

1 **Parasite-microbiota interactions potentially affect intestinal communities in wild mammals**

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14 **Summary**

15

16 Detecting interaction between species is notoriously difficult, and disentangling species associations in host-  
17 related gut communities is especially challenging. Nevertheless, due to contemporary methods, including  
18 metabarcoding and 16S sequencing, collecting observational data on community composition has become  
19 easier and much more common. We studied the previously collected data sets of intestinal microbiota and  
20 parasite compositions within longitudinally followed mouse lemurs by analysing the potential interactions  
21 with diversity metrics and novel joint species distribution modelling. Both methods showed consistent  
22 statistical association between certain parasite species and microbial composition. Both unicellular *Eimeria*  
23 sp. and cestode *Hymenolepis diminuta* had an effect on diversity of gut microbiota. These parasite species  
24 also had negative associations with several bacterial orders. In comparison, closely related species *H. nana*  
25 did not have an effect on diversity, and it had positive associations with several bacterial orders. Our results  
26 reveal potential interactions between some, but not all, intestinal parasites and gut microbiota. While  
27 environmental variables explained almost half of the total variation, of which almost half could be explained  
28 by traits of parasites and microbiota, there were no clear patterns regarding mouse lemur individual variables  
29 explaining variation in the occurrence patterns of parasite and microbiota significantly. Our results provide  
30 new hypothesis for interactions between and among parasites and microbiota to be tested further with  
31 experimental studies.

32 **Key words:** species distribution modelling, primates, *Microcebus*, helminths

### 33 **Introduction**

34 Interaction between species is one of the key determinants of the spatial and temporal dynamics of species  
35 communities (Ings *et al.* 2009). Communities within host individuals are no exception: the multitude of  
36 interactions between intestinal organisms, both beneficial and detrimental to their hosts, affect both ecology  
37 and evolution of these symbiont communities (Petney & Andrews 1998; Pedersen & Fenton 2007; Rigaud,  
38 Perrot-Minnot & Brown 2010; Glendinning *et al.* 2014).

39 Microbiota normally exceed macroscopic parasites in number, species diversity and biomass. Thus, it is not  
40 only plausible, but probable, that microbiota interacts with intestinal parasites in many ways, affecting both  
41 invasions of new parasite species and their ability to colonize the intestine and within-host dynamics of  
42 parasites (Hayes *et al.* 2010; Berrilli *et al.* 2012). Indeed, e.g., a nematode parasite of mice, *Trichuris muris*,  
43 requires microbiota interactions for successful establishment in host intestine (Hayes *et al.* 2010).

44 Interactions between different parasite species have been studied extensively in laboratory experiments  
45 (Graham 2008; Knowles 2011), but it is notoriously difficult to identify between-species interactions in  
46 observational studies (Fenton, Viney & Lello 2010; Fenton *et al.* 2014). While many studies have found  
47 random parasite assemblages indicating no interactions (Poulin 1996; Behnke 2008), some studies have also  
48 found interactions between parasites, both positive and negative (Lello *et al.* 2004). One successful way of  
49 combining an experimental approach to free-living host communities is community perturbation  
50 experiments, which have revealed parasite interactions (Knowles *et al.* 2013; Pedersen & Fenton 2015).

51 The experimental study of interactions between microbiota and parasites has become more feasible in recent  
52 years (Reynolds, Finlay & Maizels 2015). There has also been some observational studies both in humans  
53 and in wildlife which have looked into the interaction between microbiota and parasites — some studies  
54 have not found any microbiota changes on infection (Cooper *et al.* 2013; Baxter *et al.* 2015), while others did  
55 (Lee *et al.* 2014; Kreisinger *et al.* 2015; Baxter *et al.* 2015; Maurice *et al.* 2015). In all of the cases with  
56 significant changes, infections have been linked to higher diversity of microbiota, but the effects on bacterial  
57 OTUs have been parasite species specific.

58 We studied parasite and microbiota occurrence in free-living mammals longitudinally, acquiring several  
59 samples from same individuals to increase the reliability of the sampling. While both intestinal bacterial and  
60 parasite communities have been studied for a long time, new sequencing technologies have brought  
61 unprecedented resolution and coverage to identifying intestinal community composition. Nevertheless,  
62 intestinal communities have not been studied as a whole, but rather in different taxonomical groups. These  
63 groups relate to different research methods used to identify virome (Breitbart *et al.* 2003), microbiota  
64 (Eckburg *et al.* 2005), unicellular eukaryotes (Bass *et al.* 2015) or helminth communities (Tanaka *et al.*  
65 2014; Avelo *et al.* 2015). Our work combines two separate metabarcoding approaches, nematodes and  
66 microbiota, and supplements it with morphologically identified parasites: other helminths (cestodes),  
67 unicellular eukaryotes (*Eimeria*) and ectoparasites (lice and ticks).

68 Many community ecological questions, such as the strengths and directions of species interactions, require  
69 the joint analysis of organismal abundances, and if these organisms are identified using modern tools like  
70 metabarcoding, the number of taxa in a community can be in the thousands. Nevertheless, it is possible to  
71 fully specify joint statistical models by using multivariate extensions of generalized linear mixed models  
72 (Warton *et al.* 2015). Modern joint species distribution modelling approaches allow the study of association  
73 patterns between species, while also studying their environmental responses, and thus teasing the two apart.  
74 Latent variable models are an especially exciting tool that has recently been applied for ordination as well as  
75 studying the factors driving co-occurrence (Warton *et al.* 2015). In our study, we use a Bayesian hierarchical  
76 generalized linear modelling framework to analyse species environmental responses (Ovaskainen *et al.*  
77 2016a; b). Our models include latent variables, which model the residual co-occurrence patterns in our focal  
78 communities, quantifying hypothetical species association patterns, as well as specific parameters modelling  
79 the effects of species traits on the species responses to their environment, accounting also for phylogenetic  
80 relationships between the species (Abrego, Norberg & Ovaskainen 2016b).

81 Our aim was to explore associations within parasite species and between parasites and microbiota by mark-  
82 recapturing the wild rufous mouse lemur (*Microcebus rufus*) population and collecting fecal samples and  
83 related metadata. Our specific research questions were: 1) are parasites associated with each other, 2) are

84 different parasite species associated with different microbiota compositions, and 3) which, if any, host  
85 variables affect the parasite community and microbiota composition.

