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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Keywords: Spillover, Western Ghats, Small mammals, Vector-borne pathogens

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 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 a forest-plantation mosaic

88 and a relatively undisturbed habitats within a protected area in this landscape to address
89 the following questions – i) How are small mammal communities structured in a forest-
90 plantation mosaic and relatively less disturbed protected area? ii) Is there any difference in
91 *Bartonella* prevalence between these two communities? iii) What are the main drivers of
92 *Bartonella* prevalence and transmission in the landscape? (iv) and finally, do we find
93 evidence for spillover, detected as shared *Bartonella* haplotypes between members of these
94 communities? We began with the expectation that the forest-plantation mosaic would have
95 higher small mammal densities and *Bartonella* prevalence than the protected area [9,15].
96 We also expected that *Bartonella* haplotypes would be specific to host species [25] with
97 limited spillover (vector-mediated spillover) among hosts that occupy the same habitats
98 [26].
99

100 ## 2. Methods

101 **(a) Field sampling**

102 We carried out this study in two sites in the central Western Ghats: i) Kadamane, a mixed-
103 use, forest-plantation mosaic in the Karnataka state in India, and ii) Kudremukh National
104 Park, a relatively less disturbed protected area in the same state **(Figure 1)**. We sampled
105 small mammals (rodents and shrews) from different land-use types in Kadamane and
106 Kudremukh between 2016 and 2021. Kadamane was sampled three times during this period
107 (2016, 2017, and 2018) and Kudremukh was sampled once in 2021. In Kadamane, we
108 sampled in forest fragments, grasslands, tea plantations, rested plantations (unmanaged
109 cardamon and coffee), and human habitation. In Kudremukh, we sampled forests,
110 grasslands, and human habitation (a partially occupied township adjacent to 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 occupied by each land-use type. 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 [27–29]. 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 released at the site of capture after sample collection (except in 2018; individuals were sacrificed for harvesting organs to screen for various pathogens).

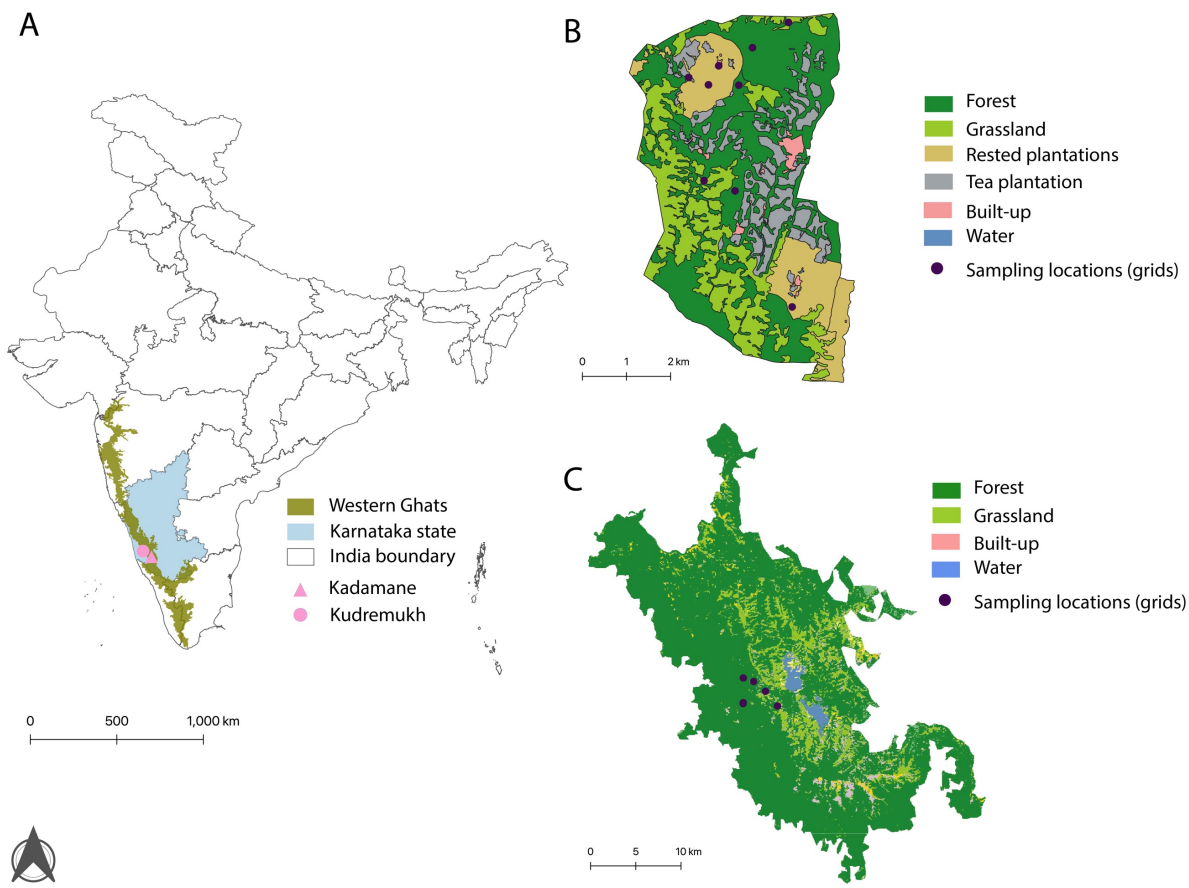


Figure 1: Map of the study area. A) Map of India with Karnataka state, Western Ghats and study sites marked. (B) Land-use map of Kadamane forest-plantation mosaic with sampling locations marked. (C) Land-use map of Kudremukh National Park with sampling locations marked.

(b) Small mammal community

DNA was extracted from ear clippings using DNeasy Blood & Tissue Kit (Qiagen), following which 1140 bp of Cytochrome b (*Cytb*) gene was amplified and sequenced [30]. Maximum likelihood phylogenetic trees were constructed to identify cryptic *Mus* species and validate morphological species identification. All the other species were identified morphologically and validated using BLAST (blast.ncbi.nlm.nih.gov) similarity scores of *Cytb* sequences.

