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Co-infections and environmental conditions drive the distributions of blood parasites in wild birds

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2 in wild birds

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24 **Abstract**

25 1. Patterns of pathogen co-occurrence can affect the spread or severity of disease.

26 Yet due to difficulties distinguishing and interpreting co-infections, evidence
27 for the presence and directionality of pathogen co-occurrences in wild hosts is
28 rudimentary.

29 2. We provide empirical evidence for pathogen co-occurrences by analysing
30 infection matrices for avian malaria (*Haemoproteus* and *Plasmodium* spp.)
31 and parasitic filarial nematodes (microfilariae) in wild birds (New Caledonian
32 *Zosterops* spp.).

33 3. Using visual and genus-specific molecular parasite screening, we identified
34 high levels of co-infections that would have been missed using PCR alone.
35 Avian malaria lineages were assigned to species level using morphological
36 descriptions. We estimated parasite co-occurrence probabilities, while
37 accounting for environmental predictors, in a hierarchical multivariate logistic
38 regression.

39 4. Co-infections occurred in 36% of infected birds. We identified both positive
40 and negatively correlated parasite co-occurrence probabilities when
41 accounting for host, habitat and island effects. Two of three pairwise avian
42 malaria co-occurrences were strongly negative, despite each malaria parasite
43 occurring across all islands and habitats. Birds with microfilariae had elevated
44 heterophil to lymphocyte ratios and were all co-infected with avian malaria,
45 consistent with evidence that host immune modulation by parasitic nematodes
46 facilitates malaria co-infections. Importantly, co-occurrence patterns with
47 microfilariae varied in direction among avian malaria species; two malaria

48 parasites correlated positively but a third correlated negatively with
49 microfilariae.

50 5. We show that wildlife co-infections are frequent, possibly affecting infection
51 rates through competition or facilitation. We argue that combining multiple
52 diagnostic screening methods with multivariate logistic regression offers a
53 platform to disentangle impacts of environmental factors and parasite co-
54 occurrences on wildlife disease.

55

56 Key words: avian malaria, *Haemoproteus*, heterophil to lymphocyte ratio, filarial
57 parasite, immune modulation, parasite co-occurrence

58

59 **Introduction**

60 How pathogens are distributed and how changing environments cause disease spill-
61 over across species or geographic barriers are key questions in ecology (Wood *et al.*
62 2012; Hoberg & Brooks 2015; Plowright *et al.* 2015; Wells *et al.* 2015). While the
63 environment undoubtedly influences pathogen infections (Budria & Candolin 2013;
64 Sehgal 2015), hosts often carry multiple pathogens whose interactions can alter
65 infection dynamics (Cattadori, Boag & Hudson 2008; Johnson & Hoverman 2012).
66 Infection with one pathogen can increase a host's susceptibility to other pathogens or
67 to harmful disease (Bordes & Morand 2011). For example, chickens infected with
68 *Staphylococcus aureus* bacteria develop more severe disease when inoculated with
69 influenza than those without co-occurring bacteria (Kishida *et al.* 2004). Pathogen
70 interactions might also be antagonistic. In leaf-cutting ants, competition between
71 fungal pathogen strains leads to decreased overall pathogen transmission (Hughes *et*
72 *al.* 2004). Yet while interactions such as competition and facilitation form the

73 foundations of ecology (Dayton 1971), detecting wildlife pathogen associations is
74 challenging due to (1) difficulties distinguishing co-infections (Valkiūnas *et al.* 2006;
75 Tompkins *et al.* 2011) and (2) a lack of statistical approaches to disentangle impacts
76 of environmental predictors (Muturi *et al.* 2008; Fenton *et al.* 2014). Hierarchical
77 multivariate approaches overcome this hurdle by assessing both environmental
78 influences and interspecific co-occurrences in joint distribution models (Ovaskainen,
79 Hottola & Siitonen 2010; Kissling *et al.* 2012). We use one such tool, multivariate
80 logistic regression, to describe the presence and directionality of blood parasite co-
81 occurrences in wild birds.

82 Haematozoan blood parasites, including haemosporidians (*Plasmodium* and
83 *Haemoproteus* spp.; collectively referred to here as ‘malaria’ parasites to avoid
84 confusing ‘haemosporidian’ and ‘haematozoan’) and microfilaria (blood stages of
85 filarial nematodes), are vector-transmitted parasites that often exist in co-infection
86 (Bush 2001; Atkinson, Thomas & Hunter 2008; Clark, Clegg & Lima 2014). Because
87 both parasites are important disease agents, understanding factors that drive their
88 transmission and occurrence is vital to unravel their impacts on hosts (Muturi *et al.*
89 2008; Griffiths *et al.* 2015). Haematozoans are strongly driven by environmental
90 factors, such as temperature and habitat, that can limit parasite development or vector
91 distributions (Rogers *et al.* 2002; Santiago-Alarcon, Palinauskas & Schaefer 2012;
92 Freed & Cann 2013; Sehgal 2015). However, haematozoan infections may also be
93 influenced by biotic parasite interactions (Su *et al.* 2005; Telfer *et al.* 2010).
94 Experimental work in mammals shows that parasitic nematodes can modulate
95 immune responses of hosts by depressing antigen-recognising lymphocytes while
96 increasing neutrophils, potentially increasing concomitant malaria transmission
97 (Nacher *et al.* 2001; Graham *et al.* 2005; Su *et al.* 2005; Muturi *et al.* 2008).

98 Competition between malaria strains can also occur and is likely to influence within-
99 host progression (Bell *et al.* 2006). Yet despite increasing evidence for parasite
100 associations in model mammalian hosts (Telfer *et al.* 2010; Fenton *et al.* 2014),
101 evidence from non-model hosts is primarily experimental and remains limited by a
102 paucity of co-infection data (Jackson *et al.* 2006; Knowles 2011; Tompkins *et al.*
103 2011).

104 We assess the importance of environmental variables and interspecific
105 associations on haematozoan parasite occurrences in four avian species (family
106 Zosteropidae) in New Caledonia. We examine a possible mechanism for within-host
107 parasite interactions by asking if infections result in altered host immune profiles.
108 Birds are an ideal study system as avian haematozoans are common and co-infections
109 are abundant (Sehgal, Jones & Smith 2005; Atkinson, Thomas & Hunter 2008;
110 Marzal *et al.* 2011; Marzal 2012; Oakgrove *et al.* 2014; van Rooyen *et al.* 2014; Lutz
111 *et al.* 2015; Goulding *et al.* 2016). In New Caledonia, *Zosterops* spp. are commonly
112 infected with a diversity of avian malaria parasites (Ishtiaq *et al.* 2010; Olsson-Pons *et*
113 *al.* 2015). Possible associations between *Zosterops* spp. and filarial parasites have not
114 been studied.

115 Based on evidence for parasite competition in mammals (Bell *et al.* 2006;
116 Telfer *et al.* 2010; Hellard *et al.* 2015), we predicted that distinct avian malaria
117 parasites would exhibit negatively correlated infection probabilities when accounting
118 for environmental drivers, indicating possible parasite competition. We predicted that
119 malaria species would positively correlate with microfilaria, based on experimental
120 evidence that immune-modulating nematodes can facilitate malaria co-infections
121 (Druilhe, Tall & Sokhna 2005; Su *et al.* 2005).

122

123 **Methods**124 *Field sampling and laboratory methods*

125 New Caledonia is a sub-tropical Pacific archipelago consisting of four main islands
126 (Fig. 1a). The archipelago supports four *Zosterops* spp., including the regionally
127 widespread *Z. lateralis*, the New Caledonian endemic *Z. xanthochrous*, and two
128 single-island endemics, *Z. minutus* and *Z. inornatus* (both of which only occur on the
129 island of Lifou; Dutson 2012). All four species are omnivorous passerines that occur
130 in mixed-species flocks. We captured *Zosterops* spp. with mistnets on the four main
131 islands from Jan. to March 2014. Sites were chosen to represent the three primary
132 forested habitats in New Caledonia, namely dry lowland forest (Grand Terre, Ouvéa),
133 lowland rainforest (Ouvéa, Lifou and Maré) and montane rainforest (Grand Terre; see
134 supporting information Fig. S1 for site map). Blood samples were collected from each
135 bird ($n = 275$). Blood smears were also taken for 245 birds.

136 Avian malaria PCR screening and sequencing followed Clark *et al.* (2015),
137 with the following variations. Sequences suggested amplification bias towards
138 *Plasmodium* spp. when co-occurring with *Haemoproteus* spp., with clean
139 *Plasmodium* sequences (i.e. absence of double peaks) retrieved from 16 confirmed
140 *Plasmodium*/*Haemoproteus* co-infections (see below for smear screening). Eight
141 known co-infections produced *Haemoproteus* sequences, while a further six produced
142 double peaks (re-sequencing of all six producing clean *Plasmodium* sequences).
143 *Haemoproteus* lineages were therefore characterised using genus-specific primers
144 designed from sequences recovered in Australasian hosts (Clark & Clegg 2015; Clark,
145 Clegg & Klaassen 2016). These primers successfully amplified *Haemoproteus* DNA
146 from all visually observed *Plasmodium*/*Haemoproteus* co-infections. A Bayesian
147 phylogeny was constructed to estimate malaria relationships, following Clark *et al.*

148 (2015). For malaria lineages presenting all developmental stages in corresponding
149 single-infection smears, we identified parasites to species (see supporting information
150 for parasite identifications). For microfilaria, we screened samples by amplifying
151 782bp of the parasite large subunit rDNA. GenBank accessions for parasite lineages
152 are XXXX and XXXX, respectively. Malaria lineages are also deposited in the
153 MalAvi database (Bensch, Hellgren & Pérez-Tris 2009). PCR protocols, phylogenetic
154 methods and the malaria consensus phylogeny (Fig. S2) are presented in supporting
155 information.

156 The proportion of heterophils (avian equivalent of neutrophils) relative to
157 lymphocytes (heterophil to lymphocyte ratio; H/L) is a reliable indicator of avian
158 immune responses (Davis, Maney & Maerz 2008) and a useful metric to observe
159 whether parasites modulate host immune systems. Because filarial parasites can
160 decrease a host's ability to produce immune cells (lymphocytes in this case;
161 Chatterjee *et al.* 2015) in response to antigens, while also increasing inflammatory
162 neutrophils, we may expect microfilaria infection to lead to increased H/L ratios if
163 such immune modulation occurs in birds. To visually screen for parasites and
164 characterise H/L ratios, we examined blood smears. Smears were fixed in methanol
165 and stained with 10% Giemsa. The entire smear was screened at 200× for microfilaria.
166 We screened at least 100 fields at 1,000× to identify malaria parasites and to calculate
167 H/L ratios by categorising the first 100 white blood cells observed as heterophil,
168 lymphocyte, eosinophil or monocyte.

169

170 *Analysis of parasite distributions and co-occurrence probabilities*

171 We combined data with published malaria data from 174 New Caledonian *Zosterops*
172 individuals (Olsson-Pons *et al.* 2015) for a total of 449 birds (Table 1a). The final

173 dataset included 82 haematozoan co-infections, 16 from published data and 66 from
174 the 2014 data. Note however that observed co-infection occurrences are likely
175 underestimates, as only the 2014 samples were screened with both smears and genus-
176 specific primers. We gathered infection data from four parasite groups (*H. zosteropsis*,
177 *H. killangoi*, *Plasmodium* spp. and microfilaria; see supporting information for
178 descriptions and molecular barcoding of *H. zosteropsis* and *H. killangoi*) across 17
179 sites [46 birds in montane rainforest (Grand Terre), 111 in open lowland forest (Grand
180 Terre and Ouvéa) and 292 in lowland rainforest (Maré, Lifou and Ouvéa); Fig. 1a; Fig.
181 S1].

182 In addition to *Zosterops* spp., we included abundance data from other avian
183 species (485 individuals in total) that were also captured across the 17 sites. Host
184 availability can vary such that some hosts are in low abundance in particular habitats,
185 and this variation could influence parasite distributions (Wells *et al.* 2012).
186 Abundance data from additional avian families was therefore used in conjunction with
187 *Zosterops* abundance data to assess the influence of *Zosterops* spp. proportional
188 abundance on parasite occurrences. This parameter is warranted as *Zosterops* spp. are
189 the most common hosts for many New Caledonian avian malaria lineages (Ishtiaq *et*
190 *al.* 2010; Olsson-Pons *et al.* 2015), indicating that local *Zosterops* abundances could
191 influence transmission (Moens *et al.* 2016; Ricklefs *et al.* 2016). Moreover, *Zosterops*
192 spp. are the only hosts recorded for lineages belonging to the *Haemoproteus* spp.
193 tested here, a pattern supported by morphological data ranging from Africa to
194 Australasia (Valkiūnas 2005). Thus, Zosteropidae hosts likely represent the only
195 available ‘habitat’ for *H. zosteropsis* and *H. killangoi* to asexually develop. *Zosterops*
196 spp. sample sizes ranged from three to 105 and proportional abundance ranged from
197 19.4 - 100% across sites.