## 86 **Materials and methods**

### 87 *Sample collection*

88 We followed rufous mouse lemur (*Microcebus rufus*) population in Ranomafana National Park in  
89 southeastern Madagascar (21°16' S latitude and 47°20' E longitude). The national park consist of lowland to  
90 montane rainforest between 500 and 1500 meters of elevation. We collected samples and data for nematodes,  
91 cestodes, eimeriids and ectoparasites from September to December 2011 and 2012 and microbiota from  
92 September to December 2012 along previously described protocol (Aivelo *et al.* 2015; Aivelo, Laakkonen &  
93 Jernvall 2016) Shortly, we trapped mouse lemurs nightly on two transects, the first within the park  
94 boundaries in secondary forest and the second in peripheral zone in highly degraded campsite area. We  
95 measured mouse lemurs, collected samples and tagged previously unseen mouse lemurs for later  
96 identification with microchips. Animal handling procedures were approved by the trilateral commission  
97 (CAFF/CORE) in Madagascar (permits: 115/10/MEF/SG/DGF/DCB.SAP/SCBSE,  
98 203/11/MEF/SG/DGF/DCB.SAP/SCBSE and 203/12/MEF/SG/DGF/DCB.SAP/SCBSE) and the research  
99 ethics committee at Viikki campus, University of Helsinki.

100 We quantified the number of ectoparasites on mouse lemur ears. The fecal sample was divided into four  
101 parts: the first was used to identify nematodes, the second for microbiota analysis, the third for eimeriid  
102 quantification and the fourth for cestodes. We placed the cestode sample on flotation liquid (saturated  
103 MgSO<sub>4</sub> solution, c. 0.38 kg/l) and used McMaster's chamber for the quantification. We took photos on  
104 cestode eggs and identified the morphospecies. We placed eimeriids in open vials in 2.5% potassium  
105 dichromate solution to allow their sporulation, moved to flotation liquid 10 days later and quantified them.  
106 We took photos of the eimeriids for identification of morphospecies. The collection and analysis of  
107 nematode and microbiota data has been previously described in detail (Aivelo *et al.* 2015, 2016,  
108 respectively). Shortly, we isolated nematodes with Baermann's method, isolated their DNA and amplified a  
109 part of their 18S gene. These amplicons were sequenced with 454 sequencing (454 Life Sciences, Bradford,

110 CT, USA), grouped in operational taxonomic units with Séance pipeline (Medlar, Aivelo & Löytynoja 2014)  
111 and processed into putative parasite species. For microbiota, we amplified V1-V2 region of 16S gene,  
112 sequenced it with MiSeq (Illumina Inc., San Diego, CA, USA) and used mothur MiSeq SOP (Kozich *et al.*  
113 2013) to identify the OTUs.

#### 114 *Microbiota diversity*

115 We analysed the effects of different parasite species on both microbiota alpha diversity (as measured by  
116 inverse Simpson index) and richness (number of OTUs) by modelling these variables with different parasites  
117 species, host sex, trapping site, host age (divided into three age classes by protocol set by Zohdy *et al.* 2014),  
118 host condition (as in Rafalinirina *et al.* 2015), host aggressiveness, amplification batch, sequencing batch and  
119 sampling week as being explanatory variables. We used linear mixed models with the R package ‘nlme’  
120 (Pinheiro *et al.* 2013) with mouse lemur individual as random variable and started with the complete model.  
121 If the sequencing or amplification batches did not have a significant effect, we dropped them from the model  
122 as they are strongly correlated with sampling week. We also dropped non-significant variables one-by-one  
123 from the model to see if our models were robust. This did not affect which variables were statistically  
124 significant. We also explored the effects of parasites on beta diversity and included this analysis in Appendix  
125 2.

#### 126 *Joint species distribution modelling with latent variables*

127 We fitted a statistical joint species distribution model, combining information on environmental covariates,  
128 species traits and phylogenetic constraints, as well as the sampling study design. We fitted four models in  
129 total. Using only the parasite data for years 2011 and 2012, we fitted *i*) a model constrained with  
130 environmental covariates, species phylogenies and traits, and the sampling design included as latent  
131 variables, and *ii*) an unconstrained model, with only sampling unit level latent variable. Using the combined  
132 parasite and microbiota data for only 2012, we fitted *iii*) a model constrained with environmental covariates,  
133 species phylogenies and traits, and the sampling design as latent variables, and *iv*) an unconstrained model,  
134 with only sampling unit level latent variable. In all cases, we modelled the response community data matrix  
135 using the Bernoulli distribution and the probit link function. We fitted all the models with Bayesian

136 inference, using the posterior sampling scheme of Abrego *et al.* (2016b). More details and applications of the  
137 modelling framework used can be found also in (Abrego *et al.* 2016a; Ovaskainen *et al.* 2016a; b). We  
138 provide the the full description of the model, including assessment of model fit, in Appendix 3, as well as the  
139 prior distributions assumed in the Bayesian analysis. Below we describe the variables used in the different  
140 models.

#### 141 Parasites

142 For models *i)* and *ii)*, we used the presences and absences of the parasites found in the mouse lemurs during  
143 years 2011-2012 as the response matrix. For model *i)*, as environmental covariates we included the sex, age,  
144 aggressiveness and general condition of the lemurs, and with males we also accounted for the size of their  
145 testis (and considered females as individuals with extremely small testis size). We also included the time of  
146 sampling (week) and its quadratic form (week<sup>2</sup>) to account for the effect of seasonality. As species traits, we  
147 included whether the parasite has a direct or non-direct life cycle and whether it is an endo- or ectoparasite.  
148 In order to account for possible phylogenetic correlations in the species responses to their environment, we  
149 included species phylogenetic constraints in the model (for details, see Abrego *et al.* (2016b) and Appendix  
150 3). We constructed the phylogenetic relationships from the taxonomic tree with five levels: domain,  
151 kingdom, superphylum, phylum and species, assuming equal branch lengths. Finally, we included random  
152 effects, which also model the co-occurrence among species, at the levels of individual lemurs, transects and  
153 year of sampling, using a latent factor approach (Abrego *et al.* 2016b; Ovaskainen *et al.* 2016a).

#### 154 Microbiota and parasites combined

155 For models *iii)* and *iv)* we used the presences and absences of both parasites and microbiota found in the  
156 lemurs in year 2012 as the response matrix. To avoid overrepresentation of very rare OTUs, we considered  
157 only OTUs with >9 amplicons as presences. Then to avoid sequencing and OTU picking errors, we  
158 considered the OTUs present, if there were in total >99 amplicons in at least two lemur individuals. After  
159 this, we constructed the final response matrix as presence and absence at the level of orders. For model *iii)*,  
160 as environmental covariates we included the same characteristics of the lemurs as with the parasite model *i)*,  
161 and in addition, we included whether the taxon is a parasite or part of the microbiota and microbiota was

162 considered as having neither direct nor indirect life cycle. We constructed the phylogenetic relationships with  
163 five levels assuming equal branch lengths: domain, kingdom, phylum, class and order. Since the occurrences  
164 were modelled at the level of orders for the microbiota, but at the level of species for the parasites, we set the  
165 phylogenetic distance between the two hymenolepidid species in the phylogenetic correlation matrix  $C$  to  
166 0.99. We included latent random effects at the levels of individual lemurs and transects.

