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## Comparative analysis of spatial genetic structure in an ant-plant symbiosis reveals a tension zone and highlights speciation processes in tropical Africa

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1 **Original article**

2 **Comparative analysis of spatial genetic**  
3 **structure in an ant-plant symbiosis reveals a**  
4 **tension zone and highlights speciation**  
5 **processes in tropical Africa**  
6

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30

## 31 **ABSTRACT**

### 32 **Aim**

33 Pleistocene climatic oscillations induced range fluctuations in African rain forest organisms and  
34 may have shaped species diversification through allopatric speciation events. We compared the  
35 spatial genetic structure of two forest species that live in obligate symbiosis and thus must have  
36 experienced the same range fluctuations, as a means to discriminate incipient speciation from  
37 transient differentiation simply resulting from past divergence.

### 38 **Location**

39 Western central Africa.

### 40 **Methods**

41 We genotyped 765 individuals of the tree *Barteria fistulosa* and 605 colonies of its symbiotic ant  
42 *Tetraponera aethiops* at 12 and 13 microsatellite loci respectively. We compared the spatial genetic  
43 structure of the two symbionts by using Bayesian clustering algorithms, isolation-by-distance  
44 analyses and clines of synthetic alleles. We used species niche modelling (climatic and soil  
45 variables) to investigate ecological variables associated with genetic discontinuities in tree  
46 populations.

### 47 **Results**

48 The trees and the ants showed congruent patterns of spatial genetic structure. However, the trees  
49 showed a very steep genetic discontinuity between groups north and south of latitude 1°N, which  
50 was much weaker in the ants. There was no evidence for effective gene flow between the two tree

51 lineages in contact at the transition zone, despite the presence of a few hybrids. Niche modelling did  
52 not predict the occurrence of northern trees south of this genetic transition, and vice versa.

### 53 **Main conclusions**

54 The genetic discontinuity near latitude 1°N is inferred to be a tension zone resulting from  
55 reproductive incompatibilities between previously allopatric tree lineages. This tension zone may  
56 have stabilized at a climatic transition (between boreal and austral seasonal regimes), and matches  
57 patterns of genetic structure previously observed in other forest plant species. Our results illustrate  
58 independent speciation between two species that live in specific and obligate symbiosis and suggest  
59 that a tension zone may separate lineages of several central African forest plants near the thermal  
60 equator.

### 61 **KEYWORDS**

62 Africa, *Barteria*, climatic oscillations, Guineo-Congolian rainforest, incipient speciation,  
63 phylogeography, Pleistocene, symbiosis, tension zone, *Tetraoponera*.

64

## 65 INTRODUCTION

66           During the Pleistocene, Central African populations of forest-dependent species experienced  
67 cycles of contraction and expansion (Maley, 1996). Because there are few mountainous areas in this  
68 zone, populations of forest species had little opportunity to escape climate changes by altitudinal  
69 shifts. As a consequence, the spatial extent of forest contraction/expansion cycles must have led to  
70 strong population reduction and fragmentation, and species extinctions, possibly explaining the  
71 lower biodiversity observed in tropical Africa than in tropical America and Southeast Asia (Morley,  
72 2000). Extinctions may however be balanced by speciation events, as populations occupying  
73 fragmented forest cover may evolve in isolation for considerable periods of time. This process may  
74 account for several plant species radiations that were shown to have occurred during the Pliocene  
75 and the Pleistocene (Harris, 2000; Wieringa & Gervais, 2003; Plana *et al.*, 2004; Couvreur *et al.*,  
76 2011).

77           The spatial genetic structure of many plant and animal species of the tropical rain forest has  
78 recently been described in the domain of Lower Guinea (i.e., western Central Africa) in order to  
79 investigate the types and locations of putative forest refugia during the Pleistocene (plants: Hardy *et al.*  
80 *et al.*, 2013; Dauby *et al.*, 2014; Heuertz *et al.*, 2014; Ley *et al.*, 2014; Duminil *et al.*, 2015; Faye,  
81 2015; animals: Smith *et al.*, 2000; Telfer *et al.*, 2003; Bowie *et al.*, 2006; Anthony *et al.*, 2007;  
82 Gonder *et al.*, 2011; Nicolas *et al.*, 2011). Notably, 10 of the 11 plant species investigated present a  
83 genetic discontinuity between populations north and south of latitude  $\sim 1\text{-}3^\circ\text{N}$ . A similar genetic  
84 discontinuity is reported in the tree *Barteria fistulosa* Mast. (Passifloraceae) (Peccoud *et al.*, 2013).  
85 This recurrent genetic discontinuity is even more intriguing because the region does not show any  
86 obvious physical barrier. It is therefore tempting to assume the existence of a reproductive barrier  
87 between north and south lineages of several Central African forest plants, indicating a broad pattern  
88 of incipient allopatric speciation. However, strong genetic discontinuities not matching any obvious  
89 physical barrier within continuous species ranges may simply constitute remnants of past

90 divergences that subsequent gene flow may erode. Addressing this uncertainty requires a detailed  
91 investigation of the contact zone between genetic groups.