198 To model individual infection probabilities, we used a hierarchical
 199 multivariate logistic regression to decompose variation due to environment (specified
 200 by covariates) and interspecific parasite co-occurrences (specified by a
 201 variance/covariance matrix). Here, a positive correlation signifies parasites that co-
 202 occurred more often than expected by chance given their respective environmental
 203 affinities, while a negative correlation signifies the opposite. Note that positive or
 204 negative correlations do not necessarily represent explicit within-host parasite
 205 interactions, as infection intensity and, ideally, experimental infections would be
 206 needed to confirm mechanisms underlying correlations.

207 We assumed the observed presence-absence $y(p, i)$ of parasite species p in host
 208 individual i captured at site s is a random sample of the population, conditional on
 209 host identity, the surrounding environment and individual infection status with other
 210 parasites:

$$211 \quad y(p, i) \sim \text{Bernoulli}[\Psi(p, i)] \quad (\text{eqn. 1})$$

212 Using a logit-link, we modelled infection probability $\Psi(p, i)$ of each host individual
 213 with parasite p as:

$$214 \quad \text{logit}(\Psi(p, i)) \sim \beta_0^P + \beta^P_{\text{HostSp}(i)} + \beta^P_{\text{Island}(s)} + \beta^P_{\text{Forest}(s)} + \gamma_A^P A_{\text{zost.scale}(s)} +$$

$$215 \quad E(p, i) \quad (\text{eqn. 2})$$

216 Here, β_0^P is the parasite-specific intercept, while coefficients β^P_{HostSp} , β^P_{Island} , and β^P_{Forest}
 217 estimate variation in infection probability due to host species, island and forest
 218 type, respectively (categorical variables; β -values estimated for each level).

219 Superscript '^P' is used as coefficients were estimated independently for each parasite
 220 species. Coefficient γ_A^P estimates the effect of *Zosterops* proportional abundance A_{zost} ,
 221 estimated as proportion of *Zosterops* individuals from all captured birds at each site.

222 To account for unequal sampling across sites, we modelled A_{zost} as a binomial

223 function of total mistnet captures (all species; N_{total}) and *Zosterops* spp. total
224 abundance N_{zost} :

$$225 \quad N_{zost}(s) \sim \text{Binomial}(A_{zost}(s), N_{total}(s)) \quad (\text{eqn. 3})$$

226 Estimates for A_{zost} were centred and standardised in each iteration ($A_{zost.scale}$).

227 The term $E(p,i)$ captures variance-covariance relationships in parasite
228 occurrence in relation to the presence of all parasite species in host individuals
229 (O'Brien & Dunson 2004; Pollock *et al.* 2014). This matrix of random effects is
230 modelled as a zero-centred multivariate normal distribution:

$$231 \quad E(p,i) \sim \text{MVN}(0, \Omega) \quad (\text{eqn. 4})$$

232 Here, Ω comprises a variance-covariance matrix for which the conjugate prior is a
233 scaled inverse Wishart distribution. The matrix elements describe whether a given
234 parasite pair co-occurs more or less often than expected by chance (based on residual
235 correlations), after accounting for environmental β^P coefficients in *eqn. 2*. The two
236 parameters of the inverse Wishart are degrees of freedom df and a positive-definite
237 scale matrix of dimension $p \times p$ (p = total number of parasite species). We set $df = p +$
238 1 to place a uniform distribution on pairwise correlations, such that values between -1
239 and 1 were equally likely (Gelman & Hill 2007). To generate correlation estimates,
240 we scaled off-diagonal covariance elements by the diagonals. Standard deviations and
241 correlations in the $p \times p$ matrix were estimated by multiplying variances of diagonal
242 elements by scaling factors drawn from a *Uniform(0,100)* distribution (Gelman & Hill
243 2007).

244 The model was fit in a Bayesian framework with Markov Chain Monte Carlo
245 (MCMC) sampling based on the Gibbs sampler in the freeware JAGS, using the R
246 interface 'rjags' (Plummer 2003). We used normal priors with variance = 2.71 for
247 intercepts and regression coefficients. This prior gives close approximation to a

248 logistic distribution and is appropriate for estimates on a logit scale when prior
249 information is limited (Lunn *et al.* 2012). To estimate $A_{zost}(s)$, we used a $Beta(2,2)$
250 distribution truncated between 0.05 and 0.9 (based on observed range limits for
251 $A_{zost}(s)$). For categorical covariates (β^P_{HostSp} , β^P_{Island} , and β^P_{Forest}), we used
252 redundancy coefficients to improve convergence and scale estimates (Gelman & Hill
253 2007). For example, coefficient $\beta^P_{HostSp}^*$ was calculated for parasite species p in host
254 species h as:

$$255 \quad \beta^P_{HostSp}^*(h) = \beta^P_{HostSp}(h) - \text{mean}(\beta^P_{HostSp})$$

256 Convergence was assessed visually and posterior predictive checks assessed if
257 model assumptions were good approximations of the data generating process.

258 Bayesian p -values around 0.5 indicate good fit whereas values near 0 or 1 indicate a
259 discrepancy between predictions and observed data (Gelman, Meng & Stern 1996).

260 While all *Zosterops* individuals were screened for malaria, only 275 birds (from 2014)
261 were screened for microfilaria (note all combinations of host / habitat / island were
262 sampled for microfilaria). Microfilaria data for remaining samples were set as 'NA'
263 (i.e. missing data), allowing the sampler to make inferences from its posterior
264 distribution as if these values were omitted (Lunn *et al.* 2012). This approach ensured
265 inferences were made using the full dataset, rather than excluding individuals or
266 assigning random values, and is appropriate in Bayesian contexts where model-based
267 inference of host-parasite interactions generates less bias than direct data inference
268 (Wells & O'Hara 2013). Where *Haemoproteus* DNA was amplified but no sequence
269 generated and no blood smears existed ($n = 16$), *H. zosteropsis* and *H. killangoi* were
270 also specified as NA.

271 We ran two chains for 750 000 iterations, discarding 250 000 iterations as
272 burn-in, with a thinning interval of 1000. Results are given as 95% highest posterior

273 credible intervals (CI). We used odds ratios (OR) to compare strength of change in
274 infection probabilities for levels of categorical covariates. We considered credible
275 intervals that did not overlap with zero or with those from other covariates as
276 ‘significant’.

277

278 *Analysis of host heterophil to lymphocyte ratios*

279 We tested for relationships between H/L ratios and infection status for 166 birds from
280 three *Zosterops* spp. (no infections occurred in *Z. inornatus*; this species was omitted
281 from H/L analysis) using linear regressions. The response variable was logit-
282 transformed H/L ratios with assumed normal error distribution. Fixed predictors were
283 microfilaria, *Haemoproteus*, and *Plasmodium* status (binary variables: infected or
284 uninfected). Separate models tested each combination of two-way parasite
285 interactions (triple infections were too rare to test three-way interactions). As time of
286 day can influence H/L ratios (Banbura *et al.* 2013), we included ‘time’ as a
287 continuous predictor. We included ‘island’ and ‘host species’ as random grouping
288 variables, allowing the intercept to vary among groups. A conservative model was
289 also fit in which *Haemoproteus* and *Plasmodium* infections were combined
290 (‘malaria’). For model comparisons, we used Akaike’s Information Criterion (AIC),
291 assuming that a change in AIC of >2 indicates a change in model performance.

292 Data was analysed in R version 3.2.1 (R Core Team, 2008; R: A language and
293 environment for statistical computing). Data and R code used to perform analyses are
294 presented in supporting information.

295

296 **Results**

297 *Environmental influences on parasite infection probabilities*

298 In total, 228 of 449 *Zosterops* individuals were infected with haematozoans, including
299 191 *Haemoproteus*, 88 *Plasmodium* and 41 microfilaria infections (Table 1b; Fig. 1a,
300 b). Nine avian malaria lineages were morphologically identified to species level for
301 the first time, including three lineages of *H. killangoi* and four of *H. zosteropsis* (Figs.
302 S2 - S4). Each of the four focal parasites occurred on all islands, with the exception of
303 microfilariae (absent from Lifou; Table 1b; Fig. 1b). The multivariate logistic
304 regression obtained good fit (Bayesian $p = 0.56$). Estimated prevalence across all
305 individuals (β_0) was highest for *H. zosteropsis* (CI: 14 - 45%), followed by microfilaria
306 (5 - 22%), *Plasmodium* spp. (4 - 18%) and *H. killangoi* (2 - 11%).

307 'Forest type' explained 15 - 63% of environmental variation in occurrence
308 probability for microfilaria, 3 - 65% for *Plasmodium* spp. and 1 - 28% for *H.*
309 *zosteropsis*, with each parasite less likely to occur in montane rainforest than the two
310 lowland forest categories (OR: 0.02 - 0.27 for microfilaria, 0.05 - 0.65 for
311 *Plasmodium* spp. and 0.04 - 0.75 for *H. zosteropsis*). Infection patterns differed across
312 lowland forest categories, with *H. zosteropsis* and microfilaria more likely to occur in
313 lowland rainforest (OR: 2.1 - 13.8 and 2.1 - 12.8, respectively) and *Plasmodium* spp.
314 infections more likely in open lowland forest (OR: 2.1 - 14.5).

315 'Island' explained 7 - 53% of environmental variance in occurrence
316 probability for microfilaria, 2 - 28% for *H. zosteropsis* and 1 - 68% for *H. killangoi*.
317 Both *H. zosteropsis* and microfilaria were more likely on Maré than remaining islands
318 (OR: 3.7 - 37.3 and 1.9 - 13.1, respectively; Fig. 1c). Infections with *H. killangoi* were
319 more likely on Ouvéa (OR: 1.1 - 12.1; Fig. 1c). In addition to island and habitat
320 effects, *H. zosteropsis* occurrence was negatively influenced by *Zosterops* spp.
321 'proportional abundance' [explaining 6 - 91% of variation in infection probability

322 (OR: 0.01 - 0.69)]. Variance explained by ‘host species’ overlapped with zero for all
323 parasites and credible intervals overlapped among different host species.

324

325 *Co-infections and parasite co-occurrence probabilities*

326 A total of 82 parasite co-infections were observed, accounting for 35.9% of all
327 infected birds and representing all pairwise parasite combinations (Table 1c). We
328 observed 13 *H. zosteropsis*/*Plasmodium*/Microfilaria triple infections and one *H.*
329 *killangoi*/*H. zosteropsis*/*Plasmodium* triple infection. After accounting for
330 environmental covariates, estimated covariances revealed ‘significantly’ correlated
331 infection probabilities for all parasite pairs apart from *H. zosteropsis* / *Plasmodium* spp.
332 (Fig. 2). Infection probabilities for two of three pairwise avian malaria combinations
333 were negatively correlated, with the third showing a non-significant negative trend
334 (Fig. 2). All observed microfilariae co-occurred with malaria (Table 1), and
335 microfilaria infections correlated positively with occurrences of *Plasmodium* spp. and
336 *H. zosteropsis*, but negatively with *H. killangoi* (Fig. 2). In fact, thirty-three of 44
337 observed microfilaria infections co-occurred with *H. zosteropsis*, while co-infections
338 of any parasite with *H. killangoi* were rare (accounting for five of 52 observed *H.*
339 *killangoi* infections; Table 1c).

340

341 *Relationship between parasite infections and host heterophil to lymphocyte ratios*

342 Microfilariae were associated with increased H/L ratios when accounting for time and
343 presence of other parasites (Δ AIC without microfilaria: +11.17; Fig. 3). This
344 elevation was driven by increased heterophils (mean with microfilaria: 12.73 ± 2.21 ;
345 without: 5.03 ± 0.45) and decreased lymphocytes (mean with microfilaria: $74.93 \pm$
346 2.28 ; without: 82.68 ± 0.85). Neither *Haemoproteus* nor *Plasmodium* spp. influenced

347 H/L ratios, either as separate variables or combined (Δ AIC without *Haemoproteus*: -
348 2.91; without *Plasmodium*: -2.82; without 'malaria': -1.11; Fig. 3).

349

350 **Discussion**

351 We provide a rare demonstration of apparent biotic associations between wildlife
352 parasites. Two widespread *Haemoproteus* parasites had dissimilar co-infection
353 patterns and a negative co-occurrence probability, a pattern indicative of competition
354 between parasites that utilise the same host resources. Birds with microfilariae had
355 elevated H/L ratios and two avian malaria parasites (*H. zosteropsis* and *Plasmodium*
356 spp.) had positive co-occurrence probabilities with microfilaria, consistent with
357 evidence that nematode-induced immune modulation may facilitate malaria co-
358 infections (Druilhe, Tall & Sokhna 2005). Our results indicate that interspecific
359 associations are an important but overlooked mechanism influencing wildlife parasite
360 infections.