#### 167 Unconstrained models

168 As a point of comparison, for both data sets, we fitted unconstrained models *ii*) and *iv*), where we only  
169 included a sampling unit random effect, which models the variation in species occurrences and co-  
170 occurrences at the level of individual samples, obtained from individual lemurs, and no environmental  
171 covariates, phylogenetic constraints, nor traits. Thus, the variance across sampling units in the species  
172 responses is explained with the latent variables. By comparing the results for the constrained and  
173 unconstrained models, we can separate the associations that are solely due to the (dis)similar habitat  
174 requirements (e.g. when two species share the same habitat preferences, and hence co-occur more often than  
175 expected by random) or hidden by the (dis)similar habitat requirements (e.g. when two species share the  
176 same habitat preferences, but even after accounting for this, they still co-occur more often than expected by  
177 random) from the associations immune to the effects of the explanatory variables (i.e. we see the same  
178 association patterns regardless of the inclusion of the explanatory variables). This approach is analogous to  
179 comparing a constrained and an unconstrained ordination, with the difference of our approach being model-  
180 based (see e.g. Hui et al. 2015, Warton et al. 2015).

#### 181 Variance partitioning

182 Variance partitioning provides means to assess the explanatory power of different explanatory variables in  
183 relation to the same response variables, and hence give insight to which environmental variables are the most  
184 influential ones (Borcard, Legendre & Drapeau 1992). For the constrained models *i*) and *iii*), we partitioned  
185 the variation explained by the model into the part explained with fixed effects and random effects. Moreover,  
186 we separated among the fixed effects the variation explained with covariates related to lemurs and to



187 seasonality, as well as the share of variation explained by the traits. We also differentiated between the  
188 variation explained at different levels of random effects.

## 189 **Results**

190 We collected complete parasite and metadata for 281 samples in two years, 2011 and 2012, and combined  
191 parasite and microbiota data for 80 samples from 2012. Prevalences for different parasites varied from 1 to  
192 72% (Table 1). All observed lice were *Lemurpediculus verruculosus*, and all ticks belonged to  
193 *Haemaphysalis lemuris*. We identified cestodes based on shape of eggs to two distinct species *Hymenolepis*  
194 *diminuta* and *H. nana*. Two genotyped adult specimens also validated the identification of cestode species  
195 (Voitto Haukisalml, pers. comm.). Eimeriids belonged to one morphospecies and nematode putative species  
196 were grouped as in Aivelo et al. (2015).

197 Neither microbiota alpha diversity nor richness was related to host variables or most of the parasite  
198 presences. The only significant variable was *Eimeria* presence for both diversity (with significance of  $p =$   
199 0.038 and the coefficient: 7.8) and richness ( $p = 0.011$ , coef.: 24.5) (Figure 1). For beta diversity, *H.*  
200 *diminuta* and ectoparasites presence both had significant effects on two of the four metrics (Appendix 2).

201 All the parameter estimates, including associations between species, presented in the following chapters as  
202 ‘significant’ have statistical support based on the 90% central credible interval, unless otherwise stated. A  
203 positive association between two species means that they occur together more often than expected based on  
204 their (dis)similar habitat preferences and purely by random, whereas negative associations implies that they  
205 occur together less often than expected based on their habitat preferences or by random.

206 *Model i) and ii): responses to the environment and associations between parasites species*

207 In model i), all significant associations between parasite species at the level of individual lemurs were  
208 positive (Figure 2a). Cestode *Hymenolepis diminuta* had strong ( $R > 0.79$ , Appendix 4, Figure A2a) positive  
209 associations between putative nematodes species 1 and 2, which both had in turn particularly strong positive  
210 association with putative nematode species 4. *Eimeria* and *H. diminuta* had a strong association ( $R = 0.84$ ,  
211 Appendix 4, Figure A2b) at the level of transects. At the temporal level (Figure 2b), there were both negative

212 and positive associations, meaning that some parasites were co-occurring during the same year (positive) or  
213 occurring during different years (negative). These associations coincide with differences in parasite  
214 prevalence (Table 1): cestodes were less prevalent in 2011 whereas the prevalence of ectoparasites was more  
215 similar between years, with a high prevalence of lice and low of ticks.

216 There were a few significant explanatory variables for presences of parasite species. *Eimeria* was more  
217 probable to be present when the host lemur had better body condition (Appendix 4 Table A1). Lice and ticks  
218 were more probable to occur in males, while the occurrence probability of lice was negatively correlated  
219 with testis size. Both PS1 and PS4 were negatively correlated with higher age, whereas PS1 was also  
220 positively correlated with body condition. Neither the mode of parasite infection – indirect or direct – nor  
221 ecto- or endoparasitism significantly explained the differences in responses to parasite species.

222 In the unconstrained model ii), both the amount of significant associations and the amount of interactive  
223 species was the same as with the constrained model at the level of sampling units (Figure 2c) and years  
224 (Figure 2b). *Eimeria* and PS5 showed unconstrained association patterns with several nematode species and  
225 ticks, but these did not exist after accounting for their habitat requirements (Figure 2a-b). No associations  
226 changed directions: positive associations were positive in both models at all levels, as were the negative  
227 associations. All the significant constrained associations at the level of individual lemurs (Figure 2a) were  
228 also visible in the unconstrained model (Figure 2c), implying that some of the unconstrained associations  
229 were due to (dis)similar habitat requirements.

230 After partitioning the variation explained by the model i), the covariates related to the lemurs accounted for  
231 49% of the total variation explained by the model, whereas the covariates related to seasonality accounted for  
232 9.5% (Appendix 4, Fig. A3a). Species traits explained 46% of the total variation captured with fixed effects  
233 (which was 58.8% of the total variation explained by the model). Random effects accounted for 18% at the  
234 scale of lemurs, 9.8% at the scale of transects and 13% at the scale of years of the total variance explained by  
235 the model.

236 No traits had significant effects, but there was a strong phylogenetic signal in the species responses to their  
237 environment (0.92, see Appendix 3 for details).

238 *Model iii) and iv): responses to the environment and associations between parasites and microbiota*

239 There were three parasite species with significant associations with bacterial families at the individual mouse  
240 lemur level: *Eimeria* sp., *Hymenolepis diminuta* and *H. nana* (Figure 3). Whilst *Eimeria* and *H. diminuta* had  
241 a positive association ( $R = 0.79$ ; Appendix 4 Figure A2b), *H. nana* and *H. diminuta* had a negative  
242 association ( $R = -0.89$ ). Consequently, *H. diminuta* and *Eimeria* sp. had mostly negative associations with  
243 bacterial families ( $R = -0.85$  —  $-0.98$  and  $R = -0.78$  —  $-0.79$ , while the associations of *H. nana*  
244 positive ( $R = 0.83$ —  $0.90$ ). All bacterial families, which had positive or negative association with parasite  
245 species, had positive associations with each other ( $R = 0.79$ —  $0.94$ ). *Eimeria* had in addition two negative  
246 associations, with Lactobacillales and Pasteurellales. Only Enterobacteriales had negative association with  
247 *H. nana*, while it did not have significant positive association with *H. diminuta*. No explanatory variables  
248 were significant, except that of Anaeroplasmatales, which were more abundant in males, nor were there  
249 associations at the level of transects.