92         The tree *B. fistulosa* offers a unique opportunity to test whether the observed north-south  
93 discontinuity is a sign of incipient speciation or a transient imprint of past divergence. Indeed, this  
94 tree lives in obligate and specific symbiosis with the ant *Tetraponera aethiops* Smith, 1877  
95 (Hymenoptera: Formicidae), which means that both organisms experienced the exact same range  
96 fluctuations. Habitat fragmentation should therefore leave comparable genetic discontinuities  
97 between the two partners. However, symbionts and hosts usually have very different life history  
98 characteristics (e.g., generation time, dispersal range, mutation rate) so that their genetic structures  
99 may erode at different rates (Nieberding & Olivieri, 2007). Symbiotic partners may also differ in  
100 respect to the strength of reproductive barriers that have evolved during geographical isolation, and  
101 which govern the maintenance of genetic discontinuities if secondary contact occurs. As a result,  
102 comparing genetic discontinuities in populations of obligate symbionts may inform on the presence  
103 of such reproductive barriers and thus, on the evolutionary consequences of forest fragmentation  
104 due to climatic fluctuations. This symbiosis is widely distributed in the lowlands of central Africa,  
105 though restricted to the tropical rainforest, such that populations of the two species have  
106 experienced fragmentation, isolation and recent expansion that are expected to have created spatial  
107 genetic structure. Moreover, the two species reproduce by outcrossing, which allows interpreting  
108 population genetic results in the same way for both. Finally, the association has to be re-established  
109 anew at each generation, which ensures that signatures of population dynamics at neutral markers  
110 are independent between the two species.

111         Here, we distinguish between reproductive isolation and transient differentiation resulting  
112 simply from past divergence by confronting the spatial genetic structures of populations of the two  
113 obligate symbionts, *B. fistulosa* and *T. aethiops*. We particularly sampled along a transect in a  
114 region of supposed contact between two previously identified genetic groups of *B. fistulosa*

115 (Peccoud *et al.*, 2013).

## 116 **MATERIALS AND METHODS**

### 117 **Study system**

118 *Barteria fistulosa* trees have swollen and hollow branches presenting cavities called domatia in  
119 which nests the large black ant *T. aethiops*. A closely related ant species, *T. latifrons* Emery, 1912,  
120 is occasionally associated with *B. fistulosa*, although much less frequently than *T. aethiops*. The two  
121 ant species can be distinguished unambiguously and *T. latifrons* is not considered in this paper.  
122 Each tree is occupied by a single colony (Yumoto & Maruhashi, 1999; Blatrix and Djiéto-Lordon,  
123 unpublished field observations). The ant has a powerful sting and protects its host tree from  
124 herbivorous insects (Janzen, 1972; Dejean *et al.*, 2008) and vertebrates (McKey, 1974). It also  
125 prunes surrounding vegetation (Janzen, 1972; Yumoto & Maruhashi, 1999). In return, the ant  
126 colony gets most of its food resources from the tree through exploitation of extrafloral nectar and of  
127 hemipterans reared within domatia (Bequaert, 1922; Janzen, 1972). Fungi grown within domatia  
128 constitute an additional food source for the ants (Blatrix *et al.*, 2012).

### 129 **Study area and sampling**

130 Trees of *B. fistulosa* and associated *T. aethiops* ants were sampled in western Central Africa, across  
131 the Lower Guinea domain (Cameroon, Gabon, Republic of Congo) (see Appendices S1 and S2 in  
132 Supporting Information). Leaf samples were dried with silica gel immediately upon collection.  
133 Additional samples were obtained from the National Herbarium of the Netherlands (WAG) and the  
134 Royal Botanic Garden Edinburgh (E), Scotland. Samples of the symbiotic ant *T. aethiops* were  
135 stored in 70% ethanol or dried with silica gel. Since each *B. fistulosa* tree is occupied by a single  
136 colony of *T. aethiops*, we used only one individual ant from each tree for genetic analyses.

## 137           **Molecular methods**

138 DNA was extracted from ~0.2 g of dry leaf for each tree using the DNeasy Plant Mini Kit (Qiagen,  
139 Venlo, Netherlands), and from the head of each ant with the DNeasy Tissue Kit (Qiagen), or with  
140 the Extract-N-Amp PCR ReadyMix (Sigma-Aldrich, St. Louis, USA) for both ant and tree  
141 specimens, following the manufacturers' instructions. Each individual of *B. fistulosa* was genotyped  
142 for 12 microsatellite markers (Bar6, Bar16, Bar31, Bar50, Bar51, Bar53, Bar56, Bar57, Bar58,  
143 Bar62, Bar64, Bar69) following a protocol described previously (Molecular Ecology Resources  
144 Primer Development Consortium *et al.*, 2012b). Each individual of *T. aethiops* was genotyped for  
145 13 microsatellite markers (Molecular Ecology Resources Primer Development Consortium *et al.*,  
146 2012a). We used a total of 765 and 605 individuals for the tree and the ant respectively, including  
147 358 tree individuals that were genotyped for 11 loci by Peccoud *et al.* (2013).