361

362 *Correlated infection probabilities: evidence of parasite competition and facilitation?*

363 We identified negative parasite co-occurrence probabilities between *H. zosteropsis* / *H.*
364 *killangoi* and between *H. killangoi* / *Plasmodium* spp., supporting our prediction that
365 interspecific malaria infections would be negatively correlated. Only two co-
366 infections were observed for each of the above parasite pairs, despite each parasite
367 occurring on all islands and habitats. Considering that *H. zosteropsis* and *H. killangoi*
368 are avian host specialists that appear restricted to Zosteropidae (Valkiūnas 2005;
369 Clark & Clegg 2015), our results may be evidence of interspecific competition. We
370 also found a striking difference in likelihoods of microfilaria co-infection for the two
371 *Haemoproteus* species. We predicted malaria infections would positively correlate

372 with microfilaria; yet, while no filarial parasites occurred in birds free from avian
373 malaria, birds carrying *H. killangoi* rarely carried microfilaria. In comparison, birds
374 carrying *H. zosteropsis* had increased likelihood of carrying microfilaria when
375 accounting for their similar environmental affiliations. Contrasting patterns for host-
376 specialist *Haemoproteus* parasites suggest associations with immune-modulating
377 nematodes are uneven between rival malaria species, a fascinating finding that
378 deserves further attention in field and laboratory studies.

379 Explaining patterns of co-occurrence for vector-borne parasites requires
380 careful consideration of the role of vectors. Similarly to previous studies, we found
381 important environmental influences on blood parasite distributions (Lachish *et al.*
382 2011; Oakgrove *et al.* 2014; Sehgal 2015). Despite wide CIs owing to uncertainty, we
383 identified habitat and island infection patterns that likely reflect distributions of
384 arthropod vectors (Rogers *et al.* 2002; Santiago-Alarcon, Palinauskas & Schaefer
385 2012). Both *Haemoproteus* and microfilaria are known to use Ceratopogonid midges
386 as vectors, and evidence suggests that different *Haemoproteus* parasites can use
387 different Ceratopogonid species (Santiago-Alarcon, Palinauskas & Schaefer 2012).
388 Associations between *H. zosteropsis* and microfilaria could be evidence of a shared
389 vector, while a different vector may transmit *H. killangoi*, perhaps reducing co-
390 infections. This hypothesis adds to the growing need for future studies of
391 haematozoan vectors (Clark, Clegg & Lima 2014; Bobeva *et al.* 2015; Žiegytė &
392 Valkiūnas 2015; Bernotienė & Valkiūnas 2016). In addition to environmental effects,
393 a surprising finding was the negative influence of *Zosterops* spp. proportional
394 abundance on *H. zosteropsis* occurrence. The idea that hosts reach higher abundance
395 where infections are lower touches on exciting evolutionary questions, such as host-

396 parasite interactions driving taxon cycles (Ricklefs *et al.* 2016) or shaping host
397 dispersal patterns (Poulin *et al.* 2012; Aharon-Rotman *et al.* 2016).

398 Our data was not complete, as only samples from 2014 were subject to smear
399 and genus-specific PCR screening, adding to uncertainty in our estimates and
400 emphasising the need for greater scrutiny of co-occurring wildlife pathogens (Petney
401 & Andrews 1998; Knowles 2011). In addition to incomplete data, some parasite
402 associations seen here could have been inflated by missing covariates (Pollock *et al.*
403 2014), as we lacked microhabitat data such as temperature and moisture that can
404 influence local transmission (Zamora-Vilchis, Williams & Johnson 2012; Cornuault
405 *et al.* 2013; Sehgal 2015). Due to complex environmental influences and the inherent
406 uncertainty in pathogen observations, we propose that multivariate logistic regression
407 combined with appropriate covariate data provides a useful platform for analyses of
408 wildlife pathogen associations.

409

410 *Altered heterophil to lymphocyte ratios in malaria/microfilaria coinfections*

411 Though often overlooked, haematozoan co-infections are important, as they may
412 compound effects on host condition and survival (Valkiūnas *et al.* 2006; Palinauskas
413 *et al.* 2011; Oakgrove *et al.* 2014; Dimitrov *et al.* 2015). Yet identifying mechanisms
414 that drive wildlife parasite associations is challenging (Cattadori, Boag & Hudson
415 2008; Tompkins *et al.* 2011). Our finding of altered H/L ratios during microfilaria
416 infection identifies immune modulation as a possible mechanism by which parasitic
417 nematodes may facilitate co-occurring malaria. Microfilariae led to decreased
418 lymphocytes and increased heterophils, changes that could decrease a host's ability to
419 regulate pathogens through antigen recognition (Pedersen & Fenton 2007; Bordes &
420 Morand 2011). We did not observe changes in H/L ratios in birds carrying malaria but

421 not microfilaria, consistent with prior studies (Ricklefs & Sheldon 2007) and
422 suggesting the presence of parasitic nematodes drove these changes. This pattern
423 supports laboratory evidence that microfilariae depress adaptive immune pathways
424 responsible for identifying infections while increasing neutrophil-associated
425 inflammation (Druilhe, Tall & Sokhna 2005).

426 Increases in disease have been observed for many pathogens that co-occur
427 with nematodes, including HIV in humans (Bentwich *et al.* 1999). However this
428 relationship is not always facilitatory, as some nematodes depress co-occurring
429 malaria by reducing target cell densities (Griffiths *et al.* 2015). While positive
430 correlations between *H. zosteropsis* and microfilaria may indicate interspecific
431 facilitation, we stress that experimental perturbations and assessment of host
432 immunity are necessary to clarify within-host interactions (Sheldon & Verhulst 1996;
433 Johnson & Buller 2011; Knowles *et al.* 2013). In addition, data that takes into account
434 changes in parasite density during co-infection could provide clues as to how
435 coinfections alter disease progression (Metcalf *et al.* 2016). Although we cannot
436 speculate on within-host dynamics, our results contribute to a growing recognition
437 that parasitic nematodes are important components of pathogen epidemiology (Petney
438 & Andrews 1998; Nacher *et al.* 2001).

439

440 *Conclusions*

441 We present evidence that biotic associations play important roles in the occurrences
442 and infection likelihoods of haematozoan parasites. Our description of parasite co-
443 occurrence patterns provides critical new insights into disease ecology, as parasite
444 associations are expected across many host systems (Bell *et al.* 2006; Pérez-Tris *et al.*
445 2007; Johnson & Buller 2011; Vaumourin *et al.* 2015), yet evidence from wildlife is

446 biased towards mammalian hosts (Lello *et al.* 2004; Tompkins *et al.* 2011; Hellard *et*
447 *al.* 2015). Additionally, we show that co-infections are difficult to identify using PCR
448 alone, a finding demonstrated for many host-pathogen systems (Valkiūnas *et al.* 2006;
449 Dyachenko *et al.* 2010; Grybchuk-Ieremenko *et al.* 2014; Moustafa *et al.* 2016). We
450 overcame this hurdle by combining traditional and molecular parasitology methods, a
451 multidisciplinary approach that we recommend for future work on wildlife co-
452 infections.

453

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465

466 **Data accessibility**

467 Malaria lineages will be deposited in GenBank and the MalAvi database. Microfilaria
468 LSU lineages will be deposited in GenBank. Data and R code used for analyses will
469 be deposited to Dryad upon acceptance.

470

471 **References**

- 472 Aharon-Rotman, Y., Buchanan, K.L., Clark, N.J., Klaassen, M. & Buttemer, W.A.
473 (2016) Why fly the extra mile? Using stress biomarkers to assess wintering
474 habitat quality in migratory shorebirds. *Oecologia*, DOI: 10.1007/s00442-
475 00016-03679-00441.
- 476 Atkinson, C.T., Thomas, N.J. & Hunter, D.B. (2008) *Parasitic diseases of wild birds*.
477 John Wiley & Sons, Inc, Wiley Online Library.
- 478 Banbura, J., Skwarska, J., Banbura, M., Gladalski, M., Holysz, M., Kalinski, A.,
479 Markowski, M., Wawrzyniak, J. & Zielinski, P. (2013) Spatial and temporal
480 variation in heterophil-to-lymphocyte ratios of nestling passerine birds:
481 comparison of blue tits and great tits. *Plos One*, **8**, e74226.
- 482 Bell, A.S., De Roode, J.C., Sim, D., Read, A.F. & Koella, J. (2006) Within-host
483 competition in genetically diverse malaria infections: parasite virulence and
484 competitive success. *Evolution*, **60**, 1358-1371.
- 485 Bensch, S., Hellgren, O. & Pérez-Tris, J. (2009) MalAvi: a public database of malaria
486 parasites and related haemosporidians in avian hosts based on mitochondrial
487 cytochrome *b* lineages. *Molecular Ecology Resources*, **9**, 1353-1358.
- 488 Bentwich, Z., Kalinkovich, A., Weisman, Z., Borkow, G., Beyers, N. & Beyers, A.D.
489 (1999) Can eradication of helminthic infections change the face of AIDS and
490 tuberculosis? *Immunology today*, **20**, 485-487.
- 491 Bernotienė, R. & Valkiūnas, G. (2016) PCR detection of malaria parasites and related
492 haemosporidians: the sensitive methodology in determining bird-biting insects.
493 *Malaria Journal*, **15**, 1-8.
- 494 Bobeva, A., Zehindjiev, P., Ilieva, M., Dimitrov, D., Mathis, A. & Bensch, S. (2015)
495 Host preferences of ornithophilic biting midges of the genus *Culicoides* in the
496 Eastern Balkans. *Medical and Veterinary Entomology*, **29**, 290-296.
- 497 Bordes, F. & Morand, S. (2011) The impact of multiple infections on wild animal
498 hosts: a review. *Infection Ecology & Epidemiology*, **1**,
499 10.3402/iee.v3401i3400.7346.
- 500 Budria, A. & Candolin, U. (2013) How does human-induced environmental change
501 influence host-parasite interactions? *Parasitology*, **141**, 1-13.
- 502 Bush, A.O. (2001) *Parasitism: the diversity and ecology of animal parasites*.
503 Cambridge University Press.
- 504 Cattadori, I., Boag, B. & Hudson, P. (2008) Parasite co-infection and interaction as
505 drivers of host heterogeneity. *International Journal for Parasitology*, **38**, 371-
506 380.
- 507 Chatterjee, S., Clark, C.E., Lugli, E., Roederer, M. & Nutman, T.B. (2015) Filarial
508 Infection Modulates the Immune Response to Mycobacterium tuberculosis

- 509 through Expansion of CD4+ IL-4 Memory T Cells. *The Journal of*
510 *Immunology*, **194**, 2706-2714.
- 511 Clark, N.J. & Clegg, S.M. (2015) The influence of vagrant hosts and weather patterns
512 on the colonization and persistence of blood parasites in an island bird.
513 *Journal of Biogeography*, **42**, 641-651.
- 514 Clark, N.J., Clegg, S.M. & Klaassen, M. (2016) Migration strategy and pathogen risk:
515 non-breeding distribution drives malaria prevalence in migratory waders.
516 *Oikos*, DOI: 10.1111/oik.03220.
- 517 Clark, N.J., Clegg, S.M. & Lima, M.R. (2014) A review of global diversity in avian
518 haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new
519 insights from molecular data. *International Journal for Parasitology*, **44**, 329-
520 338.
- 521 Clark, N.J., Olsson-Pons, S., Ishtiaq, F. & Clegg, S.M. (2015) Specialist enemies,
522 generalist weapons and the potential spread of exotic pathogens: malaria
523 parasites in a highly invasive bird. *International Journal for Parasitology*, **45**,
524 891-899.
- 525 Cornuault, J., Khimoun, A., Harrigan, R.J., Bourgeois, Y.X., Milá, B., Thébaud, C. &
526 Heeb, P. (2013) The role of ecology in the geographical separation of blood
527 parasites infecting an insular bird. *Journal of Biogeography*, **40**, 1313-1323.
- 528 Davis, A., Maney, D. & Maerz, J. (2008) The use of leukocyte profiles to measure
529 stress in vertebrates: a review for ecologists. *Functional Ecology*, **22**, 760-772.
- 530 Dayton, P.K. (1971) Competition, disturbance, and community organization: the
531 provision and subsequent utilization of space in a rocky intertidal community.
532 *Ecological Monographs*, **41**, 351-389.
- 533 Dimitrov, D., Palinauskas, V., Iezhova, T.A., Bernotienė, R., Ilgūnas, M.,
534 Bukauskaitė, D., Zehtindjiev, P., Ilieva, M., Shapoval, A.P. & Bolshakov, C.V.
535 (2015) *Plasmodium* spp.: An experimental study on vertebrate host
536 susceptibility to avian malaria. *Experimental Parasitology*, **148**, 1-16.
- 537 Druilhe, P., Tall, A. & Sokhna, C. (2005) Worms can worsen malaria: towards a new
538 means to roll back malaria? *Trends in Parasitology*, **21**, 359-362.
- 539 Dutson, G. (2012) *Birds of Melanesia: Bismarcks, Solomons, Vanuatu, and New*
540 *Caledonia*. A&C Black.
- 541 Dyachenko, V., Kuhnert, Y., Schmaeschke, R., Etzold, M., Pantchev, N. &
542 Dauschies, A. (2010) Occurrence and molecular characterization of
543 *Cryptosporidium* spp. genotypes in European hedgehogs (*Erinaceus*
544 *europaeus* L.) in Germany. *Parasitology*, **137**, 205-216.
- 545 Fenton, A., Knowles, S.C.L., Petchey, O.L. & Pedersen, A.B. (2014) The reliability
546 of observational approaches for detecting interspecific parasite interactions:
547 comparison with experimental results. *International Journal for Parasitology*,
548 **44**, 437-445.