Based on species identification, we created a species abundance matrix for each land-use type, followed by a distance matrix using the Morisita-Horn index, a standard beta diversity measure to calculate dissimilarity [31]. The beta diversity index was plotted following hierarchical clustering to visualise the dissimilarity between small mammal communities in different land-use types and sampling grids. We also calculated the relative abundance of each small mammal species in each land-use type to understand species-habitat associations. The community ecology package Vegan [32] in R was used for this analysis.

We used a Capture-Mark-Recapture approach [33] to estimate densities of the four most common small mammals by creating individual capture histories in forest and grassland. Since rested plantations and forests had similar vegetation structure and small mammal communities, we grouped them under 'forests' for this analysis (to obtain adequate sample size). We assumed a closed population with varying capture probability to estimate population size following a customised log-linear model [34]. Density (number of individuals per hectare) for each species was estimated after accounting for the number of grids sampled in each habitat. We used R package *Rcapture* for this analysis [34].

(c) Ectoparasite community

Ectoparasites were brought back to the laboratory in 100% ethanol. Mites, ticks, and fleas were segregated using a stereo-zoom microscope. We identified ticks and fleas to the genus level under a high-resolution microscope with the help of taxonomic keys and monographs [35–37]. Mites were mounted on a slide for observation under the microscope and identified through descriptions in a monograph [38]. We serially named and counted individual ectoparasites that belonged to the same morphotypes (potentially species).

169 We constructed a distance matrix based on ectoparasite abundance per individual small
170 mammal species [39]. We used this matrix for a Principal Coordinate Analysis (PCoA) in R
171 [40], and plotted the clustering using ggplot2 [41] to understand the overlap in ectoparasite
172 communities between small mammal species (the first two PCoA axes). We also estimated
173 ectoparasite density, or the mean number of each morphotype per host species in forests
174 (including rested plantations), grasslands, and built-up areas. The infestation rate, i.e.,
175 frequency of ectoparasite (mites, ticks, and fleas) occurrence in each host species and
176 habitat, was recorded as aggregated ectoparasite load.
177
178 **(d) *Bartonella* screening and prevalence estimation**
179 To detect *Bartonella* in small mammals, DNA was extracted using QIAamp DNA Mini Kit
180 (Qiagen) from the dried blood spots (DBS) collected in 2018 (Kadamane) and 2021
181 (Kudremukh), and screened for *Bartonella* specific *rpoB* gene (825 bp) using conventional
182 PCR [42]. *Bartonella* prevalence in the Kadamane samples was reported previously [43]. To
183 detect *Bartonella* in ectoparasites, ectoparasite were pooled, mechanically homogenised,
184 and DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen). A pool contained 1-10
185 individual ectoparasites (mean = 3.06) belonging to the same species (previously called
186 morphotypes) collected from the same individual small mammal. We used PCR reagents as
187 follows- 25 µl reaction mixture composed of 6 µl of template DNA, 12.5 µl of 1X HotStarTaq
188 Master Mix (Qiagen), 1 µl each of 5 µM primers and 4.5 µl of nuclease-free water. PCR
189 products were visualized on a 1.5% agarose gel stained with GelRed Nucleic Acid Gel Stain
190 (Biotium Inc) with reference to negative and positive controls set along the test samples.
191 Positive samples were purified using AMPure XP magnetic beads (Beckman Coulter) and
192 sequenced. Sequences were verified for *Bartonella* after comparing with the reference

193 database (NCBI) using BLAST [44]. All *Bartonella* sequences generated in this study have
194 been deposited in GenBank under accession numbers OR574780-OR574826.
195
196 Prevalence was calculated as the proportion of positive individuals against the number of
197 individuals tested. Small mammal species with low sample sizes were grouped as 'others' in
198 the prevalence calculation. While calculating the overall prevalence, we included two forest
199 grids from Kudremukh twice (randomly selected) to compensate for additional trapping
200 effort in Kadamane (rested plantations) to make the sites comparable. We calculated 95%
201 confidence intervals (Wilson interval) associated with the prevalence using the R package
202 *Hmisc* [45]. Prevalence in ectoparasite pools was represented as the proportion of positive
203 pools among the total number of ectoparasite pools tested.
204
205 **(e) Ecological correlates of *Bartonella* prevalence**
206 We used a generalized linear model (GLM) to understand drivers of *Bartonella* prevalence
207 among small mammals in the landscape. We modelled prevalence as the incidence of
208 *Bartonella* (presence/absence) in each individual small mammal as a function of the
209 sampling site, aggregated ectoparasite load (the proportion of all individuals of the species
210 with any ectoparasite presence - mites, ticks, and fleas - within the relevant habitat-site
211 combination), and the density of the small mammal species itself. We assumed a binomial
212 error distribution as our response consisted of 0s and 1s. We restricted this analysis to the
213 10 species-habitat-site combinations that contained data from more than three individuals
214 of the species, together comprising 196 individuals of five species. We also examined host
215 species identity and habitat type as covariates but dropped them because the models did
216 not converge. The covariate 'aggregated ectoparasite load' is the frequency of ectoparasite

217 occurrence in each species and habitat, and is not ectoparasite density per individual small
218 mammal. We considered this a more reliable measure than raw ectoparasite densities since
219 it is possible to occasionally encounter individuals with heavy infestation in the community
220 and this might potentially influence our inference.
221
222 **(f) Phylogenetic analyses**
223 We generated a consensus of forward and reverse sequences for the partial *rpoB* sequence.
224 These sequences were aligned with known *Bartonella* species sequences from the NCBI
225 database and sequences reported in Ansil et al 2021 (accession number MT787671-
226 MT787733) [43]. A 773 bp alignment was created using MUSCLE in MEGA 7.0.26 [46].
227 Sequences generated from the ectoparasites were also included in this alignment.
228 Alignment was codon-optimized and tested for the appropriate nucleotide substitution
229 models using PartitionFinder 2.1.1 [47]. We performed a Bayesian phylogenetic analysis
230 using this alignment in BEAST 1.8.4 [48]. The phylogeny was run for 10^8 Markov Chain
231 Monte Carlo (MCMC) cycles. Effective sample sizes (ESS) and parameter convergence of
232 MCMC sampling were assessed using Tracer 1.6.0 [49]. The resulting trees were summarised
233 into a consensus tree after 25% burnin, and were visualized and annotated using FigTree
234 1.4.4 [50]. The phylogenetic tree was rooted using *Brucella melitensis* (AY562181) as an
235 outgroup. Internal nodes of the tree are marked and coloured based on the Posterior
236 Probability (PP).
237
238 **(g) Host- *Bartonella* association**