## 148           **Spatial genetic structure**

149 To assess spatial genetic structure, we first used the Bayesian clustering algorithm implemented in  
150 the software STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), which uses the genotypic data alone.  
151 STRUCTURE was run 10 times for each number of groups assumed ( $K$  from 1 to 10), considering  
152 admixture and independent allele frequencies between groups (Falush *et al.*, 2003), with the  
153 admixture parameter  $\alpha$  inferred from the data and given a uniform prior. Each run consisted of  
154 1 000 000 iterations, including a burn-in of 200 000. Runs for each value of  $K$  were summarised  
155 using CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007). The most likely number of groups was  
156 determined using the method of Evanno *et al.* (2005) implemented in STRUCTURE HARVESTER (Earl  
157 & vonHoldt, 2012).

158           To take spatial coordinates of sampled individuals into account, we also used the Bayesian  
159 clustering algorithm implemented in the software TESS 2.3 (François *et al.*, 2006). This approach is  
160 less likely than STRUCTURE to identify genetic discontinuities that may result from uneven  
161 sampling. TESS was run 10 times for each  $K$  from 2 to 10, using the admixture BYM model. Each



162 run consisted of 200 000 iterations, including a burn-in of 50 000. The interaction parameter was set  
163 to 0.6. The most likely number of groups was determined by comparing the deviance information  
164 criterion (DIC) among values of  $K$ . Congruence between the groups defined by STRUCTURE and  
165 those defined by TESS was evaluated by computing the proportion of individuals that were not  
166 assigned to the same group in the two analyses.

167       To assess the reality of the genetic discontinuities identified by the Bayesian clustering  
168 algorithms, pairwise kinship coefficients (Loiselle *et al.*, 1995) were computed for pairs of  
169 individuals within groups, pairs among groups and all pairs. In the presence of a genetic  
170 discontinuity, it is expected that kinship between individuals of different groups is lower than that  
171 between individuals within the same group, for the same geographical distance. Kinship coefficients  
172 were computed using SPAGEDI 1.4 (Hardy & Vekemans, 2002) for each of 10 categories of spatial  
173 distances defined so that the number of pairwise comparisons was similar across categories.  
174 Jackknife standard errors were computed over loci. For each level of partition of the pairwise  
175 kinship coefficients—within groups, among groups and all pairs—we drew either a logarithmic or a  
176 linear regression curve, whichever best fitted the data. Best fit was determined by comparing the  
177 coefficients of determination computed by SPAGEDI. To test for spatial genetic structure, the slope  
178 of the regression was tested by 9 999 random permutations of locations. Pairwise  $F_{ST}$  values among  
179 groups were calculated with SPAGEDI using the ANOVA approach (Weir & Cockerham, 1984).

180       We then focused on the north-south genetic discontinuity already noticed in a previous study  
181 (Peccoud *et al.*, 2013), which proved to be congruent between the ant and the tree in the present  
182 study. We compared tree and ant genetic data in respect to the abruptness of the discontinuity,  
183 independently of differences in allelic frequencies, by building synthetic alleles (Bierne *et al.*, 2002)  
184 and considering  $K = 2$  genetic groups for both species. In each species, allelic frequencies were  
185 obtained from SPAGEDI for each locus and for each of the two groups. Each allele was attributed to  
186 the group in which its frequency was higher, and was then replaced by this group name in the

187 individual genotypes, creating a synthetic allele. This allowed computing the frequency of each  
188 synthetic allele for each individual (averaged across loci), which we call  $y$ . A generalized logistic  
189 function was fitted using the following formula:  $y = a + (b-a)/(1+e^{c(x-d)})$ , where  $x$  is the individual's  
190 coordinates along a transect perpendicular to the genetic discontinuity,  $a$  is the low asymptote,  $b$  the  
191 high asymptote,  $c$  the slope of the tangent at the inflexion point, and  $d$  the abscissa of the inflexion  
192 point. Parameters  $a$ ,  $b$  and  $c$  describe the shape of the regression and allow comparing the  
193 abruptness of the genetic discontinuity between the tree and the ant.

194 As we highlighted a particularly abrupt genetic discontinuity for the tree, we searched for  
195 potential hybrids using the Bayesian method implemented in NEWHYBRIDS 1.1 (Anderson &  
196 Thompson, 2002). Individuals were assigned to one of the five following categories: each of the  
197 two purebred parents, F1 and each of the two first-generation backcrosses. Uniform priors were  
198 used. The Markov chain was run for 1 000 000 iterations, including a burn-in of 500 000.

199 To assess whether the difference in strength of the genetic discontinuity between ants and  
200 trees could be explained by different dispersal abilities, we compared degrees of isolation-by-  
201 distance of the two species. To limit the influence of genetic discontinuities that may interfere with  
202 the estimate of isolation-by-distance, we restricted this analysis to within genetic groups (for two  
203 groups, including the most geographically widespread), that were congruent between the tree and  
204 the ant. For pairs of individuals of this group, average pairwise kinship coefficients (Loiselle *et al.*,  
205 1995) established with SPAGED1 were plotted on 10 categories of spatial distances defined so that the  
206 number of pairwise comparisons was approximately constant across categories.