250 All the unconstrained associations (Figure 3b) were also visible with the constrained model (Figure 3a).  
251 There were fewer significant associations when the environmental covariates were not included in the model,  
252 meaning that some associations were not observable before removing the effect of the (dis)similar habitat  
253 requirements. Of all the parasites, only *H. diminuta* expressed unconstrained associations (Figure 3b). The  
254 negative associations between *H. diminuta* and several bacterial families were present regardless of the  
255 inclusion of the environmental constrains, but all the associations between *H. nana* and the bacterial families  
256 as well as its negative association with *H. diminuta* were not visible in the unconstrained associations. The  
257 species that exhibited any associations with the unconstrained model did so also with the constrained model,  
258 with the exception of *Eimeria* and *H. nana*. No associations changed directions between unconstrained and  
259 constrained models.

260 The covariates related to the lemurs accounted for 56% of the total variation explained, whereas the  
261 covariates related to seasonality accounted for 15% (Appendix 4 Fig. A3). Species traits explained 44% of  
262 the total variation explained with fixed effects (which was 71% of the total variation explained with the

263 model). Random effects accounted for 19% at the scale of lemurs, and 11% at the scale of transects of the  
264 variance explained by the model.

265 No traits had significant effects, but there was a strong phylogenetic signal in the species responses to their  
266 environment (with posterior mean 0.94; for more details, see Appendix 3).

## 267 **Discussion**

268 Our results show that some intestinal macroparasite species were associated more often than predicted by  
269 chance with other helminths, and that they are also associated with differences in microbiota composition.  
270 The presence of *Hymenolepis diminuta* – but not the closely related species *Hymenolepis nana* – is correlated  
271 with markedly different parasite and microbiota community (Figures 2, 3;. permutational manova: Appendix  
272 2). In more detail, *H. diminuta* presence is negatively associated with bacterial orders Enterobacteriales,  
273 Lactobacillales, Campylobacteriales, Pasteurellales, Bacilliales and Neisseriales (Figure 3), whilst it has  
274 positive association with nematode species, putatively *Strongyloides* and *Caenorhabditis* (Figure 2a). In  
275 addition, *Eimeria sp.* was positively associated with *H. diminuta* and thus also negatively associated with  
276 Pasteurellales and Lactobacillales (Figure 3). Surprisingly, host variables did not have significant effects on  
277 microbiota, while there were some significant variables affecting the parasite presence.

278 Two previous studies have looked specifically for the effect of *Hymenolepis sp.* infection: a laboratory study  
279 on rats and *H. diminuta* showed a reduction in Lactobacillales and Bacillales and increase in Bacteroidales  
280 and Clostridiales, while not showing differences in alpha and beta diversity (McKenney *et al.* 2015), and  
281 observational study on wild mice showed both increase and decrease OTUs belonging to Bacteroidales and  
282 Clostridiales (Kreisinger *et al.* 2015). Thus, our study is partly consistent with previously found results.  
283 Nevertheless, collating OTU data on order level can mask the changes in lower levels: if there has been  
284 actually both decrease and increase in different OTUs within an order, say Bacteroidales or Clostridiales,  
285 these might not show in upper taxonomic level.

286 The associations are not necessarily a sign of direct interactions (such as competition) between species, but  
287 they can also be driven by indirect causation, like host immune response (host type 2 immunity) towards  
288 parasites driving the changes in microbiota or microbiota immunomodulation affecting colonization success

289 of parasites (Ramanan *et al.* 2016). *Eimeria*, unlike other surveyed parasites, is a single-celled intracellular  
290 parasite, and the immune reaction normally is type 1 -biased (Cornelissen *et al.* 2009). Thus, it is possible,  
291 that *Eimeria* affects microbiota by direct competition, while others have indirect effects. *Eimeria* was the only  
292 parasite, which affected the alpha diversity of microbiota (Figure 1). In poultry *Eimeria* reduces the alpha  
293 diversity (Stanley *et al.* 2014; Wu *et al.* 2014), while it is less often studied in mammals. Evidence so far  
294 seems to indicate that they can either increase or decrease alpha diversity (Bär *et al.* 2015; Ras *et al.* 2015).

295 Our previous study has shown that there is pervasive within-individual variation in mouse lemur microbiota  
296 (Aivelo *et al.* 2016). Thus, it is understandable why we did not identify statistically significant host variables  
297 in microbiota variation (Appendix 4 Table A2). Our larger dataset, model i), on parasite occurrence did find  
298 host traits which affect parasite presence: better body condition seemed to lead to more probable infection  
299 with *Eimeria* and PS1 (putative *Strongyloides*) (Appendix 4 Table A1). This is in line with previous studies  
300 with mouse lemurs (Rafalinirina *et al.* 2007). Males more often had ectoparasites, which have been also  
301 previously noted (Durden, Zohdy & Laakkonen 2010; Zohdy *et al.* 2012), likely due to more common social  
302 interaction between males. Lice prevalence decreased with higher testis volume, which was a surprise, as  
303 higher testis volume correlates with higher testosterone levels which in turn can be immunocompromising  
304 (Zohdy 2012). Age seemed to lead to lower abundance for two nematode species, which might be caused for  
305 example by immunity acquisition (Turner & Getz 2010) or more infected individuals dying younger  
306 (Hayward 2013).

307 The associations between parasites within host individuals were positive in model i) (Figure 2a).  
308 *Hymenolepis diminuta* again had associations, though this time positive, with other parasite species, whereas  
309 *H. nana* did not have significant associations. This analysis did not find a negative association between *H.*  
310 *diminuta* and *H. nana*, nor a positive association between *H. diminuta* and *Eimeria* sp., due to having larger  
311 data set than in combined parasite and microbiota analysis (model iii). The year-level associations were  
312 positive between endoparasites on the one hand and ectoparasites on the other hand, while the associations  
313 between these groups were negative (Figure 2b). This indicates that endoparasites and ectoparasites have  
314 differing dynamics, i.e., when ectoparasites are more common, the endoparasites are less common and other  
315 way round. This means that some other factors not captured by our variables, can modulate the parasite

316 prevalence. These could be phenology of insects (Atsalis 2008), which are intermediate hosts for  
317 endoparasites, and ambient temperature, which affects sleeping patterns and thus nesting site sharing for  
318 mouse lemurs (Schmid & Ganzhorn 2009; Karanewsky & Wright 2015).