- 549 Freed, L.A. & Cann, R.L. (2013) Vector movement underlies avian malaria at upper
550 elevation in Hawaii: implications for transmission of human malaria.
551 *Parasitology Research*, **112**, 3887-3895.
- 552 Gelman, A. & Hill, J. (2007) *Data analysis using regression and*
553 *multilevel/hierarchical models*. Cambridge University Press Cambridge.
- 554 Gelman, A., Meng, X.L. & Stern, H. (1996) Posterior predictive assessment of model
555 fitness via realized discrepancies. *Statistica Sinica*, **6**, 733-760.
- 556 Goulding, W., Adlard, R.D., Clegg, S.M. & Clark, N.J. (2016) Molecular and
557 morphological description of *Haemoproteus (Parahaemoproteus) bukaka*
558 (species nova), a haemosporidian associated with the strictly Australo-Papuan
559 host subfamily Cracticinae. *Parasitology Research*, DOI: 10.1007/s00436-
560 00016-05099-x.
- 561 Graham, A.L., Lamb, T.J., Read, A.F. & Allen, J.E. (2005) Malaria-filaria coinfection
562 in mice makes malarial disease more severe unless filarial infection achieves
563 patency. *Journal of Infectious Diseases*, **191**, 410-421.
- 564 Griffiths, E.C., Fairlie-Clarke, K., Allen, J.E., Metcalf, C.J.E. & Graham, A.L. (2015)
565 Bottom-up regulation of malaria population dynamics in mice co-infected with
566 lung-migratory nematodes. *Ecology Letters*, DOI: 10.1111/ele.12534.
- 567 Grybchuk-Ieremenko, A., Losev, A., Kostygov, A.Y., Lukeš, J. & Yurchenko, V.
568 (2014) High prevalence of trypanosome co-infections in freshwater fishes.
569 *Folia Parasitologica*, **61**, 495-504.
- 570 Hellard, E., Fouchet, D., Vavre, F. & Pontier, D. (2015) Parasite-parasite interactions
571 in the wild: how to detect them? *Trends in Parasitology*, **31**, 640-652.
- 572 Hoberg, E.P. & Brooks, D.R. (2015) Evolution in action: climate change, biodiversity
573 dynamics and emerging infectious disease. *Philosophical Transactions of the*
574 *Royal Society B: Biological Sciences*, **370**, 20130553.
- 575 Hughes, W.O., Petersen, K.S., Ugelvig, L.V., Pedersen, D., Thomsen, L., Poulsen, M.
576 & Boomsma, J.J. (2004) Density-dependence and within-host competition in a
577 semelparous parasite of leaf-cutting ants. *Bmc Evolutionary Biology*, **4**, 45.
- 578 Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F. & Sheldon, B.C.
579 (2010) Biogeographical patterns of blood parasite lineage diversity in avian
580 hosts from southern Melanesian islands. *Journal of Biogeography*, **37**, 120-
581 132.
- 582 Jackson, J.A., Pleass, R.J., Cable, J., Bradley, J.E. & Tinsley, R.C. (2006)
583 Heterogenous interspecific interactions in a host-parasite system.
584 *International Journal for Parasitology*, **36**, 1341-1349.
- 585 Johnson, P.T. & Buller, I.D. (2011) Parasite competition hidden by correlated
586 coinfection: using surveys and experiments to understand parasite interactions.
587 *Ecology*, **92**, 535-541.

- 588 Johnson, P.T.J. & Hoverman, J.T. (2012) Parasite diversity and coinfection determine
589 pathogen infection success and host fitness. *Proceedings of the National*
590 *Academy of Sciences*, **109**, 9006-9011.
- 591 Kishida, N., Sakoda, Y., Eto, M., Sunaga, Y. & Kida, H. (2004) Co-infection of
592 *Staphylococcus aureus* or *Haemophilus paragallinarum* exacerbates H9N2
593 influenza A virus infection in chickens. *Archives of Virology*, **149**, 2095-2104.
- 594 Kissling, W.D., Dormann, C.F., Groeneveld, J., Hickler, T., Kühn, I., McNerny, G.J.,
595 Montoya, J.M., Römermann, C., Schiffers, K., Schurr, F.M., Singer, A.,
596 Svenning, J.-C., Zimmermann, N.E. & O'Hara, R.B. (2012) Towards novel
597 approaches to modelling biotic interactions in multispecies assemblages at
598 large spatial extents. *Journal of Biogeography*, **39**, 2163-2178.
- 599 Knowles, S.C. (2011) The effect of helminth co-infection on malaria in mice: a meta-
600 analysis. *International Journal for Parasitology*, **41**, 1041-1051.
- 601 Knowles, S.C., Fenton, A., Petchey, O.L., Jones, T.R., Barber, R. & Pedersen, A.B.
602 (2013) Stability of within-host-parasite communities in a wild mammal
603 system. *Proceedings of the Royal Society of London B: Biological Sciences*,
604 **280**, 20130598.
- 605 Lachish, S., Knowles, S.C.L., Alves, R., Wood, M.J. & Sheldon, B.C. (2011)
606 Infection dynamics of endemic malaria in a wild bird population: parasite
607 species-dependent drivers of spatial and temporal variation in transmission
608 rates. *Journal of Animal Ecology*, **80**, 1207-1216.
- 609 Lello, J., Boag, B., Fenton, A., Stevenson, I.R. & Hudson, P.J. (2004) Competition
610 and mutualism among the gut helminths of a mammalian host. *Nature*, **428**,
611 840-844.
- 612 Lunn, D., Jackson, C., Best, N., Thomas, A. & Spiegelhalter, D. (2012) *The BUGS*
613 *Book: A practical introduction to Bayesian analysis*. CRC Press.
- 614 Lutz, H.L., Hochachka, W.M., Engel, J.I., Bell, J.A., Tkach, V.V., Bates, J.M.,
615 Hackett, S.J. & Weckstein, J.D. (2015) Parasite prevalence corresponds to
616 host life history in a diverse assemblage of Afrotropical birds and
617 haemosporidian parasites. *Plos One*, **10**, e0121254.
- 618 Marzal, A. (2012) Recent advances in studies on avian malaria parasites. *Malaria*
619 *Parasites* (ed. O.O. Okwa), pp. 135-158. InTech.
- 620 Marzal, A., Ricklefs, R.E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C.,
621 Czirjak, G.A., Ewen, J., Hellgren, O., Horakova, D., Iezhova, T.A., Jensen, H.,
622 Krizanauskienė, A., Lima, M.R., de Lope, F., Magnussen, E., Martin, L.B.,
623 Møller, A.P., Palinauskas, V., Pap, P.L., Pérez, J., Sehgal, R.N.M., Soler, M.,
624 Eszter, S., Westerdahl, H., Zetindjiev, P. & Bensch, S. (2011) Diversity, loss,
625 and gain of malaria parasites in a globally invasive bird. *Plos One*, **6**, e21905.
- 626 Metcalf, C.J.E., Graham, A.L., Martinez-Bakker, M. & Childs, D.Z. (2016)
627 Opportunities and challenges of Integral Projection Models for modelling
628 host-parasite dynamics. *Journal of Animal Ecology*, **85**, 343-355.

- 629 Moens, M.A.J., Valkiūnas, G., Paca, A., Bonaccorso, E., Aguirre, N. & Pérez-Tris, J.
630 (2016) Parasite specialization in a unique habitat: hummingbirds as reservoirs
631 of generalist blood parasites of Andean birds. *Journal of Animal Ecology*,
632 DOI: 10.1111/1365-2656.12550.
- 633 Moustafa, M.A.M., Taylor, K., Nakao, R., Shimozuru, M., Sashika, M., Rosà, R., Thu,
634 M.J., Rizzoli, A. & Tsubota, T. (2016) Dynamics, co-infections and
635 characteristics of zoonotic tick-borne pathogens in Hokkaido small mammals,
636 Japan. *Ticks and Tick-borne Diseases*, DOI: 10.1016/j.ttbdis.2016.1004.1014.
- 637 Muturi, E.J., Jacob, B.G., Kim, C.-H., Mbogo, C.M. & Novak, R.J. (2008) Are
638 coinfections of malaria and filariasis of any epidemiological significance?
639 *Parasitology Research*, **102**, 175-181.
- 640 Nacher, M., Singhasivanon, P., Gay, F., Phumratanaprapin, W., Silachamroon, U. &
641 Looareesuwan, S. (2001) Association of helminth infection with decreased
642 reticulocyte counts and hemoglobin concentration in Thai *falciparum* malaria.
643 *The American Journal of Tropical Medicine and Hygiene*, **65**, 335-337.
- 644 O'Brien, S.M. & Dunson, D.B. (2004) Bayesian multivariate logistic regression.
645 *Biometrics*, **60**, 739-746.
- 646 Oakgrove, K.S., Harrigan, R.J., Loiseau, C., Guers, S., Seppi, B. & Sehgal, R.N.
647 (2014) Distribution, diversity and drivers of blood-borne parasite co-infections
648 in Alaskan bird populations. *International Journal for Parasitology*, **44**, 717-
649 727.
- 650 Olsson-Pons, S., Clark, N.J., Ishtiaq, F. & Clegg, S.M. (2015) Differences in host
651 species relationships and biogeographic influences produce contrasting
652 patterns of prevalence, community composition and genetic structure in two
653 genera of avian malaria parasites in southern Melanesia. *Journal of Animal
654 Ecology*, **84**, 985-998.
- 655 Ovaskainen, O., Hottola, J. & Siitonen, J. (2010) Modeling species co-occurrence by
656 multivariate logistic regression generates new hypotheses on fungal
657 interactions. *Ecology*, **91**, 2514-2521.
- 658 Palinauskas, V., Valkiūnas, G., Bolshakov, C.V. & Bensch, S. (2011) *Plasmodium*
659 *relictum* (lineage SGS1) and *Plasmodium ashfordi* (lineage GRW2): The
660 effects of the co-infection on experimentally infected passerine birds.
661 *Experimental Parasitology*, **127**, 527-533.
- 662 Pedersen, A.B. & Fenton, A. (2007) Emphasizing the ecology in parasite community
663 ecology. *Trends in Ecology & Evolution*, **22**, 133-139.
- 664 Pérez-Tris, J., Hellgren, O., Križanauskienė, A., Waldenström, J., Secondi, J.,
665 Bonneaud, C., Fjeldså, J., Hasselquist, D. & Bensch, S. (2007) Within-host
666 speciation of malaria parasites. *Plos One*, **2**, e235.
- 667 Petney, T.N. & Andrews, R.H. (1998) Multiparasite communities in animals and
668 humans: frequency, structure and pathogenic significance. *International
669 Journal for Parasitology*, **28**, 377-393.

- 670 Plowright, R.K., Eby, P., Hudson, P.J., Smith, I.L., Westcott, D., Bryden, W.L.,
671 Middleton, D., Reid, P.A., McFarlane, R.A. & Martin, G. (2015) Ecological
672 dynamics of emerging bat virus spillover. *Proceedings of the Royal Society of*
673 *London B: Biological Sciences*, **282**, 20142124.
- 674 Plummer, M. (2003) JAGS: A program for analysis of Bayesian graphical models
675 using Gibbs sampling. *Proceedings of the 3rd International Workshop on*
676 *Distributed Statistical Computing*, pp. 125. Technische Universit at Wien
677 Wien, Austria.
- 678 Pollock, L.J., Tingley, R., Morris, W.K., Golding, N., O'Hara, R.B., Parris, K.M.,
679 Vesk, P.A. & McCarthy, M.A. (2014) Understanding co-occurrence by
680 modelling species simultaneously with a Joint Species Distribution Model
681 (JSDM). *Methods in Ecology and Evolution*, **5**, 397-406.
- 682 Poulin, R., Closs, G.P., Lill, A.W., Hicks, A.S., Herrmann, K.K. & Kelly, D.W.
683 (2012) Migration as an escape from parasitism in New Zealand galaxiid fishes.
684 *Oecologia*, **169**, 955-963.
- 685 Ricklefs, R.E. & Sheldon, K.S. (2007) Malaria prevalence and white-blood-cell
686 response to infection in a tropical and in a temperate thrush. *Auk*, **124**, 1254-
687 1266.
- 688 Ricklefs, R.E., Soares, L., Ellis, V.A. & Latta, S.C. (2016) Haemosporidian parasites
689 and avian host population abundance in the Lesser Antilles. *Journal of*
690 *Biogeography*, doi:10.1111/jbi.12730.
- 691 Rogers, D.J., Randolph, S.E., Snow, R.W. & Hay, S.I. (2002) Satellite imagery in the
692 study and forecast of malaria. *Nature*, **415**, 710-715.
- 693 Santiago-Alarcon, D., Palinauskas, V. & Schaefer, H.M. (2012) Diptera vectors of
694 avian Haemosporidian parasites: untangling parasite life cycles and their
695 taxonomy. *Biological Reviews*, **87**, 928-964.
- 696 Sehgal, R.N. (2015) Manifold habitat effects on the prevalence and diversity of avian
697 blood parasites. *International Journal for Parasitology: Parasites and Wildlife*,
698 DOI: 10.1016/j.ijppaw.2015.1009.1001.
- 699 Sehgal, R.N., Jones, H.I. & Smith, T.B. (2005) Molecular evidence for host
700 specificity of parasitic nematode microfilariae in some African rainforest birds.
701 *Molecular Ecology*, **14**, 3977-3988.
- 702 Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: costly parasite defences
703 and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, **11**,
704 317-321.
- 705 Su, Z., Segura, M., Morgan, K., Loredó-Osti, J.C. & Stevenson, M.M. (2005)
706 Impairment of protective immunity to blood-stage malaria by concurrent
707 nematode infection. *Infection and immunity*, **73**, 3531-3539.