## 207 **Species distribution modelling**

208 To explore the possibility that the main spatial genetic discontinuity in *B. fistulosa* could match with  
209 an environmental discontinuity (genotype-environment association), we built a species distribution  
210 model based on environmental variables for each of the two genetic groups independently and we  
211 checked whether each group was predicted to occur on the opposite side of the genetic

212 discontinuity. We used the R package ‘biomod2’ (Thuiller *et al.*, 2013) with the “ensemble  
213 modelling” approach. We selected seven climatic and five soil variables (see below) out of the 19  
214 and seven from WorldClim (Hijmans *et al.*, 2005) and Africa Soil Profiles Database  
215 (<http://www.isric.org/data/soil-property-maps-africa-1-km>) respectively. Variables from the Africa  
216 Soil Profiles Database contained values for six soil depths that we averaged for this study. The  
217 purpose of variable selection was to remove strong correlation among variables used in the  
218 modelling procedure. Among correlated variables, we selected the one that appeared the most  
219 meaningful biologically according to our knowledge of the organisms. After selection, pairwise  
220 correlations between variables were  $< 0.7$ . Variables used were (names in parentheses are names of  
221 the variables in their respective databases): annual mean temperature (BIO1), maximum  
222 temperature of warmest month (BIO5), minimum temperature of coldest month (BIO6),  
223 precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of  
224 warmest quarter (BIO18), precipitation of coldest quarter (BIO19), pH (PHIHO5), soil organic  
225 carbon (ORCDRC), sand content (SNDPPT), silt content (SLTPPT) and bulk density (BLD). All  
226 variables were used at a 10 min. resolution. We used presence data for the focal genetic group only.  
227 Presence data of the non-focal group were coded as *no data*, like all the cells for which we lacked  
228 presence data. As there were no true absence data, five repetitions of 100 pseudo-absences were  
229 generated following the *Surface Range Envelope* strategy. Nine different distribution models were  
230 built (GLM, GBM, GAM, ANN, SRE, CTA, RF, MARS and FDA; for definition of the models see  
231 reference manual of ‘biomod2’ at <https://cran.r-project.org/web/packages/biomod2/biomod2.pdf>).  
232 Model evaluation was based on a five-fold cross-validation by randomly splitting the data set into  
233 two subsets, one for calibrating the model (70 % of the presence data) and one for testing the model  
234 (30 % of the presence data). After removing modelling runs with an evaluation TSS score  $< 0.7$ ,  
235 individual models were combined with the committee averaging algorithm (i.e., model probabilities  
236 are transformed into binary data which are averaged over models). The resulting meta-model was

237 projected on the current variables. To determine which environmental variables were most  
238 important in explaining the distribution of the focal genetic group we normalized the relative  
239 contributions of each variable to each run of each model and plotted the average over the 25 runs of  
240 each model for each variable (five independent runs  $\times$  five repetitions of generation of pseudo-  
241 absences).

242 Statistical analyses and figures, unless specified otherwise, were performed using R 3.1.0 (R  
243 Core Team, 2014).

## 244 **RESULTS**

245 Individual genotypes and collection details are presented in Appendices S1 and S2.

### 246 **Spatial genetic structure**

247 For the tree, DIC values obtained with TESS began to stabilize at  $K = 4$  groups (Appendix S3).  
248 However, the  $\Delta K$  value was highest for  $K = 2$ . Thus, both values were investigated. For the ant, both  
249 DIC values and  $\Delta K$  indicate that the most likely number of groups is four (Appendix S3). For both  
250 the tree and the ant, the genetic groups defined by TESS and STRUCTURE for  $K = 4$  were highly  
251 congruent (only 9 % and 7 % of the tree and ant individuals respectively were not assigned to the  
252 same group), indicating that the two algorithms gave similar results. For  $K = 2$ , there was no  
253 mismatch at all for the tree and only 0.5 % of the ant individuals mismatched. We thus chose to  
254 consider only the results obtained with STRUCTURE in the subsequent analyses (Fig. 1a). Kinship  
255 coefficients were higher for pairwise comparisons within than among groups, whatever the  
256 geographic distance (Fig. 1b), hence the genetic discontinuities are real and not the product of  
257 isolation-by-distance coupled with uneven sampling. Regression of kinship coefficient on spatial  
258 distance was significant for pairwise comparisons within groups, among groups and among all  
259 individuals for both the ant and the tree, indicating spatial genetic structure. Kinship coefficients for  
260 pairs of trees belonging to different groups (Fig. 1b, circles), and for all trees (diamonds), were

261 better fitted by a linear than by a logarithmic regression, indicating that a process other than  
262 isolation-by-distance is involved in the spatial genetic structure of *B. fistulosa* (Born *et al.*, 2008).  
263 This was not the case for *T. aethiops*.