319 As we compare the constrained and unconstrained models, some of the associations are *a*) consistent  
320 throughout the levels of observation (such as the positive associations between *Hymenolepis diminuta* and  
321 some nematode genera exhibited at all levels of observation with the parasite data set (Figure 2a-c). This  
322 implies that these are strong associations not related to the (dis)similar habitat preferences of the species, as  
323 they are captured by the model regardless of whether these preferences are accounted for or not; or that there  
324 are some unmeasured habitat covariates, to which the species respond (dis)similarly, causing the pattern.  
325 Other associations are captured only with *b*) the unconstrained model (such as some of the associations  
326 between parasites [Figure 2c]), or *ic*) the constrained model (such as many of the year-level association of  
327 parasites [Figure 2b] as well as many of the associations between parasite species and bacterial families  
328 [Figure 2a]). The latter case (*c*) implies associations hidden by the (dis)similar responses to habitat, but  
329 revealed after these are accounted for, and the former (*b*) suggests associations that are due to these  
330 responses to habitat, and disappear after they are accounted for. The associations patterns captured in the  
331 manner of *a*) and *c*) can be considered as hypotheses for species interactions, as they are non-random co-  
332 occurrence patterns even after accounting for the habitat requirements of the species. Parasite-microbiota  
333 studies are further complicated by needing to take into account interactions with the host (Kreisinger *et al.*  
334 2015; Loke & Lim 2015). With the framework used in this study, it is not possible to account for the effects  
335 that the parasites and/or microbiome most certainly has on the host directly, but by using host characteristics  
336 as explanatory variables, we are controlling for some of the effects of the host on the parasites and  
337 microbiota.

338 Parasite and microbiota traits explained almost half of the variation among the species niches (environmental  
339 covariates; Appendix 4 Figure A3). This gives support for idea of niche conservatism (Mouillot *et al.* 2006),  
340 as the phylogenetic signal in the species responses to their environment was strong. Moreover, the  
341 phylogenetic relationships between the species and the covariance structure of the latent variables at the level  
342 of lemurs correlated with each other (Appendix 3), suggesting that there are some similar patterns in the

343 phylogenetic relationships and association between species, and this could be due to some unmeasured,  
344 phylogenetically conservative traits, which determine the species niches (in and on the lemurs).

345 General problems with fecal sampling also need to be taken into account while interpreting the results. For  
346 microbiota composition, the composition of fecal matter differ in different parts of the intestinal mucosa  
347 (Eckburg *et al.* 2005; Walk *et al.* 2010) and thus fecal sampling could give only a partial picture of what is  
348 happening in the gastrointestinal tract. Nevertheless, the helminth effect on microbiota is not confined to  
349 helminth niche as such (Kreisinger *et al.* 2015). Parasite prevalence cannot be definitely assessed by non-  
350 terminal sampling, as there could be parasites in the intestine, but they might not be laying eggs for a number  
351 of reasons (Gillespie 2006; Jorge *et al.* 2013). Our observational sampling also limits how strong statements  
352 we can give about causations within the system. Our model is further limited by the low prevalence of many  
353 of the parasites, including ticks and putative nematode species 3-6 (Table 1). This influences the estimation  
354 of the associations, which is sensitive to the rarity of focal taxa, whereas the estimation of the fixed effects is  
355 more robust, as information is being borrowed from other taxa (Ovaskainen & Soininen 2011; Abrego *et al.*  
356 2016b).

357 In conclusion, we found associations, which can be considered as hypothesis for interactions between  
358 intestinal parasites and gut microbiota in observational data set on wild-living mammal. While the presence  
359 of unicellular *Eimeria* was linked to a higher alpha diversity, the betadiversity was modulated by ectoparasite  
360 and cestode *Hymenolepis diminuta* presence. We also explored the use of novel joints species distribution  
361 modelling for identifying interactions between multiple parasite species and microbiota, and found differing  
362 responses on microbiota orders by closely related two cestode species and *Eimeria* sp. This investigation  
363 should be followed by experimental work in order to establish the causative reasons for variation in the  
364 microbiota.

#### 365 **Author contributions**

366 T.A. conceived and designed the study and collected and processed the samples, A.N. conducted the joint  
367 species distribution modelling and both authors analysed and interpreted the data and wrote the article.

#### 368 **Acknowledgments**

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374

### 375 **Data accessibility**

376 All metadata used has been uploaded to Figshare: doi: 10.6084/m9.figshare.3385573 (Aivelo & Norberg  
377 2016). These are also described in detail in Appendix 1. All sequence data has been deposited to SRA  
378 (accession numbers: SRP042187 and SRP063971) and the correspondence to samples can be found in  
379 metadata in Figshare. The code for beta diversity analysis is uploaded to GitHub:  
380 <https://github.com/aivelo/lemur-community> and the code for species modelling can be acquired from  
381 authors.

### 382 **References**

383 Abrego, N., Dunson, D., Halme, P., Salcedo, I. & Ovaskainen, O. (2016a) Wood-inhabiting fungi with tight  
384 associations with other species have declined as a response to forest management. *Oikos*, (**accepted**).

385 Abrego, N., Norberg, A. & Ovaskainen, O. (2016b) Measuring and predicting the influence of traits on the  
386 assembly processes of wood-inhabiting fungi. (**submitted**).

387 Aivelo, T., Laakkonen, J. & Jernvall, J. (2016) Population and individual level dynamics of intestinal  
388 microbiota of a small primate. *Applied and Environmental Microbiology*, AEM.00559–16.

389 Aivelo, T., Medlar, A., L ytynoja, A., Laakkonen, J. & Jernvall, J. (2015) Tracking year-to-year changes in  
390 intestinal nematode communities of rufous mouse lemurs (*Microcebus rufus*). *Parasitology*, **142**, 1095–  
391 1107.

392 Aivelo, T. & Norberg, A. (2016) Data from: Parasite-microbiota interactions potentially affect intestinal



- 393 communities in wild mammals.
- 394 Atsalis, S. (2008) *A Natural History of the Brown Mouse Lemur*. Pearson Education, Upper Saddle River,  
395 NJ, USA.
- 396 Bass, D., Stentiford, G.D., Littlewood, D.T.J. & Hartikainen, H. (2015) Diverse applications of  
397 environmental DNA methods in parasitology. *Trends in parasitology*, **31**, 499–513.
- 398 Baxter, N.T., Wan, J.J., Schubert, A.M., Jenior, M.L., Myers, P. & Schloss, P.D. (2015) Intra- and  
399 interindividual variations mask interspecies variation in the microbiota of sympatric *Peromyscus*  
400 populations. *Applied and Environmental Microbiology*, **81**, 396–404.
- 401 Behnke, J.M. (2008) Structure in parasite component communities in wild rodents: predictability, stability,  
402 associations and interactions .... or pure randomness? *Parasitology*, **135**, 751–766.
- 403 Berrilli, F., Di Cave, D., Cavallero, S. & D'Amelio, S. (2012) Interactions between parasites and microbial  
404 communities in the human gut. *Frontiers in Cellular and Infection Microbiology*, **2**, 141.
- 405 Borcard, D., Legendre, P. & Drapeau, P. (1992) Partialling out the Spatial Component of Ecological  
406 Variation. *Ecology*, **73**, 1045–1055.
- 407 Breitbart, M., Hewson, I., Felts, B., Mahaffy, J.M., Nulton, J., Salamon, P. & Rohwer, F. (2003)  
408 Metagenomic analyses of an uncultured viral Community from human feces. *Journal of Bacteriology*,  
409 **185**, 6220–6223.
- 410 Bär, A.K., Phukan, N., Pinheiro, J. & Simoes-Barbosa, A. (2015) The interplay of host microbiota and  
411 parasitic protozoans at mucosal interfaces: implications for the outcomes of infections and diseases.  
412 *PLoS Neglected Tropical Diseases*, **9**, e0004176.
- 413 Cooper, P., Walker, A.W., Reyes, J., Chico, M., Salter, S.J., Vaca, M. & Parkhill, J. (2013) Patent human  
414 infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in the faecal  
415 microbiota. *PloS one*, **8**, e76573.
- 416 Cornelissen, J.B.W.J., Swinkels, W.J.C., Boersma, W.A. & Rebel, J.M.J. (2009) Host response to