239 We also constructed a tanglegram using sequence data from the hosts (Cytb) and *Bartonella*
240 (*rpoB*) in TREEMAP [51] to understand host- *Bartonella* haplotype association. We visually
241 analysed the resulting co-phylogeny for potential host switching (spillover) events.
242 A separate alignment was constructed using unique *Bartonella* haplotypes and considered
243 for a Bayesian phylogenetic ancestral trait reconstruction. We restricted this analysis to
244 unique haplotypes recovered from each host species. We included one additional sequence
245 from *Mus cf. fernandoni* to avoid over-representation and subsequent biases from *Rattus*
246 *satarae* sequences. We used six identified host states as traits to estimate host probabilities
247 for all ancestral nodes in BEAST 1.8.4 [48]. The resultant phylogeny was summarised and
248 annotated as described before.
249

250 ## 3. Results

251 **(a) Small mammal community assembly**

252 We recorded 11 small mammal species, of which nine were rodents, and two were shrews
253 **(Table S1).** The rodent species were *Rattus satarae*, *Rattus rattus*, *Mus cf. fernandoni*, *Mus*
254 *cf. famulus*, *Mus cf. terricolor*, *Golunda ellioti*, *Vandeleuria nilagirica*, *Platacanthomys*
255 *lasiurus*, and *Funambulus tristriatus*. The two shrew species were *Suncus niger* and
256 *Crocidura horsfieldii*. The three *Mus* species were morphologically similar and could only be
257 differentiated using their phylogenetic clustering **(Figure S1)**. The average genetic distances
258 (p distances) for *M. cf. fernandoni*, *M. cf. famulus*, and *M. cf. terricolor* from their respective
259 parent species (for example, *M. cf. fernandoni* vs *M. fernandoni*) were 0.05, 0.07 and 0.09,
260 respectively.
261

Among the various habitats sampled, grasslands had the most diverse small mammal communities with five species detected in both Kadamane and Kudremukh. Forest fragments in Kadamane also had five species of small mammals, but forests in Kudremukh only had three species. Built-up areas were less diverse with just three species captured at each site, while tea plantations were the most species-poor land-use type, with only one species (*M. cf. famulus*) captured once in 2016.

Based on the hierarchical clustering of beta diversity, forests (FR16, FR17, FR18, and FR21) and rested plantations (RP16, RP17, and RP18) showed similar community assemblages with minimal dissimilarity (<20%); the forest community from Kudremukh (FR21) was also a part of this cluster **(Figure 2A)**. The grassland cluster showed 40% dissimilarity with the forest-rested plantation cluster while maintaining moderate dissimilarity (<20%) between years (GL16, GL17, GL18) and sites (GL21). Built-up habitats were the most dissimilar (60%) to other land-use types, although they retained two species (*S. niger* and *M. cf. famulus*) detected in the other land-use types. We were able to estimate densities for four (most abundant of the eleven) common small mammal species (*Rattus satarae*, *M. cf. fernandoni*, *M. cf. famulus*, and *M. cf. terricolor*). These densities are reported in supplementary results and in **Figure S2.**