- 708 Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S. & Begon,
709 M. (2010) Species interactions in a parasite community drive infection risk in
710 a wildlife population. *Science*, **330**, 243-246.
- 711 Tompkins, D.M., Dunn, A.M., Smith, M.J. & Telfer, S. (2011) Wildlife diseases:
712 from individuals to ecosystems. *Journal of Animal Ecology*, **80**, 19-38.
- 713 Valkiūnas, G. (2005) *Avian malaria parasites and other Haemosporida*. CRC Press,
714 Boca Raton, FL.
- 715 Valkiūnas, G., Bensch, S., Iezhova, T.A., Krizanauskiene, A., Hellgren, O. &
716 Bolshakov, C.V. (2006) Nested cytochrome *b* polymerase chain reaction
717 diagnostics underestimate mixed infections of avian blood haemosporidian
718 parasites: Microscopy is still essential. *Journal of Parasitology*, **92**, 418-422.
- 719 van Rooyen, J., Jenkins, T., Lahlah, N. & Christe, P. (2014) North-African house
720 martins endure greater haemosporidian infection than their European
721 counterparts. *Journal of Avian Biology*, **45**, 450-456.
- 722 Vaumourin, E., Vourc'h, G., Gasqui, P. & Vayssier-Taussat, M. (2015) The
723 importance of multiparasitism: examining the consequences of co-infections
724 for human and animal health. *Parasites & Vectors*, **8**, 1-13.
- 725 Wells, K. & O'Hara, R.B. (2013) Species interactions: estimating per-individual
726 interaction strength and covariates before simplifying data into per-species
727 ecological networks. *Methods in Ecology and Evolution*, **4**, 1-8.
- 728 Wells, K., O'Hara, R.B., Morand, S., Lessard, J.-P. & Ribas, A. (2015) The
729 importance of parasite geography and spillover effects for global patterns of
730 host-parasite associations in two invasive species. *Diversity and Distributions*,
731 **21**, 477-486.
- 732 Wells, K., O'Hara, R.B., Pfeiffer, M., Lakim, M.B., Petney, T.N. & Durden, L.A.
733 (2012) Inferring host specificity and network formation through agent-based
734 models: tick-mammal interactions in Borneo. *Oecologia*, **172**, 307-316.
- 735 Wood, J.L.N., Leach, M., Waldman, L., MacGregor, H., Fooks, A.R., Jones, K.E.,
736 Restif, O., Dechmann, D., Hayman, D.T.S., Baker, K.S., Peel, A.J., Kamins,
737 A.O., Fahr, J., Ntiamoa-Baidu, Y., Suu-Ire, R., Breiman, R.F., Epstein, J.H.,
738 Field, H.E. & Cunningham, A.A. (2012) A framework for the study of
739 zoonotic disease emergence and its drivers: spillover of bat pathogens as a
740 case study. *Philosophical Transactions of the Royal Society B: Biological
741 Sciences*, **367**, 2881-2892.
- 742 Zamora-Vilchis, I., Williams, S.E. & Johnson, C.N. (2012) Environmental
743 temperature affects prevalence of blood parasites of birds on an elevation
744 gradient: implications for disease in a warming climate. *Plos One*, **7**, e39208.
- 745 Žiegytė, R. & Valkiūnas, G. (2015) Recent advances in vector studies of avian
746 haemosporidian parasites. *Ekologija*, **60**.
- 747

748 Table 1: (a) *Zosterops* spp. sample sizes across New Caledonian islands (numbers in
 749 italics indicate published samples) included in the multivariate logistic regression. (b)
 750 observed haematozoan parasite infections and (c) co-infections across islands. Note
 751 that 449 samples were screened for *Haemoproteus* and *Plasmodium* spp., while 275
 752 samples were screened for microfilariae.
 753

(a) <i>Zosterops</i> host species	Grand Terre	Maré	Ouvéa	Lifou
<i>Z. lateralis</i>	10 (26)	5 (20)	44 (0)	27 (20)
<i>Z. xanthochrous</i>	69 (43)	38 (20)	absent	absent
<i>Z. minutus</i>	absent	absent	absent	72 (25)
<i>Z. inornatus</i>	absent	absent	absent	10 (20)
(b) Haematozoan parasites				
<i>Haemoproteus zosteropis</i>	60	36	14	9
<i>H. killangoi</i>	28	5	11	8
<i>Plasmodium</i> spp.	76	7	3	2
Microfilaria	25	12	7	0
(c) Observed co-infections				
	<i>Plasmodium</i> spp.	Microfilaria	<i>H. killangoi</i>	
<i>H. zosteropis</i>	28	33	2	
<i>H. killangoi</i>	2	1	-	

754
 755

756 **Figure legends**

757

758 Fig. 1: (a) *Zosterops* spp. sample sizes (n) on New Caledonian islands. (b)
759 Observations of haematozoan parasite infections and co-infections. Note that only 275
760 samples were screened for microfilaria. (c) Estimated odds ratios of infection
761 probability across islands. Presented are posterior modes, 50% highest posterior
762 density credible intervals (thick lines) and 95% highest posterior density credible
763 intervals (thin lines). Colours of symbols correspond to colours of islands in (a). (Full
764 page figure, 182mm)

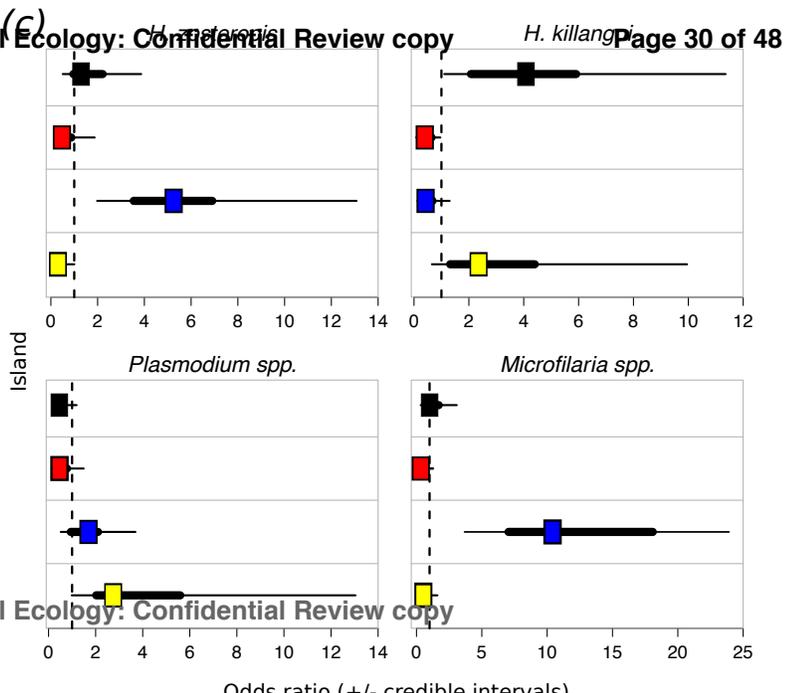
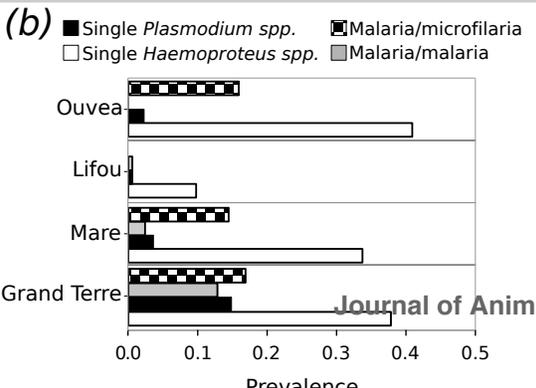
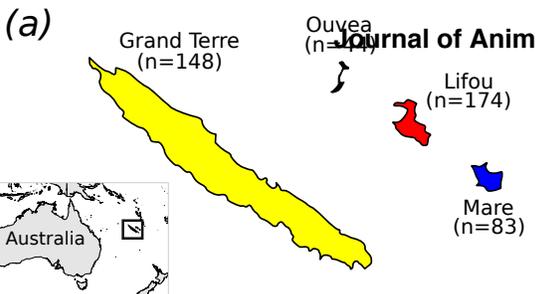
765

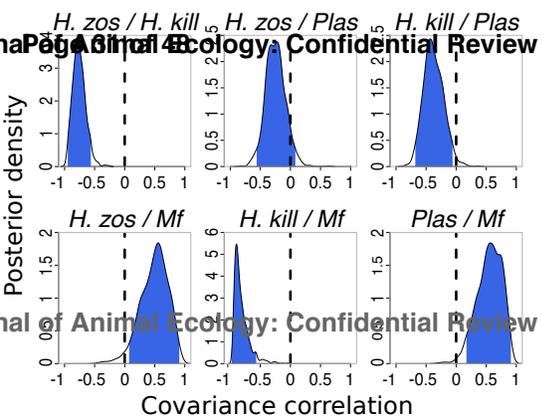
766 Fig. 2: Haematozoan parasite pairwise correlations of infection probabilities.
767 Correlations were estimated from a parasite variance-covariance matrix after
768 accounting for environmental covariates in a multivariate logistic regression. Shading
769 indicates 95% highest posterior density credible intervals. *Plas.*, *Plasmodium* spp.;
770 *Mf.*, microfilaria (Single column figure, 70mm)

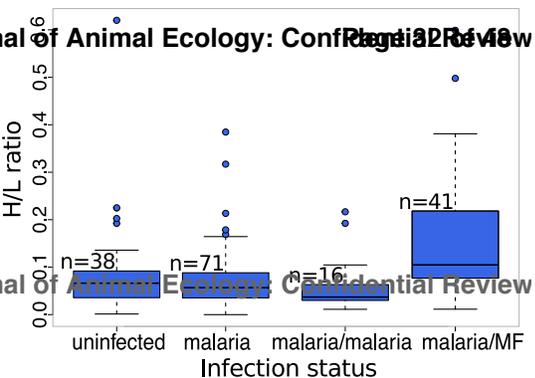
771

772 Fig. 3: Heterophil to lymphocyte ratios for *Zosterops* spp. across parasite infection
773 classes. Also presented are total sample sizes (n) for each infection. MF, microfilaria
774 (Single column figure, 70mm)

775







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Distribution of sample sites across habitats and islands in New Caledonia

Sample sites were chosen to represent the three primary forested habitats that occur in New Caledonia. These include montane rainforest (occurring in upper elevations along the central midline of Grand Terre), dry lowland forest [which occur in on the western (leeward) side of Grand Terre and on the north-western end of Ouvéa] and lowland rainforest (the principle habitat on the outer islands of Ouvéa, Lifou and Maré; Fig. S1). The final dataset included *Zosterops* infection data and *Zosterops* relative abundance data from 17 sites (Fig. S1).