- 417 simultaneous infections with *Eimeria acervulina*, *maxima* and *tenella*: A cumulation of single  
418 responses. *Veterinary Parasitology*, **162**, 58–66.
- 419 Durden, L.A., Zohdy, S. & Laakkonen, J. (2010) Lice and ticks of the eastern rufous mouse lemur,  
420 *Microcebus rufus*, with descriptions of the male and third instar nymph of *Lemurpediculus*  
421 *verruculosus* (Phthiraptera: Anoplura). *The Journal of Parasitology*, **96**, 874–878.
- 422 Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E.  
423 & Relman, D.A. (2005) Diversity of the Human Intestinal Microbial Flora. *Science*, **308**, 1635–1638.
- 424 Fenton, A., Knowles, S.C.L., Petchey, O.L. & Pedersen, A.B. (2014) The reliability of observational  
425 approaches for detecting interspecific parasite interactions: Comparison with experimental results.  
426 *International Journal for Parasitology*, **44**, 437–445.
- 427 Fenton, A., Viney, M.E. & Lello, J. (2010) Detecting interspecific macroparasite interactions from ecological  
428 data: patterns and process. *Ecology letters*, **13**, 606–15.
- 429 Gillespie, T.R. (2006) Noninvasive assessment of gastrointestinal parasite infections in free-ranging  
430 primates. *International Journal of Primatology*, **27**, 1129–1143.
- 431 Glendinning, L., Nausch, N., Free, A., Taylor, D.W. & Mutapi, F. (2014) The microbiota and helminths:  
432 sharing the same niche in the human host. *Parasitology*, **141**, 1255–71.
- 433 Graham, A.L. (2008) Ecological rules governing helminth – microparasite coinfection. *Proceedings of*  
434 *National Academies of Sciences*, **105**, 566–570.
- 435 Hayes, K.S., Bancroft, A.J., Goldrick, M., Portsmouth, C., Roberts, I.S. & Grencis, R.K. (2010) Exploitation  
436 of the intestinal microflora by the parasitic nematode *Trichuris muris*. *Science*, **328**, 1391–1395.
- 437 Hayward, A.D. (2013) Causes and consequences of intra- and inter-host heterogeneity in defence against  
438 nematodes. *Parasite Immunology*, 362–373.
- 439 Hui, F.K.C., Taskinen, S., Pledger, S., Foster, S.D. & Warton, D.I. (2015) Model-based approaches to  
440 unconstrained ordination. *Methods in Ecology and Evolution*, **6**, 399–411.

- 441 Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F., Edwards, F., Figueroa,  
442 D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M., Olesen, J.M., van Veen, F.J.F.,  
443 Warren, P.H. & Woodward, G. (2009) Ecological networks--beyond food webs. *Journal of Animal*  
444 *Ecology*, **78**, 253–69.
- 445 Jorge, F., Carretero, M. a, Roca, V., Poulin, R. & Perera, A. (2013) What you get is what they have?  
446 Detectability of intestinal parasites in reptiles using faeces. *Parasitology Research*, **112**, 4001–4007.
- 447 Karanewsky, C.J. & Wright, P.C. (2015) A preliminary investigation of sleeping site selection and sharing  
448 by the brown mouse lemur *Microcebus rufus* during the dry season. *Journal of Mammalogy*, **96**, 1344–  
449 1351.
- 450 Knowles, S.C.L. (2011) The effect of helminth co-infection on malaria in mice: a meta-analysis.  
451 *International Journal for Parasitology*, **41**, 1041–51.
- 452 Knowles, S.C.L., Fenton, A., Petchey, O.L., Jones, T.R., Barber, R. & Pedersen, A.B. (2013) Stability of  
453 within-host – parasite communities in a wild mammal system. *Proceedings of the Royal Society B:*  
454 *Biological Sciences*, **280**.
- 455 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013) Development of a dual-  
456 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq  
457 Illumina sequencing platform. *Applied and Environmental Microbiology*, **79**, 5112–20.
- 458 Kreisinger, J., Bastien, G., Hauffe, H.C., Marchesi, J. & Perkins, S.E. (2015) Interactions between multiple  
459 helminths and the gut microbiota in wild rodents. *Philosophical Transactions of the Royal Society B:*  
460 *Biological Sciences*, **370**, 20140295.
- 461 Lee, S.C., Tang, M.S., Lim, Y. a L., Choy, S.H., Kurtz, Z.D., Cox, L.M., Gundra, U.M., Cho, I., Bonneau,  
462 R., Blaser, M.J., Chua, K.H. & Loke, P. (2014) Helminth colonization is associated with increased  
463 diversity of the gut microbiota. *PLoS Neglected Tropical Diseases*, **8**, e2880.
- 464 Lello, J., Boag, B., Fenton, A., Stevenson, I.R. & Hudson, P.J. (2004) Competition and mutualism among the  
465 gut helminths of a mammalian host. *Nature*, **428**, 20–24.