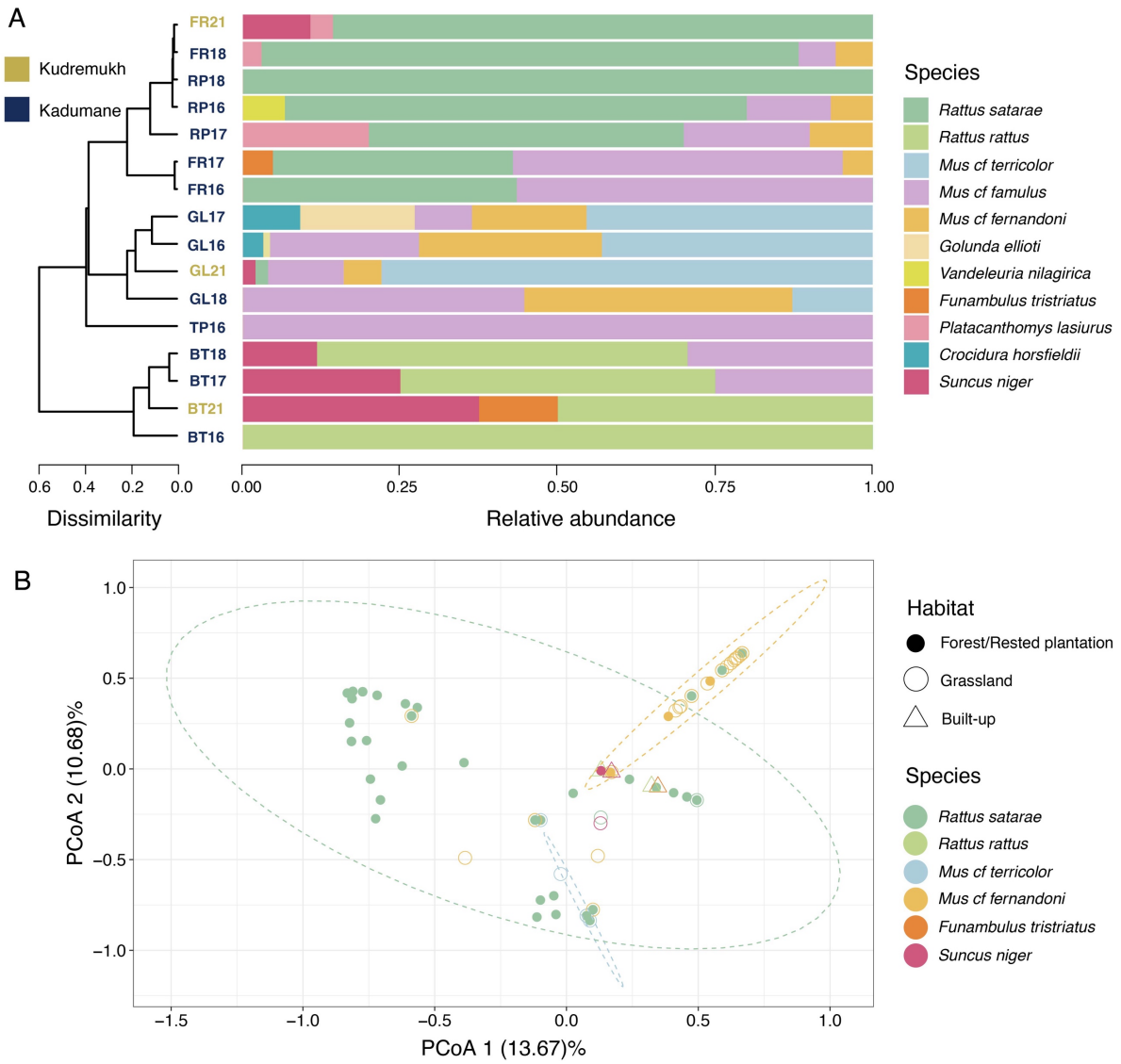


Figure 2: Community structure of small mammals and their ectoparasites. A) Left: a dendrogram showing dissimilarity between small mammal communities inhabiting different land-use types sampled and sampling years (2016, 2017, 2018, 2021). Abbreviations: FR-Forest, GL-Grassland, RP-Rested plantations, TP-Tea plantations, and BT-Built-up areas. Right: Relative abundance of different species captured in different land-use types. B) Principal Coordinate (PCoA) plot showing ectoparasite community structure across different small mammals and habitats.

(c) Ectoparasite assemblage

292 Ten ectoparasite species were recorded from the small mammal community; two species of
293 mites belonging to the genus *Laelaps*, three species of ticks belonging to the genus
294 *Rhipicephalus*, *Ixodes* and *Haemaphysalis* and five species of fleas belonging to the genus
295 *Xenopsylla* **(Table S2)**. Since more than one species was detected under *Laelaps* (mites) and
296 *Xenopsylla* (fleas), these morphotypes were named serially *(e.g. Xenopsylla sp 1, Xenopsylla*
297 *sp 2* and so on). Only one morphotype each was identified from three tick genera.
298 Interestingly, six ectoparasite species (60%) were detected in multiple hosts (generalists),
299 and six of eight small mammals sampled (75%) were polyparasitic (infested with two or
300 more ectoparasite species).
301
302 Our PCoA revealed substantial overlap and variation in ectoparasite communities hosted by
303 small mammals (**Figure 2B**). *R. satarae* showed significant variation in ectoparasite
304 community along the PCoA axis 1 and 2 (13.67 % and 10.68 % explained, respectively), while
305 *M. cf.* fernandoni predominantly displayed similar ectoparasite community (dominated by
306 *Laelaps* 1). However, Individuals from *R. rattus*, *F. tristriatus*, *M. cf. fernandoni*, *M. cf.*
307 *terricolor*, and *S. niger* also had similar ectoparasite communities to *R. satarae*. We also
308 estimated the mean density of each ectoparasite species recovered from each small
309 mammal species per habitat and this is presented in Supplementary results and in **Figure S2**.
310
311 **(d) *Bartonella* prevalence in small mammals**
312 We detected *Bartonella* in five species of small mammals in the community: *R. satarae*, *R.*
313 *rattus*, *M. cf. fernandoni*, *M. cf. famulus,* and *S. niger* (**Table 1**) with varying prevalence (0-
314 75.8%). In both sites, *R. satarae* showed high prevalence (75.8% in Kadamane and 72% in
315 Kudremukh). *M. cf. fernandoni* showed 54.5% prevalence (n=22) in Kadamane and 40%

316 (n=5) in Kudremukh. Among the other two most abundant species in the landscape, *M. cf.*
317 *famulus* showed low prevalence in Kadamane (3.8%, n=26, only one individual positive) and
318 zero prevalence in Kudremukh (n=3), while *M. cf. terricolor* showed zero prevalence at both
319 sites despite large sample size (n =6 and 37 in Kadamane and Kudremukh, respectively).
320 Other species, such as *R. rattus* and *S. niger* had low prevalence, but also had low sample
321 sizes, hence we have minimal confidence in their prevalence estimates.
322
323 We did not observe any significant site level difference in prevalence for common species
324 (*R. satarae*, *M. cf. fernandoni*, *M. cf. famulus* and *M. cf. terricolor*) and between the overall
325 communities at both sites **(Figure 3A)**. Pooled ectoparasite positivity was largely correlated
326 with prevalence in small mammals, albeit slightly lower in every case. Positivity in specific
327 ectoparasite species is provided in **Table S3.**
328
329 **Table 1:** *Bartonella* prevalence in small mammal blood and ectoparasites. Prevalence
330 reported for ectoparasites is not true prevalence since ectoparasites were pooled for
331 screening (see methods).
332

	***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)

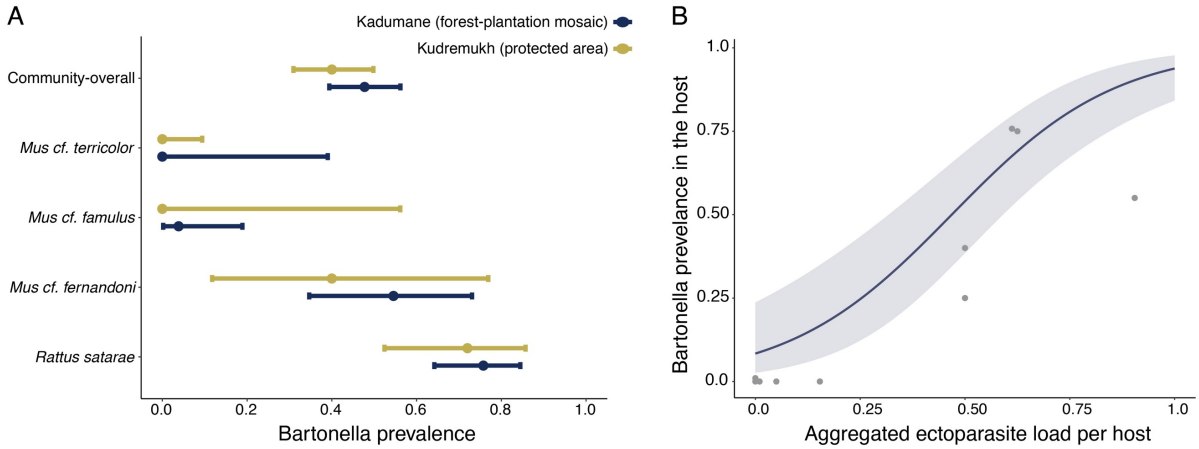


Figure 3: (A) *Bartonella* prevalence in the overall community and the four most abundant small mammals. (B) *Bartonella* prevalence positively correlated with aggregated ectoparasite densities within a small mammal species at each habitat site. The trend line indicates predicted *Bartonella* prevalence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