264 In each species, the four genetic groups are spatially segregated and their distributions appear  
265 congruent between the tree and the ant (Fig. 1a). However, the southernmost group of trees was  
266 highly differentiated from the three other ones ( $F_{ST} = 0.38$ ), congruent with results from Peccoud *et*  
267 *al.* (2013), a pattern that did not occur in the ant (Fig. 1a). In contrast, the main genetic  
268 discontinuity in the ant was that between the northernmost group and the others.

### 269 **Characterisation of the north-south genetic discontinuity**

270 The frequencies of synthetic alleles representative of the northern groups (assuming  $K = 2$  for  
271 trees and for ants) along a latitudinal transect (Fig. 2) confirm that the north-south genetic  
272 discontinuity is much more abrupt in trees. Not only do asymptotes from either side of the  
273 discontinuity represent more extreme (high or low) allelic frequencies in trees, but also the slope of  
274 the tangent at the inflexion point is steeper for the tree (parameter  $c = 6.79$ ) than for the ant  
275 ( $c = 2.81$ ).

276 The NEWHYBRIDS algorithm detected 21 individuals of *B. fistulosa* as potential hybrids  
277 between the northern and southern groups, all of which were found within a narrow contact zone of  
278 ~100 km (Fig. 3). These hybrid individuals represented 18 % of the individuals sampled in this area.  
279 No F1 hybrid was detected as only first-generation backcrosses were found.

### 280 **Isolation-by-distance within genetic groups**

281 Within two widespread genetic groups separated by the north-south genetic discontinuity,  
282 regression of pairwise kinship coefficients on spatial distance was significant both for the tree and  
283 the ant, and values were better fit by a logarithmic than a linear regression, indicating a pattern of  
284 isolation-by-distance for both organisms (Fig. 1a). In the southern (blue) groups, the decline in

285 kinship coefficients and the fit of regression are similar in ants ( $r^2 = 0.2452$ ) and trees ( $r^2 = 0.2427$ )  
286 (Fig. 4a). In the northern (orange) group, the steeper decline in kinship coefficients and the better fit  
287 of regression in ants ( $r^2 = 0.0766$ ) than in trees ( $r^2 = 0.0018$ ) showed that isolation-by-distance  
288 within this widespread genetic group is more pronounced in ants (Fig. 4b). The same pattern was  
289 observed when we considered pairs within groups for the whole sampling of trees and ants  
290 (triangles in Fig. 1b).

### 291 **Species distribution modelling**

292 Modelling of the spatial distribution of the *B. fistulosa* genetic groups north and south, based on  
293 environmental variables, showed similar and symmetrical genotype-environment associations for  
294 the two groups, and thus, only those for the northern group are presented. The predicted distribution  
295 is restricted to the northern half of Lower Guinea. The model does not predict the northern group to  
296 occur where the southern group occurs (Fig. 5). Among the 12 soil and climatic variables used to  
297 build the models, one had a particularly strong contribution to the explanation of the distribution of  
298 the northern genetic group: precipitation during the driest quarter (Fig. 6), which is lower in the  
299 south than in the north. This was true for each of the nine modelling algorithms used in the  
300 ensemble modelling procedure.

## 301 **DISCUSSION**

302 The tree *Barteria fistulosa* and its obligate ant symbiont *Tetraoponera aethiops* both display spatial  
303 genetic structure and share the same geographic pattern of genetic discontinuities. These patterns  
304 probably result from the multiple successive events of range fragmentation through time (Maley,  
305 1996). The Last Glacial Maximum imposed particularly dry conditions in the lowlands of tropical  
306 Africa that resulted in a considerable reduction of the forest, so that most of the surface covered by  
307 the current African rainforest has been recolonized only relatively recently (Hessler *et al.*, 2010;  
308 Lezine *et al.*, 2013). Genetic discontinuities remain detectable after secondary contact, as long as

309 gene flow has not completely erased past differentiation. Obligate symbionts are expected to show  
310 spatial congruency in their genetic structures because they experienced the same range fluctuations  
311 as well as a recent episode of recolonization.