- 466 Loke, P. & Lim, Y. a L. (2015) Helminths and the microbiota: parts of the hygiene hypothesis. *Parasite*  
467 *Immunology*, **37**, 314–23.
- 468 Maurice, C.F., Cl Knowles, S., Ladau, J., Pollard, K.S., Fenton, A., Pedersen, A.B. & Turnbaugh, P.J. (2015)  
469 Marked seasonal variation in the wild mouse gut microbiota. *The ISME journal*, 1–12.
- 470 McKenney, E.A., Williamson, L., Yoder, A.D., Rawls, J.F., Bilbo, S.D. & Parker, W. (2015) Alteration of  
471 the rat cecal microbiome during colonization with the helminth *Hymenolepis diminuta*. *Gut Microbes*,  
472 **6**, 182–193.
- 473 Medlar, A., Aivelo, T. & Löytynoja, A. (2014) Séance □: reference-based phylogenetic analysis for 18S  
474 rRNA studies. *BMC Evolutionary Biology*, **14**.
- 475 Mouillot, D., Krasnov, B.R., Shenbrot, G.I., Gaston, K.J. & Poulin, R. (2006) Conservatism of host  
476 specificity in parasites. *Ecography*, **4**, 596–602.
- 477 Ovaskainen, O., Abrego, N., Halme, P. & Dunson, D. (2016a) Using latent variable models to identify large  
478 networks of species-to-species associations at different spatial scales. *Methods in Ecology and*  
479 *Evolution*, **7**, 549–555.
- 480 Ovaskainen, O., Roy, D.B., Fox, R. & Anderson, B.J. (2016b) Uncovering hidden spatial structure in species  
481 communities with spatially explicit joint species distribution models. *Methods in Ecology and*  
482 *Evolution*, **7**, 428–436.
- 483 Ovaskainen, O. & Soininen, J. (2011) Making more out of sparse data: hierarchical modeling of species  
484 communities. *Ecology*, **92**, 289–295.
- 485 Pedersen, A.B. & Fenton, A. (2007) Emphasizing the ecology in parasite community ecology. *Trends in*  
486 *Ecology & Evolution*, **22**, 133–9.
- 487 Pedersen, A.B. & Fenton, A. (2015) The role of antiparasite treatment experiments in assessing the impact of  
488 parasites on wildlife. *Trends in Parasitology*, **31**, 200–211.
- 489 Petney, T.N. & Andrews, R.H. (1998) Multiparasite communities in animals and humans: frequency ,

- 490 structure and pathogenic significance. *International Journal for Parasitology*, **28**, 377–393.
- 491 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Team, R.C. (2013) nlme: Linear and Nonlinear Mixed  
492 Effects Models. , R package version 3.1–113.
- 493 Poulin, R. (1996) Richness, nestedness , and randomness in parasite infracommunity structure. *Oecologia*,  
494 **105**, 545–551.
- 495 Rafalinirina, H.A., Aivelo, T., Wright, P.C. & Randrianasy, J. (2007) Comparison of parasitic infections and  
496 body condition in rufous mouse lemurs (*Microcebus rufus*) at Ranomafana National Park, southeast  
497 Madagascar. *Madagascar Conservation & Development*, **1**, 60–66.
- 498 Rafalinirina, H.A., Aivelo, T., Wright, P.C. & Randrianasy, J. (2015) Comparison of parasitic infections and  
499 body condition in rufous mouse lemurs ( *Microcebus rufus* ) at Ranomafana National Park, southeast  
500 Madagascar. *Madagascar Conservation & Development*, **10**, 60–66.
- 501 Ramanan, D., Bowcutt, R., Lee, S.C., Tang, M.S., Kurtz, Z.D., Ding, Y., Honda, K., Gause, W.C., Blaser,  
502 M.J., Bonneau, R.A., Lim, Y. AL, Loke, P. & Cadwell, K. (2016) Helminth infection promotes  
503 colonization resistance via type 2 immunity. *Science*, **3229**.
- 504 Ras, R., Huynh, K., Desoky, E., Badawy, A. & Widmer, G. (2015) Perturbation of the intestinal microbiota  
505 of mice infected with *Cryptosporidium parvum*. *International Journal for Parasitology*, **45**, 567–573.
- 506 Reynolds, L. a, Finlay, B.B. & Maizels, R.M. (2015) Cohabitation in the intestine: interactions among  
507 helminth parasites, bacterial microbiota, and host immunity. *Journal of Immunology*, **195**, 4059–66.
- 508 Rigaud, T., Perrot-Minnot, M.-J. & Brown, M.J.F. (2010) Parasite and host assemblages: embracing the  
509 reality will improve our knowledge of parasite transmission and virulence. *Proceedings of the Royal  
510 Society B: Biological Sciences*, **277**, 3693–3702.
- 511 Schmid, J. & Ganzhorn, J.U. (2009) Optional strategies for reduced metabolism in gray mouse lemurs. *Die  
512 Naturwissenschaften*, **96**, 737–41.
- 513 Stanley, D., Wu, S.B., Rodgers, N., Swick, R.A. & Moore, R.J. (2014) Differential responses of cecal

- 514 microbiota to fishmeal, Eimeria and Clostridium perfringens in a necrotic enteritis challenge model in  
515 chickens. *PLoS ONE*, **9**, e104739.
- 516 Tanaka, R., Hino, A., Tsai, I.J., Palomares-Rius, J.E., Yoshida, A., Ogura, Y., Hayashi, T., Maruyama, H. &  
517 Kikuchi, T. (2014) Assessment of helminth biodiversity in wild rats using 18S rDNA based  
518 metagenomics. *PloS one*, **9**, e110769.
- 519 Turner, W.C. & Getz, W.M. (2010) Seasonal and demographic factors influencing gastrointestinal parasitism  
520 in ungulates of Etosha National Park. *Journal of Wildlife Diseases*, **46**, 1108–1119.
- 521 Walk, S.T., Blum, A.M., Ewing, S.A.-S., Weinstock, J. V & Young, V.B. (2010) Alteration of the murine  
522 gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflammatory*  
523 *Bowel Diseases*, **16**, 1841–9.
- 524 Warton, D.I., Blanchet, F.G., O’Hara, R.B., Ovaskainen, O., Taskinen, S., Walker, S.C. & Hui, F.K.C.  
525 (2015) So many variables: joint modeling in community ecology. *Trends in Ecology & Evolution*, **30**,  
526 766–779.
- 527 Wu, S.B., Stanley, D., Rodgers, N., Swick, R.A. & Moore, R.J. (2014) Two necrotic enteritis predisposing  
528 factors, dietary fishmeal and Eimeria infection, induce large changes in the caecal microbiota of broiler  
529 chickens. *Veterinary Microbiology*, **169**, 188–197.
- 530 Zohdy, S. (2012) *Senescence Ecology: Aging in a Population of Wild Brown Mouse Lemurs ( Microcebus*  
531 *Rufus )*. University of Helsinki.
- 532 Zohdy, S., Gerber, B.D., Tecot, S., Blanco, M.B., Winchester, J.M., Wright, P.C. & Jernvall, J. (2014) Teeth,  
533 sex, and testosterone: aging in the world’s smallest primate (ed KE Samonds). *PLoS One*, **9**, e109528.
- 534 Zohdy, S., Kemp, A.D., Durden, L. a, Wright, P.C. & Jernvall, J. (2012) Mapping the social network:  
535 tracking lice in a wild primate (*Microcebus rufus*) population to infer social contacts and vector  
536 potential. *BMC Ecology*, **12**, 4.
- 537