(e) Factors affecting *Bartonella* prevalence

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

350 **Table 2:** Results of generalised linear model (GLM) examining drivers of *Bartonella*
351 prevalence. Significant variables are marked with (*)
352

	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

353

354

355 **(f) Phylogenetic relationship**

356 Phylogenetic analysis (*rpoB*) revealed eight distinct *Bartonella* lineages (BL1-BL8) from small
357 mammals and ectoparasites. We found that five lineages among them (BL1-BL5) were
358 phylogenetically related to known zoonotic *Bartonella* species (**Figure 4**). We observed
359 three lineages in *R. satarae* (BL1, BL6, and BL7) and two lineages in *M. cf. fernandoni* (BL4
360 and BL8). BL1 included *Bartonella* sequences from *R. satarae* and ectoparasites (*Laelaps sp*
361 *1, Rhipicephalus sp, Ixodes sp, Xenopsylla sp 1, and Xenopsylla sp 5*). Interestingly,
362 sequences from *Laelaps sp 1* and *Xenopsylla sp 5* in this lineage were not collected from *R.*
363 *satarae* but *M. cf. fernandoni* and *S. niger*, respectively. BL2 consisted of a single sequence
364 collected from *R. rattus*, and closely related to BL1. These two lineages were
365 phylogenetically related to *B. queenslandensis*. BL3 consisted of two sequences, one from *S.*
366 *niger* and the other from *Laelaps sp 1* collected from *M. cf. fernandoni*. This lineage was
367 related to *B. tribocorum*. BL4 consisted of *Bartonella* sequences from *M. cf. fernandoni*, *M.*
368 *cf. famulus,* and ectoparasites (*Laelaps sp 1, Rhipicephalus sp, Ixodes sp, Haemaphysalis sp,*
369 *and Xenopsylla sp 3*). In this lineage, the *Haemaphysalis sp* was recovered from *R. satarae*
370 and not from any of the *Mus* species. BL5 was a single sequence from *F. tristriatus* (reported

371 in Ansil et al. 2021, accession number- MT787671), forming a sister lineage to BL4. We

372 included this sequence in our analysis since it was collected during our study in Kadamane.

373 The remaining lineages (BL6-BL8) were related to non-pathogenic *Bartonella* species. BL6

374 comprised sequences from *R. satarae* (second lineage from *R. satarae*) and *Ixodes sp*

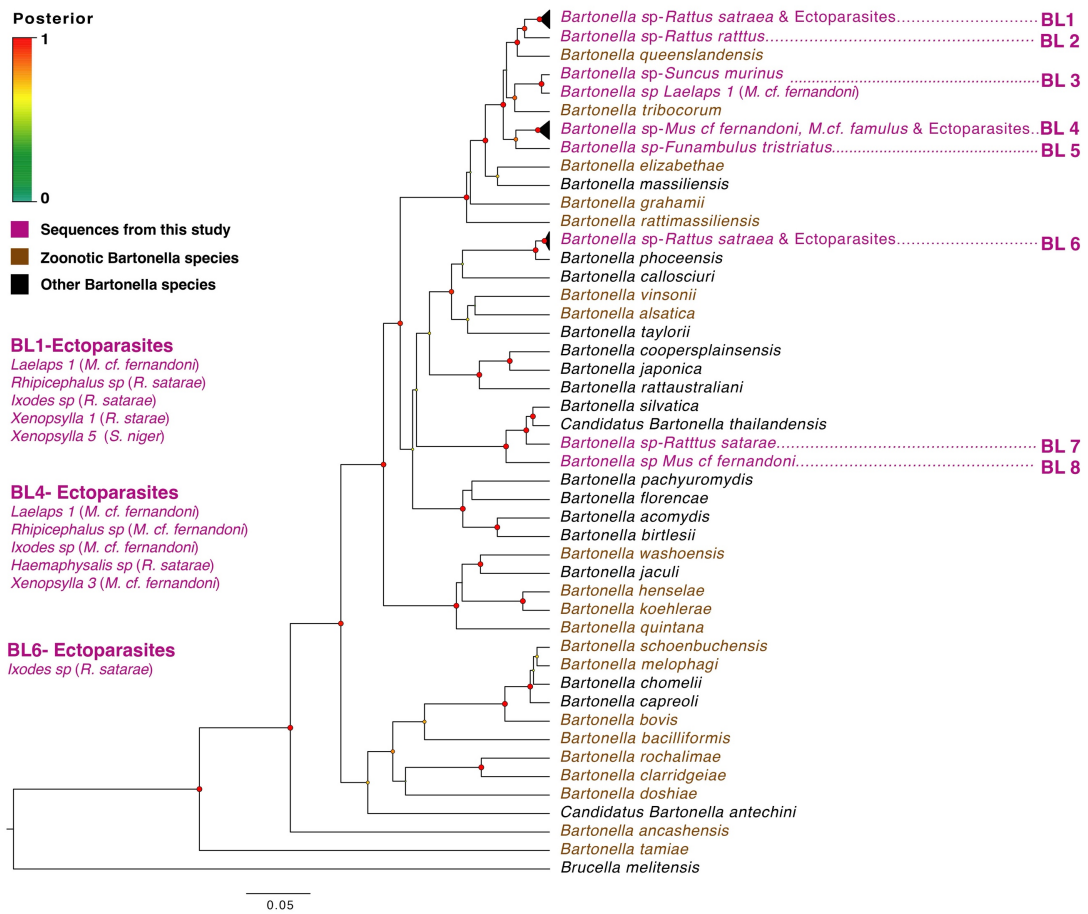
375 recovered from them. This lineage was related to *B. phoceensis*. BL7 and BL8 were two

376 distinct lineages from *R. satarae* (third lineage from *R. satarae*) and *M. cf. fernandoni*

377 (second lineage from *M. cf. fernandoni*), respectively. Both these lineages were

378 phylogenetically related to *B. thailandensis*.