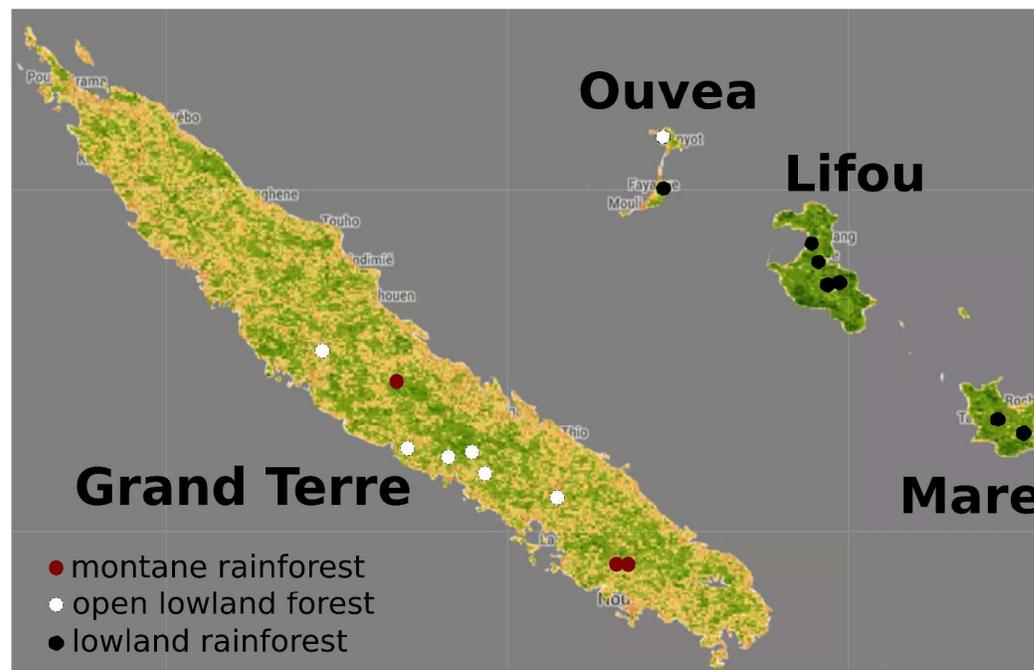


Fig. S1: Distribution of sample sites across islands and forested habitat types in New Caledonia. Colours of island landscapes represent a heat map of habitat heterogeneity, with warmer colours indicating higher heterogeneity (map accessed at <http://www.earthenv.org>)

Phylogenetic reconstruction and species identification of avian malaria lineages

Methods

We constructed a molecular phylogeny to estimate avian malaria lineage relationships. We used Akaike's information criterion in jModelTest (v 0.1.1; Posada & Crandall 1998) to determine the evolutionary model (GTR+I+G) and we used BEAST for phylogenetic reconstruction (v 1.8.1; Drummond & Rambaut 2007). We used a Yule prior for branching rates. We carried out two runs of 20 million generations, sampling at every 1000 generations for each run. We used TRACER (v 1.5; Rambaut & Drummond 2007) to test if estimated sample size (ESS) for each parameter was sufficient ($ESS > 200$) for robust estimates. We

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discarded four million generations of burn-in per run, leaving a distribution of 32000 trees. Three mammalian *Plasmodium* *cyt-b* sequences were used as outgroups. To facilitate reconstruction of relationships between morphospecies, we included malaria lineages previously detected in Australian and New Caledonian *Zosterops* spp. (Ishtiaq *et al.* 2010; Clark & Clegg 2015).

Results

We recorded 14 avian malaria lineages (six *Haemoproteus* and eight *Plasmodium*), including seven new lineages (GenBank Accessions: XXXX). Phylogenetic reconstruction revealed two distinct *Haemoproteus* clades. For Clade I, smears from four of the six lineages were inspected, with all four confirmed as the morphospecies *H. zosteropsis*. We therefore assumed that all lineages in this clade represent *H. zosteropsis* (mean within-clade divergence=1.6%; Fig. S1). The second *Haemoproteus* clade was separated from the *H. zosteropsis* clade by a mean divergence of 6.2% and was identified as *H. killangoi* (visual confirmation for three of the four lineages; Fig. S1). Both morphospecies were present on all islands.

Plasmodium lineages grouped into four clades, two of which formed the majority of *Plasmodium* lineages (12 of 16 lineages; figure S2). One of the main *Plasmodium* clades contained lineage GRW06, previously identified as *P. elongatum* by Valkiūnas *et al.* (2008). Visual inspection of smears for another lineage within this clade also identified *P. elongatum* (MYNA01P). The second major *Plasmodium* clade included lineage GRW4, previously confirmed as *P. relictum* by Beadell *et al.* (2009), and a second lineage that we confirmed as *P. relictum* (NC8P). We therefore refer to these clades as *P. elongatum* (mean within-clade divergence=2.1%) and *P. relictum* (mean within-clade divergence=1.9%).

We sequenced 41 microfilaria infections and identified three new LSU lineages (GenBank Accession numbers XXXX). Two LSU lineages occurred across the entire microfilaria distribution (ZOSMF1, ZOSMF3). The third lineage (ZOSMF2) was rare, with two recordings on Grand Terre. ZOSMF1 and ZOSMF2 were separated by a single base pair (0.1% nucleotide divergence), while ZOSMF3 was more distinct (6.0% divergence from ZOSMF1).

Sample sizes were too sparse to include each *Plasmodium* morphospecies separately in the multivariate logistic regression (MCMC chains did not reach convergence), and so *Plasmodium* infections were grouped together in a single variable. Our regression model therefore included infection data from four parasite groups (*H. zosteropsis*, *H. killangoi*, *Plasmodium* spp. and microfilaria spp.)

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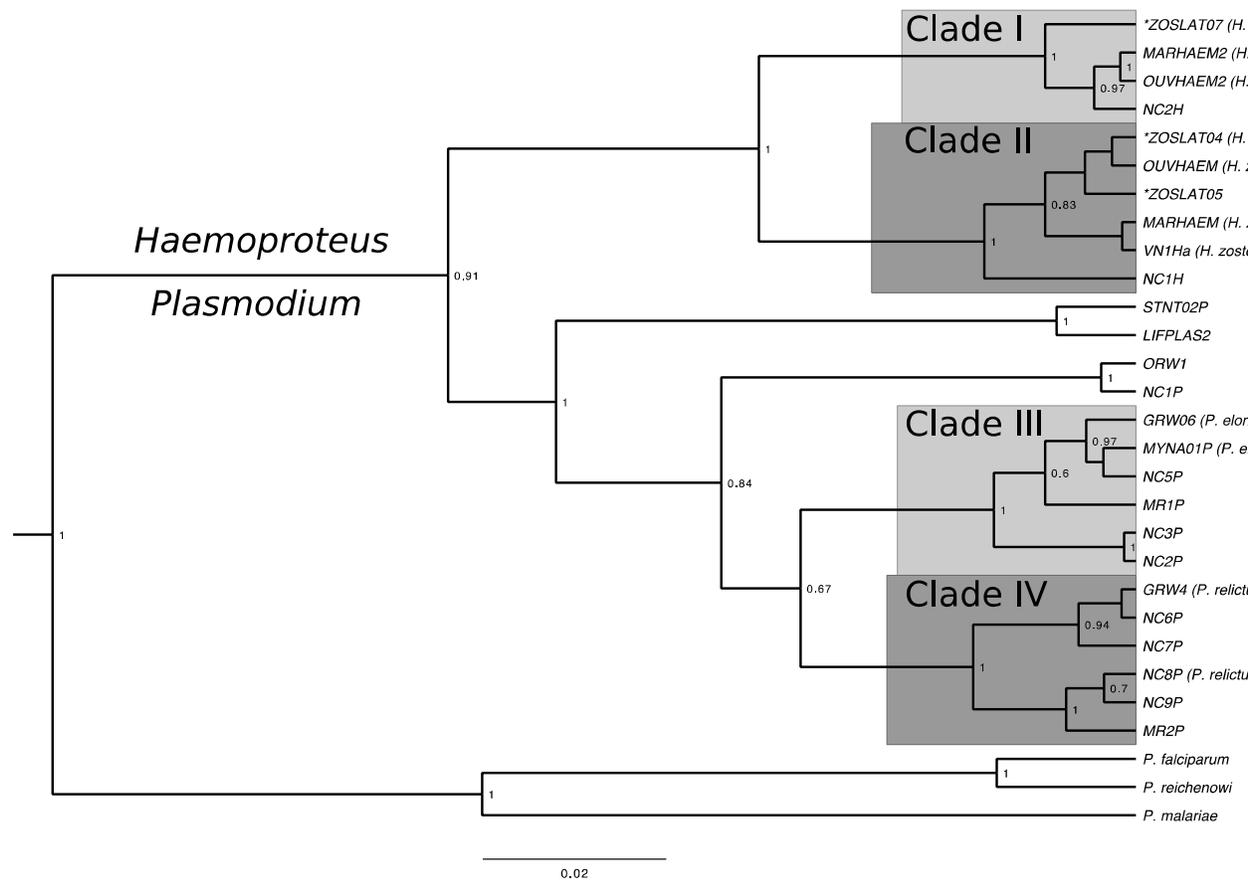


Fig. S2: Bayesian molecular phylogeny of avian malaria *cyt-b* lineages recorded in New Caledonian *Zosterops* spp. Lineages that were recorded in New Caledonian and Australian *Zosterops* spp. were included to improve phylogenetic reconstruction (Ishtiaq et al., 2010; Olsson et al., 2010). Lineages with asterisks (*) were recorded in Australian *Zosterops* spp. Shaded regions represent monophyletic clades that were visually identified as known parasite morphospecies (Clade I, *Haemoproteus killangoi*; Clade II, *H. zosteropsis*; Clade III, *Plasmodium elongatum*; Clade IV, *P. relictum*). Bootstrap values at nodes represent Bayesian posterior probabilities of branch placement.

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Parasite morphological descriptions and DNA barcoding

Haemoproteus (Parahaemoproteus) zosteropis Chakravarty and Kar, 1945

DNA sequences: Mitochondrial *cyt b* lineages ZOSLAT04, MARHAEM, OUVHAEM and VN1H (479 bp, GenBank accession numbers XXXXX).

Avian host and distribution: The lineage ZOSLAT04 has been recorded in the silvereye *Zosterops lateralis* in eastern Australia (Zamora-Vilchis, Williams & Johnson 2012; Clark & Clegg 2015; Clark *et al.* 2015). Lineage OUVHAEM was recorded in *Z. lateralis* on the island of Ouvea, New Caledonia. Lineage MARHAEM was recorded in *Z. lateralis* and the green-backed white-eye *Z. xanthochrous* on the island of Mare, New Caledonia. Lineage VN1H has been recorded in various species of white-eye throughout New Caledonia and Vanuatu (Olsson-Pons *et al.* 2015). All infections were recorded in birds caught in forested habitats using mistnets.

Site of infection: Mature erythrocytes; no other data.

Representative blood films: Voucher specimens (accession numbers XXXX) were deposited in the Queensland Museum, Brisbane, Australia. Labels data for voucher slides are: XXXX, collected by N. Clark and S. Clegg.

Young gametocytes (Fig. S3a, b) were occasionally seen in the voucher preparations, usually situated at the distal end of the infected erythrocyte; the growing gametocytes not seen in the voucher; the outline even.

Macrogametocytes (Fig. S3c–h) grow along erythrocyte nuclei and slightly enclose them with ends. Growing gametocyte usually adheres to the erythrocyte envelope and do not touch the erythrocyte nucleus forming cleft-like space between gametocyte and erythrocyte nucleus (Fig. S3c, d). Ends of medium grown gametocytes rounded (Fig. S3e). Fully-grown gametocyte filling erythrocyte up to the poles, closely appressed to the erythrocyte envelope and erythrocyte nucleus, not displace or slightly displaces the nucleus laterally (Fig. S3g, h). Dumbbell-shaped forms absent. Mature gametocytes only slightly enclose erythrocyte nuclei and never encircle them (Fig.

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S3h). The cytoplasm finely granular in appearance, frequently contains several small vacuoles usually situated next to pigment granules (Fig. S3c, g, h). The outline even. The parasite nucleus variable in shape, often roundish or oval, usually subterminal in position (Fig. S3f, g). Pigment granules are oval and roundish, of medium (0.5 to 1.0 μm) size, randomly scattered throughout the cytoplasm; their number is between 12 and 17 (on average 15.1 ± 1.0).

Microgametocytes (Fig. S3i–l). General configuration and other features are as for macrogametocytes, with usual haemosporidian sexual dimorphic characters (Valkiūnas 2005). Pigment granules roundish or rod-like of medium (0.5 to 1.0 μm) and big (1.0 to 1.5 μm) size; their number 11–17 (on average 13.8 ± 2.0).

Remarks: *Haemoproteus zosteropsis* has been frequently recorded in co-infection with *Haemoproteus killangoi* (primarily in Africa; Valkiūnas 2005), though co-infections in New Caledonia appear to be rare. *Haemoproteus zosteropsis* can be easily distinguished from *H. killangoi* based on the even outline and lack of dumbbell-shaped gametocytes. Note that the parasites from our material differ from the original neohapantotype of *H. zosteropsis* (see Valkiūnas 2005, pp. 391–394) by prominent cleft-like space between of growing macrogametocytes and erythrocyte nucleus.

Haemoproteus (Parahaemoproteus) killangoi Bennett and Peirce, 1981

DNA sequences: Mitochondrial *cyt b* lineages ZOSLAT07, OUVHAEM2, MARHAEM2 (479 bp, GenBank accession number XXXXX).

Avian host and distribution: The lineage ZOSLAT07 was recorded in the silvereye *Zosterops lateralis* in southeast Queensland, Australia. Lineage OUVHAEM2 was recorded in *Z. lateralis* on the island of Ouvea, New Caledonia. Lineage MARHAEM2 was recorded in the green-backed white-eye *Z. xanthochrous* on the island of Mare, New Caledonia. All infections were recorded in birds caught in forested habitats using mistnets.

