While our model system and investigation were specific to
550 *Bartonella*, the results and inferences extend to a broader spectrum of vector-borne
551 bacterial pathogens in multi-host communities. Our research highlights the crucial role of
552 ectoparasitism in driving *Bartonella* prevalence within these small mammal communities

553 where some hosts move across habitat boundaries. Our results also highlight the non-
554 specific association of ectoparasites with *Bartonella* diversity compared to their hosts,
555 underscoring the potential for ectoparasite-driven cross-species transmissions. Using
556 evolutionary analyses, we further demonstrate the role of historical spillover events in
557 shaping the current *Bartonella* distribution in the community and highlight the future
558 prospects of such spillover events. Collectively, our findings underscore the need for
559 comprehensive ecological studies to advance our understanding of the transmission
560 dynamics of vector-borne bacterial pathogens within multi-host communities and their
561 zoonotic implications, particularly in rapidly changing landscapes.
562
563 **Ethics statement**
564 This study and associated protocols were approved by National Centre for Biological Science
565 Institutional Animal Ethics Committee (NCBS-IAEC-2016/10-[M], NCBS-IAE-2020/ 02[N]) and
566 Institutional Biosafety Committee (TFR:NCBS:23_IBSC/2017).
567
568 **Data accessibility**
569 The manuscript and Supplementary materials present small mammal capture data and
570 *Bartonella* prevalence data from small mammals and ectoparasites. *Bartonella* sequence
571 data generated during this study are deposited in GenBank under the accession numbers
572 OR574780-OR574826.
573
574 **Author contributions**
575 ABR: conceptualization, methodology, investigation, data curation, formal analysis,
576 visualization, project administration, writing -original draft, writing-review and editing; AV:

methodology, data curation, formal analysis, visualization, writing-review and editing; VR: methodology, investigation, writing-review and editing; HMY: methodology, investigation, writing-review and editing; AS: investigation, writing-review and editing; UR: conceptualization, funding acquisition, project administration, supervision, writing-review and editing.

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