379



380

381 **Figure 4:** Bayesian phylogenetic inference of *Bartonella* based on 773 bp of *rpoB* sequences.

382 Nodes are coloured based on posterior probability, and monophyletic clades are collapsed

383 for easy visualization. Ectoparasites part of collapsed lineages are expanded on the left side.

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

385 The co-phylogeny of small mammals and *Bartonella* revealed host-specific lineages **(Figure**
386 **5A)**. However, one *Bartonella* lineage from *M. cf. fernandoni* (BL8) was observed to be
387 phylogenetically related to *Bartonella* sequences from *R. satarae* (BL7). Our phylogenetic
388 ancestral trait reconstruction revealed a high probability (0.87) of *R. satarae* being the host
389 state of the common ancestor of these lineages **(Figure 5B)**, suggesting a potential past
390 spillover event. Similarly, we also observed a moderately high probability (0.73) of *R.*
391 *satarae* being the host state for the *Bartonella* sequence from *R. rattus* and the remote
392 common ancestor (0.8) of the *Bartonella* community level phylogeny. We found no
393 evidence for contemporary *Bartonella* spillover or sharing between small mammal hosts.
394

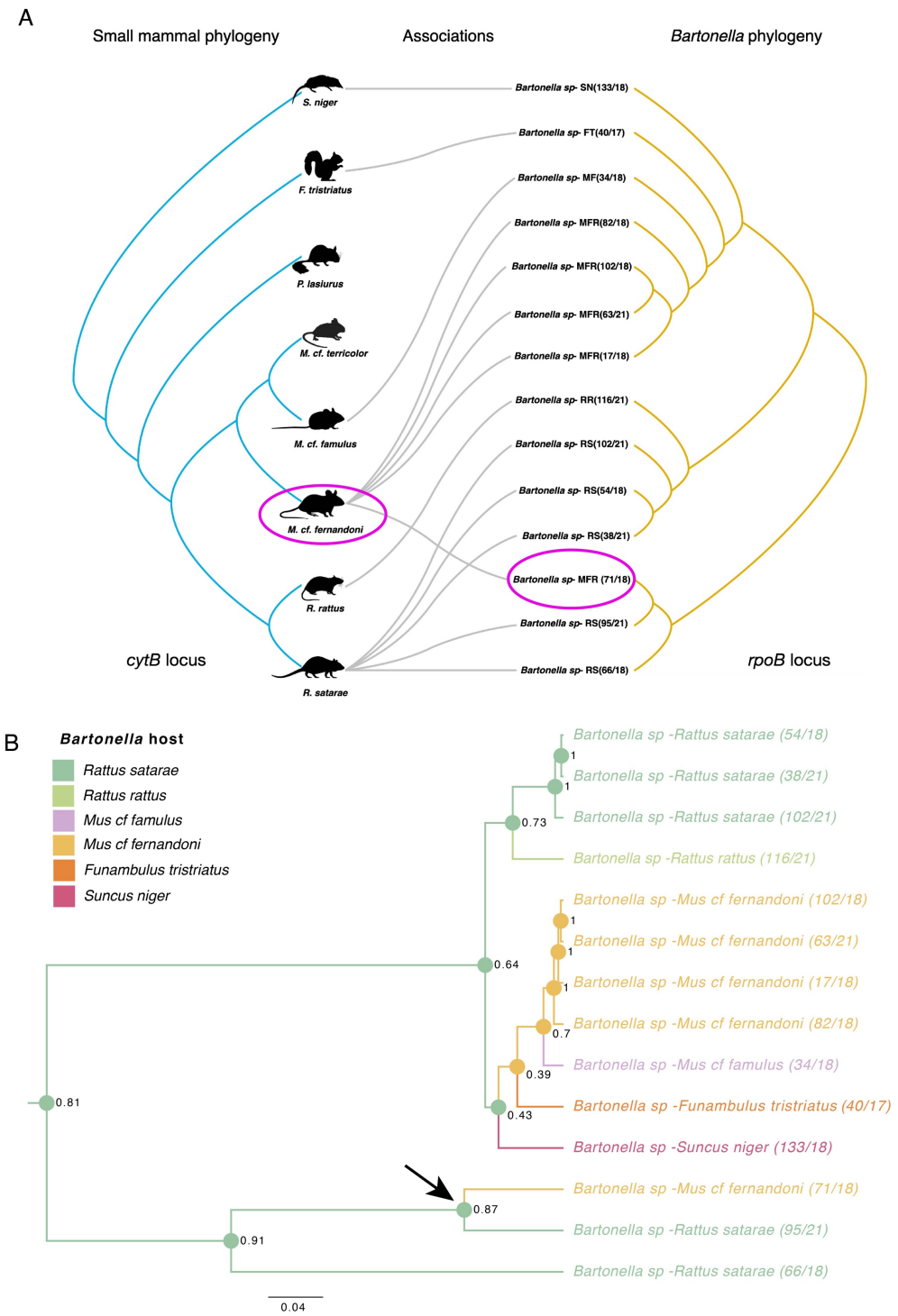


Figure 5: A) Co-phylogeny of small mammals and *Bartonella* association. One lineage of *M. cf. fernandoni* (BL8) was observed to have phylogenetic similarity with one of the *R. satarae* lineage (BL7). B) Bayesian phylogenetic inference showing ancestral host state of different *Bartonella* haplotypes.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

402 **4. Discussion**

403 We characterized small mammal communities, their ectoparasites, and associations with
404 *Bartonella* at two contrasting sites in the Western Ghats. We demonstrated that certain
405 small mammal host species occur across habitats, bringing them in contact with specialist
406 hosts with specific *Bartonella* lineages. We also demonstrate that ectoparasites can serve as
407 potential vectors, because *Bartonella* prevalence in the community is influenced by the
408 cumulative ectoparasite load in small mammals. Additionally, we present evidence of a
409 historic spillover event between two major hosts in the community and substantiate the
410 potential for future *Bartonella* sharing between hosts, including other domestic animals and
411 humans, possibly through ectoparasites.