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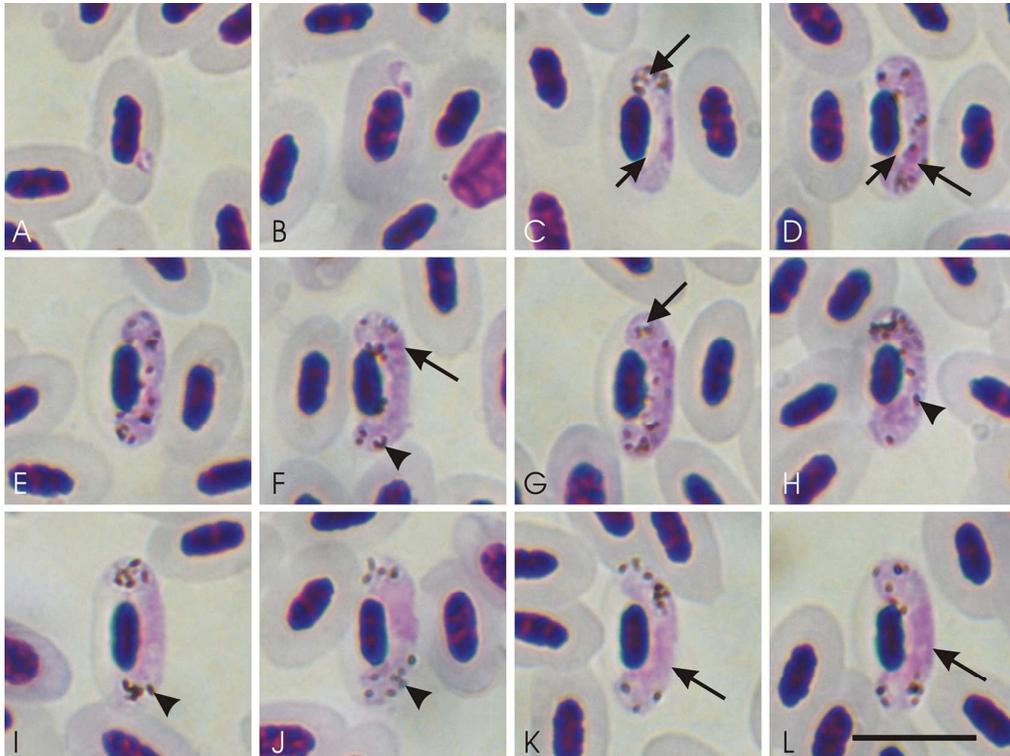


Fig. S3: Young gametocytes (a, b), macrogametocytes (c–h) and microgametocytes (i–l) of *Haemoproteus zosteropsis* from the blood of the silvereve *Zosterops lateralis*. Giemsa-stained thin blood films. Long simple arrows—nuclei of parasites; short simple arrows—unfilled spaces between gametocytes and erythrocyte nucleus; long triangle arrows—vacuoles; simple arrowheads—pigment granules. Scale bar=10 μ m

Site of infection: Mature erythrocytes; no other data.

Representative blood films: Voucher specimens (accession numbers XXXX) were deposited in the Queensland Museum, Brisbane, Australia. Labels for deposited slides are: XXXX, collected by N. Clark and S. Clegg.

Young gametocytes were not seen in voucher preparations.

Macrogametocytes (Fig. S4a–d) grow around erythrocyte nuclei and enclose them with ends but not encircle them completely. Fully grown gametocytes displace erythrocyte nucleus laterally (Fig. S4d). Enucleated erythrocytes absent. Cytoplasm finally granular, stains dark blue (basophilic) with Giemsa. Outline highly amoeboid in growing (Fig. S4a–b) and even in the fully grown gametocytes (Fig. S4d). Advanced gametocytes adhere to nuclei fill erythrocytes up to the poles, but not

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touching the central part of erythrocytes envelope causing “dip” giving dumbbell-like appearance (Fig. S4a–c). Dumbbell-shaped forms of advanced gametocytes predominate in the vouchers (80 %), but fully grown gametocyte lose dumbbell-like shape (Fig. S4d). Parasite nucleus compact, variable in shape, subterminal in position (Fig. S4b, c). Pigment granules roundish of medium size (0.5 to 1.0 μm) (Fig. S4a) and rod-like of large size (1.0 to 1.5 μm) (Fig. S4b–d); their number 7–13 (on average 10.1 ± 1.7).

Microgametocytes (Fig. S4e–h). General configuration and other features are as for macrogametocytes, with usual haemosporidian sexual dimorphic characters (Valkiūnas 2005). Pigment granules roundish of medium size (0.5 to 1.0 μm) and rod-like of large size (1.0 to 1.5 μm); their number 6–12 (on average 9.4 ± 1.4).

Remarks: *Haemoproteus killangoi* can be readily distinguished from *H. zosteropsis* based on amoeboid outlines, dumbbell shaped forms of the growing gametocytes, less number of the pigment granules and basophilic stain of the cytoplasm.

Macrogametocytes of *H. killangoi* are predominantly nucleophilic in contrast with those of *H. zosteropsis*, which closely appressed to the envelope of the erythrocyte and form prominent cleft-like space between of growing macrogametocytes and erythrocyte nucleus.

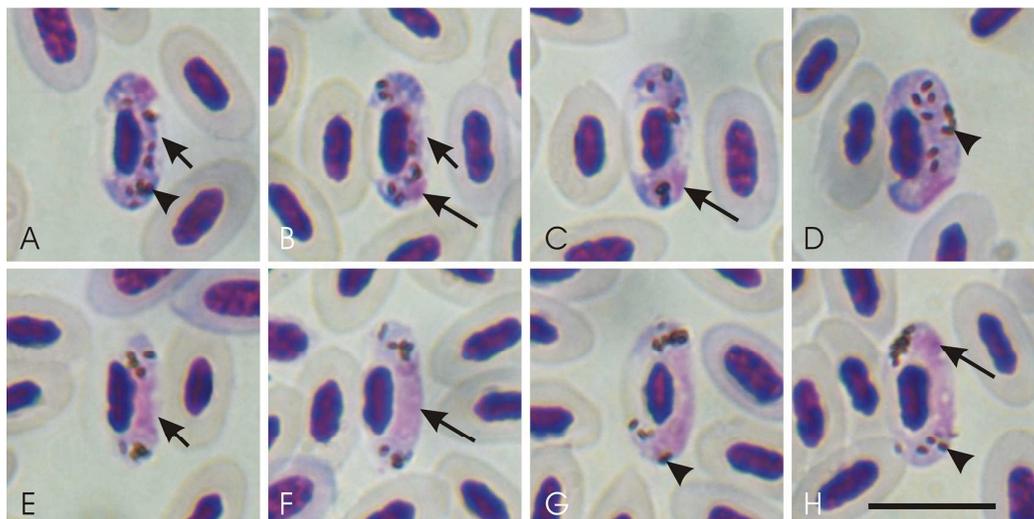


Fig. S4: Macrogametocytes (a–d) and microgametocytes (e–h) of *Haemoproteus killangoi* from the blood of the green-backed white-eye *Zosterops xanthochrous*. Giemsa-stained thin blood films. Long simple arrows—nuclei of parasites; short simple arrows—unfilled spaces between gametocytes and erythrocyte envelope; simple arrowheads—pigment granules. Scale bar=10 μm

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Table S1: Parasite PCR primers and reaction conditions. TD = touchdown (drop the anneal temperature by 1 degree per cycle)

	Primers		Volumes			Cycling Conditions		
	Forward	Reverse	Qiagen TopTaq PCR mix	Primer (each)	DNA (extraction or PCR product)	Cycle times	Annealing temps and cycles	
<i>Malaria</i>								
Nested PCR (Outside Rxn)	HAEMNF	HAEMNR 2	10uL	0.5uL	3uL (Extraction)	Denature (30 sec), Anneal (35 sec), Extend (45 sec)	60 to 55 TD (6 cycles)	53 cycles
Nested PCR (Inside Rxn)	HAEMF	HAEMR2	10uL	0.5uL	0.8uL (Nested Outside Product)	Denature (30 sec), Anneal (35 sec), Extend (45 sec)	60 to 55 TD (6 cycles)	51 cycles
<i>Haemoproteus</i> -specific Amplification	HMONLY F	HMONLY R	10uL	0.5uL	3uL (Extraction)	Denature (30 sec), Anneal (35 sec), Extend (45 sec)	60 to 55 TD (6 cycles)	51 cycles
<i>Microfilaria</i>								
LSU Screening and Amplification	Nem 1	Nem 2	10uL	0.5uL	3uL (Extraction)	Denature (30 sec), Anneal (40 sec), Extend (45 sec)	60 to 56 TD (5 cycles)	55 cycles
<i>New Primer sequences</i>								
HMONLY-F: GCAYGCYACTGGTGCTACAT								
HMONLY-R: TGCATTATCAGGATGAGCTARTGG								

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References

- Beadell, J.S., Covas, R., Gebhard, C., Ishtiaq, F., Melo, M., Schmidt, B.K., Perkins, S.L., Graves, G.R. & Fleischer, R.C. (2009) Host associations and evolutionary relationships of avian blood parasites from West Africa. *International Journal for Parasitology*, **39**, 257-266.
- Clark, N.J. & Clegg, S.M. (2015) The influence of vagrant hosts and weather patterns on the colonization and persistence of blood parasites in an island bird. *Journal of Biogeography*, **42**, 641-651.
- Clark, N.J., Olsson-Pons, S., Ishtiaq, F. & Clegg, S.M. (2015) Specialist enemies, generalist weapons and the potential spread of exotic pathogens: malaria parasites in a highly invasive bird. *International Journal for Parasitology*, **45**, 891-899.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *Bmc Evolutionary Biology*, **7**, DOI: 10.1186/1471-2148-1187-1214.
- Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F. & Sheldon, B.C. (2010) Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. *Journal of Biogeography*, **37**, 120-132.
- Nadler SA, Hudspeth, DSS. 1998 Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. *Molecular Phylogenetics and Evolution* **10**(2), 221-236.
- Olsson-Pons, S., Clark, N.J., Ishtiaq, F. & Clegg, S.M. (2015) Differences in host species relationships and biogeographic influences produce contrasting patterns of prevalence, community composition and genetic structure in two genera of avian malaria parasites in southern Melanesia. *Journal of Animal Ecology*, **84**, 985-998.
- Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.
- Rambaut, A. & Drummond, A. (2007) Tracer version 1.4. University of Edinburgh, UK.
- Sehgal RN, Jones HI, Smith TB. 2005 Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds. *Molecular Ecology* **14**(13), 3977-3988
- Waldenström J, Bensch S, Hasselquist D, Östman Ö. 2004 A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *J. Parasitol.* **90**(1), 191-194
- Zamora-Vilchis, I., Williams, S.E. & Johnson, C.N. (2012) Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. *Plos One*, **7**, e39208.

```

##Avian haematozoan parasite co-occurrences and H/L ratios analyses
library(lme4)
library(rjags)
library(MCMCpack)
library(runjags)

## Read data, set working directory
Dat <- read.csv("Clark_et_al_Supplement_RawData.csv", header=TRUE, stringsAsFactors = FALSE)

## Convert capture sessions to numeric variable
Dat$num.capture.session <- as.numeric(as.factor(Dat$Capture.session))

# Subset Zosterops data for simpler calculations of sample size and observed parasite occurrences
ZosDat<-Dat[ which(Dat$Genus=="Zosterops"),]
.d_indID <- ZosDat$Bird
.d_bird.species <- ZosDat$Species
.d_island <- ZosDat$Island
.d_capture.session <- ZosDat$num.capture.session
.d_genus <- ZosDat$Genus
.d_habitat <- ZosDat$Habitat
.d_Infected<-ZosDat$Infected
.d_infect.H.zosteropis <- ZosDat$H.zosteropis
.d_infect.H.killangoi <- ZosDat$H.killangoi
.d_Haem<-ZosDat$Haem
.d_Plas<-ZosDat$Plas
.d_infect.Microfilaria <- ZosDat$Microfilaria
.d_HLratio <- ZosDat$H.L.Ratio
.d_time <- ZosDat$Time

#####
# Model co-occurrence patterns of parasites
#####

# Data to BUGS/JAGS
island <- as.numeric(as.factor(.d_island))
habitat <- as.numeric(as.factor(.d_habitat))
hostspec <- as.numeric(as.factor(.d_bird.species))
capture.session <- as.numeric(as.factor(.d_capture.session))
nhost <- length(unique(.d_bird.species))
nisland <- length(unique(.d_island))
nhabitat <- length(unique(.d_habitat))
nind <- length(.d_indID )
ncapture.session <- length(unique(.d_capture.session))

# Calculate total number of Zosterops captured at each site using full dataset "Dat"
nzos <- rep(NA, ncapture.session)
for(i in 1:ncapture.session){
  nzos[i] <- as.numeric(length(which(Dat$num.capture.session==i & Dat$Genus=="Zosterops")))}

#Calculate total number of all birds captured at each site
ntotal <- rep(NA, ncapture.session)
for(i in 1:ncapture.session){
  ntotal[i] <- as.numeric(length(which(Dat$num.capture.session==i)))}

## Number of parasite species in modelled data
nparasite <- 4