312 Most values of genetic differentiation between groups are moderate, except that between the  
313 southernmost tree group and the others, where differentiation reaches a value ( $F_{ST} = 0.38$ ) similar to  
314 those frequently observed between plant sister species (Friar *et al.*, 2006; Yu *et al.*, 2013). We do  
315 not consider that the southern and northern populations deserve formal description as species. We  
316 rather consider they are at a stage of incipient speciation that might eventually lead to full  
317 speciation. The ant does not show such a strong north-south differentiation, neither in  $F_{ST}$  nor in the  
318 strength of shift of synthetic allele frequencies (Fig. 2). Notably, the levels of genetic differentiation  
319 between the southernmost tree group and northern groups are unaffected by geographical distance  
320 (Fig. 1a), indicating absence of significant gene flow across the north-south genetic discontinuity  
321 since the divergence of the three northern groups. Contrariwise, the southernmost group of ants is  
322 genetically closest to its parapatric group (Fig. 1a). This implies that ant genes have flowed across  
323 this boundary, not that the southernmost ant population diverged more recently, since divergence  
324 times must be the same as for the host trees. For parapatric ant populations to become genetically  
325 closer, the duration of contact must greatly exceed the effective generation time of *B. fistulosa* (~30  
326 years), and should have left enough time for gene flow between tree groups across the north-south  
327 boundary. The absence of such gene flow cannot be due to less efficient dispersal, as trees show  
328 weaker within-group isolation-by-distance than ants (Fig. 4). Considering that nothing at this  
329 contact zone is susceptible to impede seed or pollen dispersal, the only plausible barrier preventing  
330 genes flow between *B. fistulosa* groups is reproductive isolation. Incipient allopatric speciation  
331 between *B. fistulosa* group South and the other groups can explain the genetic structure of both  
332 symbiotic partners, in particular the co-occurring genetic discontinuity between northern and  
333 southern populations as the remnant of past allopatric divergence that gene flow has partially eroded

334 for the ant. Comparing spatial genetic structure between species in obligatory symbiosis (Alvarez *et*  
335 *al.*, 2010) thus proves useful for inferring the existence of reproductive barriers in contact zones.

336         The north-south transition zone between *Barteria fistulosa* genetic groups is less than  
337 100 km wide, is located near latitude 1°N and within this zone, approximately 20 % of the  
338 individuals are of hybrid origin. Absence of effective gene flow between genetic groups despite the  
339 presence of hybrids indicates that post-zygotic isolation (hybrid unfitness) is an important  
340 component of overall reproductive isolation between these tree groups. Post-zygotic isolation can  
341 freely evolve in allopatry, under the Dobzhansky-Muller-Bateson model (Coyne & Orr, 2004),  
342 resulting in intrinsic genetic incompatibilities that can kill or sterilize hybrids in many taxa  
343 (Presgraves, 2010). These incompatibilities generally do not result from the adaptation of  
344 populations to their respective environments, and thus cause “intrinsic” (environmentally-  
345 independent) post-zygotic isolation (Turelli *et al.*, 2001). Alternatively or in conjunction, hybrids  
346 may suffer from maladaptation to parental niches (ecological speciation, reviewed in Rundle &  
347 Nosil, 2005), given that the two tree groups are predicted to populate distinct environments. Known  
348 cases of hybrid ecological maladaptation result from divergent selective pressures of considerable  
349 strength that are imposed upon parental ecotypes by plainly distinct environments, such as mountain  
350 versus plain for plants (Wang *et al.*, 1997), limnetic versus benthic zones for sticklebacks (Rundle,  
351 2002) or different host species for phytophagous insects (Egan & Funk, 2009; Peccoud *et al.*, 2014).  
352 By contrast, the predicted niches of the tree groups present subtle differences that only appeared  
353 through modelling. These differences seem to us unlikely to impose selection pressures nearly as  
354 strong as those imposed upon the aforementioned ecotypes. We therefore believe that ecology  
355 would only contribute weakly, if at all, to hybrid deficiency in *B. fistulosa*, especially when it is  
356 considered that the contact zone may in fact represent an intermediate environment particularly  
357 suited to hybrids (e.g., Wang *et al.* 1997).

358         Spatial segregation of the northern and southern tree lineages, despite efficient dispersal



359 capacities, would mostly result from reciprocal exclusion by competition. Migrants from the other  
360 side of the contact zone are outcompeted by residents, as their progeny would mostly consist of  
361 deficient hybrids. The zone of contact between genetic groups can thus be seen as a “tension zone”,  
362 a term defining a cline maintained by a balance between dispersal and selection against hybrids  
363 (Barton & Hewitt, 1985). The spatial location of the tension zone should move freely according to  
364 the relative competitive ability of each lineage at the colonization front. Modelling approaches  
365 predict that the spatial location of a tension zone should stabilize on an environmental transition, a  
366 phenomenon conceptualised as “the coupling hypothesis” (Bierne *et al.*, 2011, 2013). In *Barteria*  
367 *fistulosa*, edaphic and climatic conditions would sufficiently differ between the areas north and  
368 south of latitude 1°N to explain the geographic position of the tension zone. One climatic variable  
369 contributed particularly heavily to niche differentiation: precipitation during the driest quarter,  
370 which was lower in the area occupied by the Southern group. It still remains to be tested whether  
371 southern individuals are better adapted than northern ones to a particularly pronounced dry season.

372         Genetic differentiation between groups north and south of latitude 1-3°N has been detected  
373 in many plant species in the Lower Guinea domain (see Introduction). Interestingly, these latitudes  
374 correspond to the “climatic hinge”, which is defined as the transition between the boreal and austral  
375 seasonal regimes. The tension zone we revealed between *Barteria fistulosa* lineages in that region  
376 (at 1°N) suggests that other Central African forest taxa may also have undergone incipient allopatric  
377 speciation during past episodes of forest fragmentation. This hypothesis, and the existence of other  
378 tension zones, can be tested by genetic analyses of candidate species that occur in sufficiently high  
379 densities in the equatorial region to permit adequate sampling. In combination, ecological data may  
380 inform on the environmental variables—shifts in seasonal phenology, differing soil and climate  
381 conditions—that may have a stabilizing effect on secondary contact zones, and on the extent to  
382 which these abiotic parameters explain the geographical genetic structure observed in several taxa  
383 of the African tropical rain forest.