412

413 **(a) Some small mammals have the potential to carry *Bartonella* across habitat boundaries**

414 We observed strong species-habitat associations in the focal small mammal community,
415 characterised by exclusive occurrences and high densities, mirroring several ecological
416 communities [52,53]. While species like *R. satarae* (a major *Bartonella* host with highest
417 prevalence) showed high abundance and nearly exclusive occurrence in forested habitats, *R.*
418 *rattus*, a known synanthropic species, was only detected in built-up areas, reaffirming its
419 commensal nature [54]. Similarly, *M. cf. terricolor* was exclusively recorded in grasslands in
420 both sites, albeit with higher density in Kudremukh. Other *Mus* species , *M. cf. fernandoni*
421 and *M. cf. famulus* were also abundant in grasslands, indicating a generalizable association
422 of *Mus* species with grassland ecosystems. Significantly from a disease ecology perspective,
423 few species like *M. cf. famulus* and *S. niger*, however, were recorded in multiple habitats,
424 including forests, grasslands, and built-up areas. Species like *M. cf. famulus* can also be
425 incidental hosts (as may be the case with the single positive), connecting potential reservoir

426 hosts like *R. satarae* and *M. cf. fernandoni*, with *R. rattus* (another seemingly incidental host
427 with a single positive) and humans. Similarly, *M. cf. fernandoni* was found to occur at low
428 densities in forests in addition to grasslands, bringing them in contact with *R. satarae*,
429 potentially paving the way for the historic spillover (discussed later) from *R. satarae* to *M.*
430 *cf. fernandoni*.
431
432 We did not find any notable difference between small mammal communities within
433 Kudremukh (protected area) and Kadamane (forest-plantation mosaic). One reason for this
434 might be that both landscapes (and much of the Western Ghats) have a long history of
435 human use, dampening differences that might exist between 'natural' and human
436 dominated areas. Within Kadamane, forests and rested plantations also had an analogous
437 community, dominated by *R. satarae.* [43]. Due to the similarity between these two
438 habitats, we treated them as functionally equivalent, enabling us to better estimate small
439 mammal densities and comprehensively explore habitat associations and *Bartonella*
440 prevalence. Interestingly, all *Mus* species showed variation in annual densities, indicating
441 annual population oscillations [55,56]. We could not fully capture this pattern due to the
442 relatively short duration of this study. However, understanding such variation in host
443 density is crucial as it is one of the determinants of 'zoonotic hazard', contributing to
444 spillover risk through its overlap with exposure and vulnerability of incidental hosts [57].
445 This reiterates the importance of detailed ecological studies on host communities to
446 understand spillover dynamics.
447
448 Our study suggests that small mammals in this part of the Western Ghats are in urgent need
449 of taxonomic revision. Previous studies from the landscape morphologically identified *M. cf.*

450 *fernandoni* as *M. musculus* and *M. cf. famulus* and *M. cf terricolor* as *M. booduga* [43].
451 Phylogenetic analysis and genetic distances suggest that these are distinct lineages related
452 to *M. fernandoni*, *M. famulus*, and *M. terricolor*, respectively. We urge researchers to
453 sample rodents in this landscape and resolve their taxonomy on priority, because pathogen
454 and habitat associations are often linked with species identity as we show in this study.
455
456 **(b) *Bartonella* prevalence and spillover may be mediated by ectoparasites**
457 Our results have revealed some broad ectoparasite-host associations that may have
458 implications for *Bartonella* maintenance and transmission. The observed patterns include
459 instances of polyparasitism, host sharing, and the influence of ectoparasitism on *Bartonella*
460 prevalence. In our dataset, ectoparasite diversity and density was centered around medium
461 to large-bodied, and synanthropic species. Specifically, *R. satarae* and *M. cf. fernandoni*, two
462 relatively large-bodied and abundant species with high *Bartonella* prevalence, displayed
463 polyparasitism, often with high densities of specific ectoparasites. This pattern aligns with
464 the positive correlation between body size and ectoparasitism observed in other studies
465 [58]. Potentially, habitat sharing of these species with other small mammals facilitates novel
466 ectoparasite acquisitions and driving overall ectoparasite richness on them [59,60].
467
468 Host sharing was another predominant pattern in the ectoparasite community. Several
469 ectoparasites (e.g., *Rhipicephalus sp*, *Xenopsylla sp 1*) showed a wider host range,
470 presumably due to their ability to infect multiple locally available hosts [61]. These results
471 are consistent with previous studies and indicate that host sharing among vectors is more
472 common than previously believed [62]. Such non-specific host-ectoparasite associations can

473 facilitate transmission of several pathogens, making their ecology a crucial part of vector-
474 borne pathogen dynamics [63].
475
476 Contrary to our more complex expectations based on host species identity, host density,
477 and disturbed/protected habitat, our results revealed ectoparasitism (aggregated
478 ectoparasite load per species) alone was the important predictor for prevalence.
479 Unfortunately, ecological drivers of aggregated ectoparasite load remain poorly understood.
480 As discussed earlier, longitudinal sampling targeting larger sample sizes from each
481 representative species in combination with ecological models incorporating additional
482 appropriate variables (e.g., host traits, season, etc.) might help explain variance in
483 ectoparasitism. We caution that other variables previously shown to influence pathogen
484 prevalence, like seasonality [9] and environmental stressors [64] were unavailable in our
485 study.
486
487 While we did not find the patterns we expected for *Bartonella* prevalence, our results are
488 significant from an ecological and epidemiological perspective. *Bartonella* is a vector-borne
489 pathogen known for its transmission through blood-feeding arthropods [65]. Hence,
490 elevated densities of infected ectoparasites in the environment (and on a specific host) can
491 increase transmission (density-dependant transmission) and impact prevalence and risk of
492 infection [66]. Polyparasitism and lack of host specialization suggests high chances of
493 *Bartonella* transmission between hosts through ectoparasites. In addition, known
494 synanthropic species such as *R. rattus* with multiple generalist fleas (occur in many hosts as
495 demonstrated in this study) pose risks of *Bartonella* transmission to humans and domestic
496 animals [65].