```

```

# Identity matrix for inverse Wishart prior
W.id <- diag(nparasite)

# Create array of parasite occurrence data
InfestData <- array(NA, dim =c(nind , nparasite))
InfestData[,1] <- .d_infect.H.zosteropis
InfestData[,2] <- .d_infect.H.killangoi
InfestData[,3] <- .d_Plas
InfestData[,4] <- .d_infect.Microfilaria

data2bugs_malaria <- list(
  InfestData = InfestData,
  nhost = nhost,
  nind = nind,
  nhabitat = nhabitat,
  nparasite = nparasite,
  nisland = nisland,
  ncapture.session = ncapture.session,
  nzos = nzos,
  ntotal = ntotal,
  island = island,
  habitat = habitat,
  hostspec = hostspec,
  capture.session = capture.session,
  W.id = W.id
)

#####
# BUGS/JAGS model for multi-species parasite occurrence probabilities
#####
cat(
"model {
  for (i in 1:nind){
    for (par in 1:nparasite){

## Likelihood: link data to model
InfestData[i, par] ~ dbern(p[i, par])

## Logit link function
p[i, par] <- exp(Z[i, par]) / ( exp(Z[i, par]) + 1)

## Model of environmental covariates
Mu[i, par] <- mu0[par] + b.hostsp.star[par, hostspec[i]] + b.island.star[par, island[i]]
+ b.habitat.star[par, habitat[i]] + beta1[par] * prop.zosterops.scale[capture.session[i]]
}

## Multivariate model of interspecific parasite co-occurrence in any bird
Z[i, 1:nparasite] ~ dmnorm(Mu[i, ], Tau[,])
}

## Priors.
##Normal w/ variance 2.71 (dnorm(x,0.368), appropriate for logit estimates (Lunn et al. 2012)
## mu0 mean -1 centres prev. estimates ~ 27% (inv.logit(-1)=0.27), more realistic than mean 0 (50% prev)
for(par in 1:nparasite) {
  mu0[par] ~ dnorm(-1, 0.368)
  beta1[par] ~ dnorm(0, 0.368)
}

```

```

for(h in 1:nhost){
  b.hostsp[par, h] ~ dnorm(0, 0.368)
##Redundancy parameters for covariates (.star) help speed up convergence
  b.hostsp.star[par, h] <- b.hostsp[par, h] - mean(b.hostsp[par,])
}

for(b in 1:nisland){
  b.island[par, b] ~ dnorm(0, 0.368)
  b.island.star[par, b] <- b.island[par, b] - mean(b.island[par,])
}

for(d in 1:nhabitat){
  b.habitat[par, d] ~ dnorm(0, 0.368)
  b.habitat.star[par, d] <- b.habitat[par, d] - mean(b.habitat[par,])
}
}

## Model proportion of Zosterops in each site, with possible values between 0.05 and 0.9
## Centre and standardise prop.zosterops estimates to improve interpretation of model intercept mu0
for(c in 1:ncapture.session){
  nzos[c] ~ dbinom(prop.zosterops[c], ntotal[c])
  prop.zosterops[c] ~ dbeta(2,2)T(0.05,0.9)
  prop.zosterops.scale[c] <- (prop.zosterops[c] - prop.zosterops.mean) / prop.zosterops.sd
}
prop.zosterops.mean <- mean(prop.zosterops[])
prop.zosterops.sd <- sd(prop.zosterops[])

#Scaled inverse Wishart, (equally likely with values between -1 and 1; Gelman & Hill 2007)
#df = K+ 1 sets uniform distribution on individual correlation parameters
Tau[1:nparasite, 1:nparasite] ~ dwish(W.id[, ], df)
df <- nparasite + 1
Sigma.Covar.raw[1:nparasite, 1:nparasite] <- inverse(Tau[ , ])
for(p in 1:nparasite){
  for(p.prime in 1:nparasite){
    rho[p,p.prime] <-
Sigma.Covar.raw[p,p.prime]/sqrt(Sigma.Covar.raw[p,p]*Sigma.Covar.raw[p.prime,p.prime])
  }
  sigma.sp[p] <- abs(xi.sp[p]) * sqrt(Sigma.Covar.raw[p,p])
  #scaling factor for scaled inverse Wishart
  xi.sp[p] ~ dunif(0,100)
}
#####
#Posterior predictive check
# Absolute residuals for logistic model
for (i in 1:nind){
  for (par in 1:nparasite){
    resid.infest_data[i, par] <- abs(InfestData[i, par] - p[i, par])
    Infest_sim[i, par] ~ dbern(p[i, par])
    resid.infest_sim[i, par] <- abs(Infest_sim[i, par] - p[i, par])
  }
}
fit_data <- sum(resid.infest_data[,])
fit_sim <- sum(resid.infest_sim [,])
test_fit <- step(fit_sim - fit_data)
}
"
, file=(model2bugs_malaria <- tempfile()))

```

```
#####
# RUN model via rjags
#####

param = c('b.island.star','b.habitat.star','b.hostsp.star','beta1','mu0','rho','test_fit')
na = 50000
nb = 250000
ni = 500000
nc = 2

rjags_malaria <- jags.model(model2bugs_malaria, data= data2bugs_malaria,
                           n.chains=nc , n.adapt = na)

update(rjags_malaria , nb)

out <- jags.samples(rjags_malaria, param, ni, thin=1000)

save(out, file="out-rjags_MalariaModel.RData")

#Gelman diagnostics for continuous estimates to check for mixture of chains
gelman.diag(out[["beta1"]])
gelman.diag(out[["mu0"]])
gelman.plot(out[["beta1"]])
gelman.plot(out[["mu0"]])

#Trace plots to individually check convergence for each parasite-covariate combination
plot(out$mu0[1, ,1], ylim = range(out$mu0), type='l')
lines(out$mu0[1, ,2], col =2)

plot(out$b.island.star[1,1, ,1], ylim = range(out$b.island.star), type='l')
lines(out$b.island.star[1,1, ,2], col =2)

###etc...for additional parasite/covariate combinations

##Check predictive fit ratio of 1's to 0's in the 1000 samples (500 per chain)
##Test fit of 1 indicates simulated residuals are greater than observed, 0 indicates the opposite
##A ratio of 0.4 to 0.6 indicates no discrepancy
sum(out$test_fit[, ,])/1000

#####
# Posterior processing
#####
#### Function to calculate posterior density credible intervals
hpd <- function(x, coverage)
{
  require(coda)
  x <- as.matrix(x)
  out <- matrix(NA, nrow=ncol(x), ncol=3)
  rownames(out) <- dimnames(x)[[2]]
  colnames(out) <- c("mode", "lower", "upper")
  f <- function(p) {
    if (p == density.range[2]) {
      set.coverage <- 0
    }
  }
  else {
    p.upper <- min(y.density$y[y.density$y > p])
  }
}
```

```

    p.lower <- max(y.density$y[y.density$y <= p])
    cov.upper <- sum(y.counts[y.density$y >= p.upper])/sum(y.counts)
    cov.lower <- sum(y.counts[y.density$y >= p.lower])/sum(y.counts)
    c <- (p.upper - p)/(p.upper - p.lower)
    set.coverage <- c * cov.upper + (1 - c) * cov.lower
  }
  return(set.coverage - coverage)
}
for (i in 1:ncol(x)) {
  y <- unclass(x[,i])
  y.density <- density(y, n=1024)
  m <- length(y.density$x)
  ## Find the mode
  out[i,1] <- y.density$x[which.max(y.density$y)]
  dx <- diff(range(y.density$x))/m
  breaks <- c(y.density$x[1] - dx/2, y.density$x + dx/2)
  y.counts <- hist(y, breaks=breaks, plot=FALSE)$counts
  density.range <- range(y.density$y)
  uniroot.out <- uniroot(f, density.range)
  ## Assuming that we have a single interval, find the limits
  out[i,2:3] <- range(y.density$x[y.density$y > uniroot.out$root])
  ## Check!
  if (sum(abs(diff(y.density$y > uniroot.out$root))) != 2) {
    warning("HPD set is not a closed interval for variable ",
           varnames(x)[i])
  }
}
return(out)
}

# Calculate posterior modes / 95% credible intervals for model parameters
##Change 'coverage' to 0.5 for 50% credible intervals if desired

##Calculate credible intervals for correlation matrix of parasite probabilities
Rho.95 <- array(NA, dim = c(nparasite, nparasite, 3))
for(i in 1:nparasite){
  for(j in 1:nparasite){
    Rho.95[i,j,] <- hpd(c(out$rho[i,j, , 1],
                       out$rho[i,j, , 2]), coverage = 0.95) }
}

##Calculate credible intervals for intercept (mu0) and covariates
mu0.95 <- array(NA, dim = c(nparasite, 3))
for(i in 1:nparasite){
  mu0.95[i,] <- round(hpd(c(out$mu0[i, , 1],out$mu0[i, , 2]),
                        coverage = 0.95), 3)
}

##Inverse logit function translates mu0 estimates into prevalence estimates
inverse.logit<-function(x){
  exp(x)/(1+exp(x))
}

inverse.logit(mu0.95)

b.island.95 <- array(NA, dim = c(nparasite, nisland, 3))
for(i in 1:nparasite){

```

```

    for(s in 1:nisland){
      b.island.95[i,s] <- round(hpd(c(out$b.island.star[i, s, , 1],
    out$b.island.star[i, s, , 2]), coverage = 0.95), 3)
    }

##Exponentiate coefficients for covariates to translate estimates to odds ratios
exp(b.island.95)

###etc...for additional covariates

###Calculate proportion of variance explained for each covariate
## Combine chains for each covariate
library(abind)
Burn.island<-abind(out$b.island.star[, , 1],out$b.island.star[, , 2])
Burn.habitat<-abind(out$b.habitat.star[, , 1],out$b.habitat.star[, , 2])
Burn.hostsp<-abind(out$b.hostsp.star[, , 1],out$b.hostsp.star[, , 2])
Burn.propzos<-abind(out$beta1[, , 1],out$beta1[, , 2])
Burn.totzos<-abind(out$beta2[, , 1],out$beta2[, , 2])
Burn.mu0<-abind(out$mu0[, , 1],out$mu0[, , 2])

##Function to calculate squared values
make.power <- function(n) {
  pow <- function(x) { x^n}
  pow }
square <- make.power(2)

##Function to calculate proportion of environmental variance for each covariate
variance <- function(parasite, coverage)
{
  Variance <- array(NA, dim = c(4, 3))
  rownames(Variance)<-c('Island','Habitat','Host.species','RelAbundZos')
  island.var<-vector()
  habitat.var<-vector()
  hostsp.var<-vector()
  propzos.var<-vector()
  mu.var<-vector()
  Totalvar<-vector()
  FullVar<-matrix(NA,nrow=1000,ncol=4)
  colnames(FullVar)<-c('Island','Habitat','Host.species',
    'RelAbundZos')
  for(i in 1:1000){
    island.var[i] = square(sd(Burn.island[parasite,,i]))
    habitat.var[i] = square(sd(Burn.habitat[parasite,,i]))
    hostsp.var[i] = square(sd(Burn.hostsp[parasite,,i]))
    propzos.var[i] = (Burn.propzos[parasite,i])*(Burn.propzos[parasite,i])
    Totalvar[i]=island.var[i]+propzos.var[i]+habitat.var[i]+hostsp.var[i]
    FullVar[i,1]=island.var[i]/Totalvar[i]
    FullVar[i,2]=habitat.var[i]/Totalvar[i]
    FullVar[i,3]=hostsp.var[i]/Totalvar[i]
    FullVar[i,4]=propzos.var[i]/Totalvar[i]
  }
  for(m in 1:4){
    Variance[m, ]=hpd(FullVar[, m], coverage=coverage)
  }
  return(Variance)
}

```

```
##Calculate proportions of environmental variance
h.zosvar.95<-variance(1, 0.95)

###etc... for additional parasites

#####
# Analyse variation in H/L ratios
#####

##Remove H/L ratio NAs
ZosDat2014<-ZosDat[ which(ZosDat$Data.source=="This study"),]
ZosDat2014<-ZosDat2014[!(is.na(ZosDat2014$H.L.Ratio)),]

##Logit transform H/L ratios, appropriate for skewed proportional data
HLratio.logit <- log((ZosDat2014$H.L.Ratio+0.00001)/ (1- (ZosDat2014$H.L.Ratio+0.00001)))

##Run linear regression using lmer
fullmod.logittrans <-lmer(HLratio.logit~ZosDat2014$Microfilaria+ZosDat2014$Haem*ZosDat2014$Plas+
  ZosDat2014$Time+(1|ZosDat2014$Island)+(1|ZosDat2014$Species),
  REML=F)

#Perform model comparisons, compute AIC scores
fullmod.logittrans.noMF <-lmer(HLratio.logit~ZosDat2014$Time+ZosDat2014$Haem*ZosDat2014$Plas+
  (1|ZosDat2014$Island)+(1|ZosDat2014$Species),REML=F)
anova(fullmod.logittrans,fullmod.logittrans.noMF)

###etc... for additional models
```