538 **Tables**

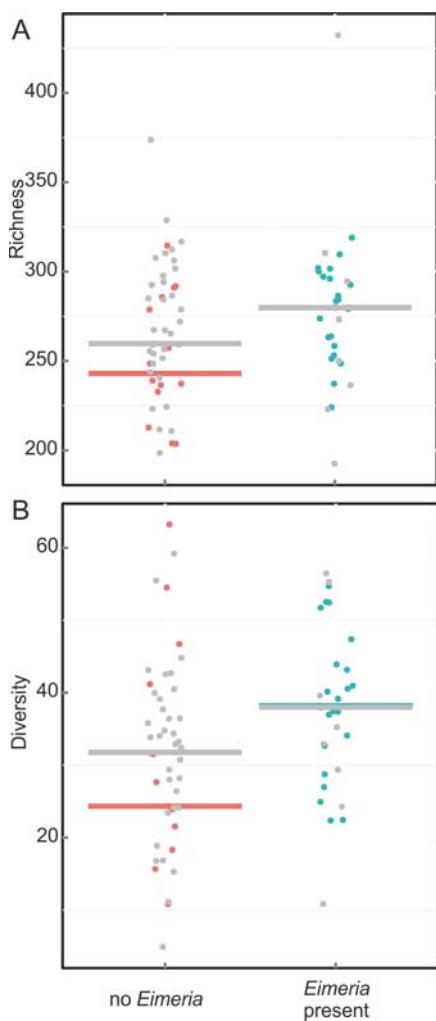
539 Table 1: Prevalence of different parasites in two years, including prevalence and absolute numbers of  
540 infected mouse lemur samples. The names of putative nematode species are based on Aivelo et al. (2015). In  
541 2011 we had a total of 100 samples from 44 individuals, while in 2012 we had 181 samples from 57  
542 individuals.

		2011		2012	
		Prevalence (%)	n	Prevalence (%)	n
Nematodes	PS1 (“ <i>Strongyloides</i> ”)	34	34	72	131
	PS2 (“ <i>Caenorhabditis</i> ”)	14	14	42	76
	PS3 (“Strongylida”)	2	2	1	2
	PS4 (“ <i>Chromadorea</i> ”)	1	1	9	16
	PS5 (“ <i>Enterobius</i> ”)	3	3	4	8
	PS6 (“ <i>Panagrellus</i> ”)	1	1	5	1
	Total	36	36	73	133
Cestodes	<i>Hymenolepis diminuta</i>	7	7	30	55
	<i>Hymenolepis nana</i>	10	10	22	39
	Total	17	17	51	93
Eimeria sp.		15	15	30	54
Ectoparasites	Lice	45	45	25	46
	Ticks	6	6	0.5	1
	Total	47	47	25	46

543

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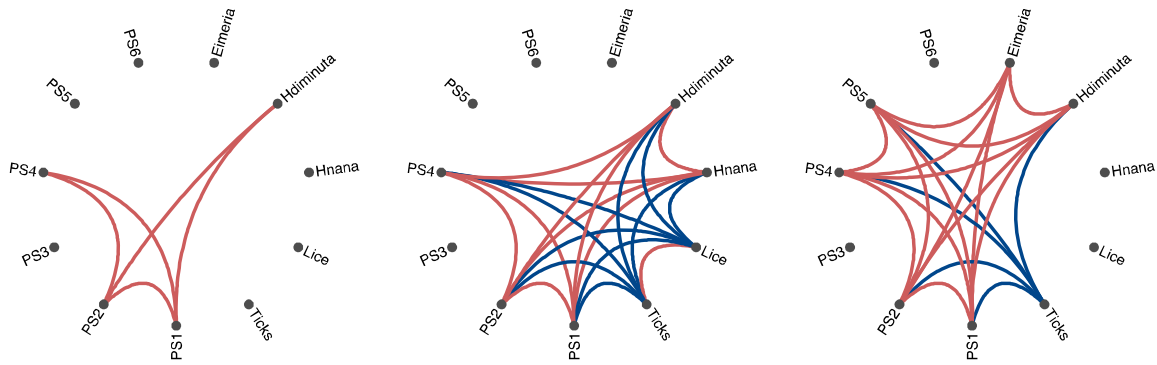
545 **Figures**



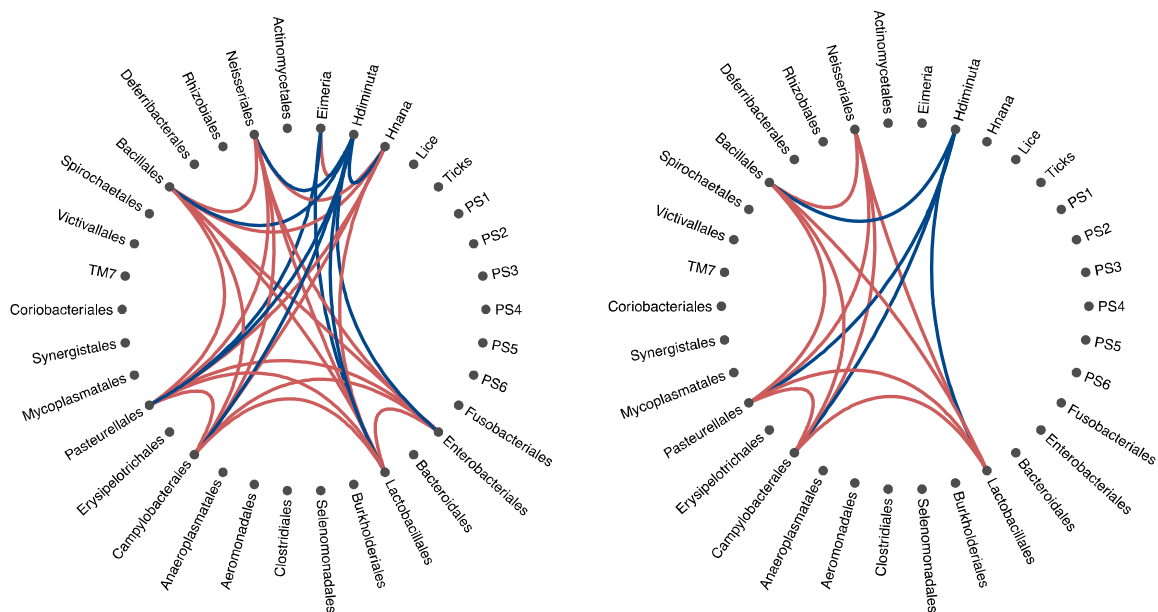
546

547 Figure 1: a) Richness and b) inverse Simpson diversity of microbiota in mouse lemurs with and without  
548 Eimeria infection. Red and blue colors represent individuals (n=11) which had both negative and positive  
549 detections of Eimeria infection, while grey points represent other individuals. The horizontal lines are  
550 medians for multiple-sampled individuals and for all individuals.





551  
552 Figure 2: Associations at the level of a) individual lemurs and b) years between different parasite species for  
553 the constrained model; and c) associations at the level of individual samples for the unconstrained model.  
554 Red lines denote positive associations and blue lines negative associations, all with significant statistical  
555 support based on the 90% central credible interval.



556  
557 Figure 3: Associations at the level of a) individual lemurs for the constrained model and b) at the level of  
558 individual samples for the unconstrained model. Red lines denote positive associations and blue lines  
559 negative associations, all with significant statistical support based on the 90% central credible interval.