384 To conclude, our results show that reproductive isolation can evolve independently between  
385 obligatory symbionts, so that diversification in ant-plant symbioses does not necessarily involve co-  
386 speciation. Although this has already been inferred from the taxonomic distribution of ant-plant  
387 symbioses and phylogenetic approaches (Davidson & McKey, 1993; Chomicki & Renner, 2015),  
388 our study constitutes the first snapshot of ongoing disjoint speciation in these symbioses.  
389 Surprisingly, we show that isolated populations of trees can evolve stronger reproductive isolation  
390 than their ant symbionts, even though insects have shorter generation times and generally present  
391 higher speciation rates than trees. This unexpected outcome of allopatric divergence illustrates the  
392 inherent stochasticity in the evolution of reproductive incompatibilities, which depends on the  
393 occurrence and location of mutations, so that reproductive barriers need not emerge in taxa that look  
394 more likely to speciate *a priori*.

395 In addition, our results provide insight into the process of incipient speciation after  
396 secondary contact. In central Africa, re-colonization is little constrained by landforms, allowing for  
397 more accurate perception of the effects of environmental factors other than topography on the  
398 spatial distribution of genetic discontinuities. The existence of a tension zone between lineages of  
399 *Barteria fistulosa* that co-localizes with an environmental transition at the climatic hinge informs us  
400 on possible ongoing speciation in other Central African forest species, and on the ecological factors  
401 that govern the location of secondary contact zones in general.

402

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417

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588

## 589 **SUPPORTING INFORMATION**

590 Additional Supporting Information may be found in the online version of this article:

591 Appendix S1 List of *Barteria fistulosa* samples and data.

592 Appendix S2 List of *Tetraoponera aethiops* samples and data.

593 Appendix S3 Selection of the number of genetic groups.

594

## 595 **BIOSKETCHES**

596 **Rumsais Blatrix** and the team at CEFÉ are interested in the functional ecology and evolutionary  
597 biology of species interactions, with a particular emphasis on ant-plant symbioses.

598 Author contributions: D.M. and R.B. conceived the ideas; R.B., D.M., C.B., C.D.-L., C.A., D.J.H.

599 and J.J.W. contributed to field work; J.P., C.B., F.P., L.B., M.S., C.D.-L. and R.B. performed lab

600 work and analysed molecular data; R.B., D.M., J.P., C.B., F.P., J.J.W. and D.J.H. interpreted the

601 results; and R.B., J.P. and D.M. wrote the paper. All co-authors revised and approved the

602 manuscript.

603

604 **Editor:** Richard Ladle

605

606 **FIGURE LEGENDS:**

607 **Figure 1:** (a) Spatial distribution of the genetic groups defined by STRUCTURE for  $K = 4$ , for the tree  
608 *Barteria fistulosa* and its symbiotic ant *Tetraoponera aethiops*. Each individual is represented by a  
609 pie chart indicating the proportions of ancestry to each of the four groups. Groups are coded by  
610 colour. Pie charts are slightly shifted from true location to reduce overlap. The map was generated  
611 using QGIS 2.8.1 with a Mercator projection. (b) Average kinship coefficient as a function of spatial  
612 distance for all pairs of individuals (black diamond), for pairs of individuals within genetic groups  
613 (triangles) and for pairs of individuals among groups (circles). Genetic groups ( $K = 4$ ) were defined  
614 using the software STRUCTURE. Dotted lines represent regression curves, either linear or logarithmic  
615 according to the best fit.

616 **Figure 2:** Variation of the frequency of the synthetic allele  $n$  (representative of the Northern group)  
617 with latitude when considering the two genetic groups defined by STRUCTURE. A generalized  
618 logistic function is fitted on the scatter-plot, the equation of which is given for each species.

619 **Figure 3:** Spatial distribution of the genetic groups of the tree *Barteria fistulosa* defined by  
620 STRUCTURE for  $K = 2$ . Potential hybrids between the two groups were identified using  
621 NEWHYBRIDS. The right panel shows a close-up of the rectangle, where all potential hybrids occur.  
622 The map was generated using QGIS 2.8.1 with a Mercator projection.

623 **Figure 4:** Average pairwise kinship coefficient as a function of geographical distance for pairs of  
624 individuals of the ant *Tetraoponera aethiops* (empty circles) and of the tree *Barteria fistulosa* (filled  
625 circles) within two genetic groups defined by STRUCTURE: (a) the southernmost group, coloured in  
626 blue in Fig. 1a, and (b) the most widespread of the northern groups, coloured in orange in Fig. 1a.