497

498 **(c) Non-specific ectoparasite-*Bartonella* associations and evidence of historic spillover**

499 **suggest future spillover risk**

500 Characterizing *Bartonella* diversity and its association with small mammals and

501 ectoparasites is crucial from a public health perspective, especially since many of the

502 *Bartonella* species are zoonotic and known to associate with small mammals and their

503 ectoparasites [67]. For instance, endemic *Bartonella* variants such as *B. bacilliformis*,

504 responsible for Carrion's disease, can be fatal and cause serious mortality [68]. Five (of

505 eight) *Bartonella* lineages identified in this study share a close evolutionary relationship with

506 known zoonotic species such as *B. queenslandensis* and *B. tribocorum*. Previous studies have

507 documented the widespread presence and global circulation of these *Bartonella* species,

508 primarily facilitated by small mammals and domestic animals [69]. However, it is important

509 to note that phylogenetic similarity alone does not confirm their ability to cause disease in

510 humans. Additional validation through molecular and *in vitro* experiments is warranted to

511 establish their pathogenicity in humans [70,71].

512

513 We observed contrasting patterns in *Bartonella* association with small mammals and

514 ectoparasites. In hosts, *Bartonella* displayed species-specific associations, as we expected

515 based on previous studies [43,72]. Remarkably, *Bartonella* showed non-specific association

516 with ectoparasites (e.g*., Xenopsylla sp* 5 collected from *S. niger* carried *Bartonella* associated

517 with *R. satarae*), leading to a higher overall diversity of haplotypes in specific ectoparasites

518 compared to their corresponding hosts. These complex patterns are not entirely

519 unprecedented, as they have been documented elsewhere [73]. Nevertheless, these non-

520 specific associations between *Bartonella* and ectoparasites have significance for cross-
521 species transmission, given vector competency.
522
523 Our phylogenetic ancestral trait reconstruction also highlighted the possibility for cross-
524 species transmission in the community. We observed *R. satarae* as the ancestral host for
525 several *Bartonella* lineages with a medium to high probability, indicating past spillover
526 events involving this species. This was evident between the BL7 and BL8 lineages, recovered
527 from *R. satarae* and *M. cf. fernandoni*, respectively. Interestingly, these two species stood
528 out as the primary *Bartonella* hosts in our dataset, displaying high prevalence, high
529 haplotype diversity, and co-occurrence in forested habitats. Such pathogen movement
530 between host species could provide an opportunity to evolve new virulent variants through
531 recombination [4], similar to those observed in other pathogen systems [6]. Although we
532 did not find instances of contemporary spillover, our results suggest past spillover events,
533 potentially through ectoparasites, emphasizing the likelihood of similar events. The
534 communities we studied include many small mammals and their ectoparasites that overlap
535 between habitats, increasing the likelihood of such inter-species transmissions [74]. In
536 addition, the movement of generalist hosts across multiple habitats carrying generalist
537 ectoparasites with non-specific *Bartonella* associations is likely to further drive pathogen
538 spillover in the landscape [75].
539

540 **5. Conclusion**

541 This study has uncovered several significant insights and nuances about the intricate
542 relationship between small mammal hosts, their ectoparasites, and bacterial agents within a
543 tropical mixed-use landscape. While our model system and investigation were specific to

544 *Bartonella*, the results and inferences extend to a broader spectrum of vector-borne
545 bacterial pathogens in multi-host communities. Our research highlights the crucial role of
546 ectoparasitism in driving *Bartonella* prevalence within these small mammal communities
547 where some hosts move across habitat boundaries. Our results also highlight the non-
548 specific association of ectoparasites with *Bartonella* diversity compared to their hosts,
549 underscoring the potential for ectoparasite-driven cross-species transmissions. Using
550 evolutionary analyses, we further demonstrate the role of historical spillover events in
551 shaping the current *Bartonella* distribution in the community and highlight the future
552 prospects of such spillover events. Collectively, our findings underscore the need for
553 comprehensive ecological studies to advance our understanding of the transmission
554 dynamics of vector-borne bacterial pathogens within multi-host communities and their
555 zoonotic implications, particularly in rapidly changing tropical landscapes.
556
557 **Ethics statement**
558 This study and associated protocols were approved by National Centre for Biological Science
559 Institutional Animal Ethics Committee (NCBS-IAEC-2016/10-[M], NCBS-IAE-2020/ 02[N]) and
560 Institutional Biosafety Committee (TFR:NCBS:23_IBSC/2017).
561
562 **Data accessibility**
563 The manuscript and Supplementary materials present small mammal capture data and
564 *Bartonella* prevalence data from small mammals and ectoparasites. *Bartonella* sequence
565 data generated during this study are deposited in GenBank under the accession numbers
566 OR574780-OR574826.
567

568 **Author contributions**

569 ABR: conceptualization, methodology, investigation, data curation, formal analysis,
570 visualization, project administration, writing -original draft, writing-review and editing; AV:
571 methodology, data curation, formal analysis, visualization, writing-review and editing; VR:
572 methodology, investigation, writing-review and editing; HMY: methodology, investigation,
573 writing-review and editing; AS: investigation, writing-review and editing; UR:
574 conceptualization, funding acquisition, project administration, supervision, writing-review
575 and editing.

576

577 **Acknowledgement**

578 This study was primarily supported by the Department of Atomic Energy, Government of
579 India (Project Identification RTI 4006) awarded to UR. We are grateful to Mr. K.M. Cariappa
580 of Kadamane for facilitating the field study; Karnataka Forests and Wildlife Department and
581 Kudremukh Wildlife Division for granting permission for small mammal sampling;
582 Kudremukh Iron Ore Company for providing accommodation in Kudremukh. We thank S.
583 Yashas, Vikrant Jathar, S. Vijayakumar, K. Kamaraj, Tamil Selvan, Subramanian, and C.
584 Karnan for their help in field sampling. We also acknowledge the support of NCBS Sanger
585 sequencing facility and museum and field Stations facility. ABR was supported by the SPM
586 fellowship by CSIR-HRDG, Government of India. ABR also acknowledges the support from
587 the Tata Institute for Genetics and Society during the manuscript preparation. AS was
588 supported by fellowship from the Trivedi School of Biosciences, Ashoka University.

589

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