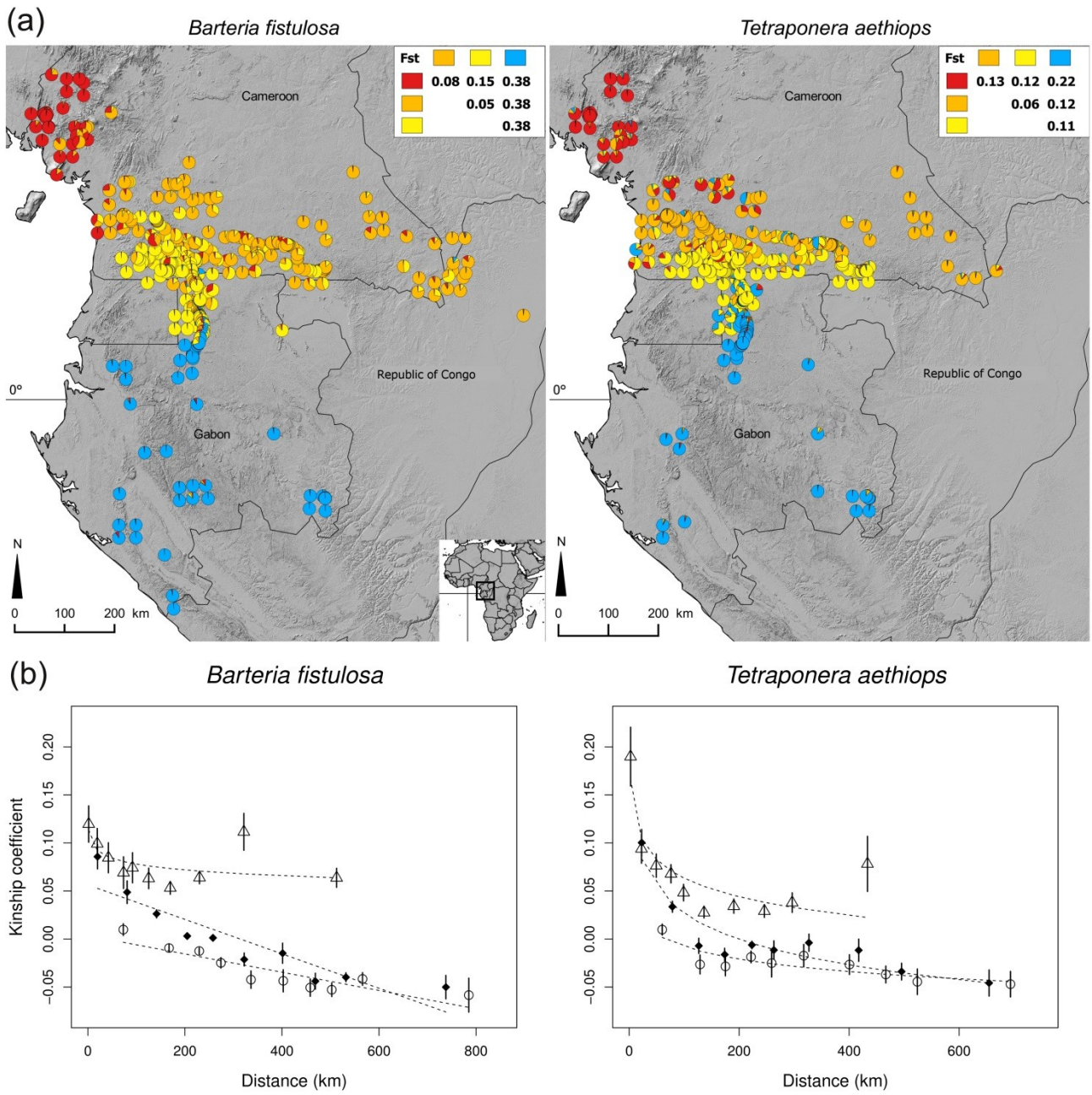
627 **Figure 5:** Projection of the consensus model (committee averaging over nine modelling algorithms)  
628 of spatial distribution of the *Barteria fistulosa* genetic group north. Points represent all the *Barteria*  
629 *fistulosa* individuals sampled. Individuals classified in group north by STRUCTURE for  $K = 2$  were  
630 used as presence data for building the distribution model. Distribution of group south was also

631 modelled and results for both groups were similar and symmetrical. Thus, only those for group  
632 north are presented. The map was generated using QGIS 2.8.1 with a Mercator projection.

633 **Figure 6:** Normalized relative contributions of each of 12 environmental variables to nine different  
634 models of species distribution run for the northern genetic group of the tree *Barteria fistulosa*  
635 defined by STRUCTURE for  $K = 2$ . Variables are: sand content (SNDPPT), silt content (SLTPPT), pH  
636 (PHIHO5), soil organic carbon (ORCDRC), and bulk density (BLD), annual mean temperature  
637 (BIO1), maximum temperature of warmest month (BIO5), minimum temperature of coldest month  
638 (BIO6), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17),  
639 precipitation of warmest quarter (BIO18) and precipitation of coldest quarter (BIO19).

640

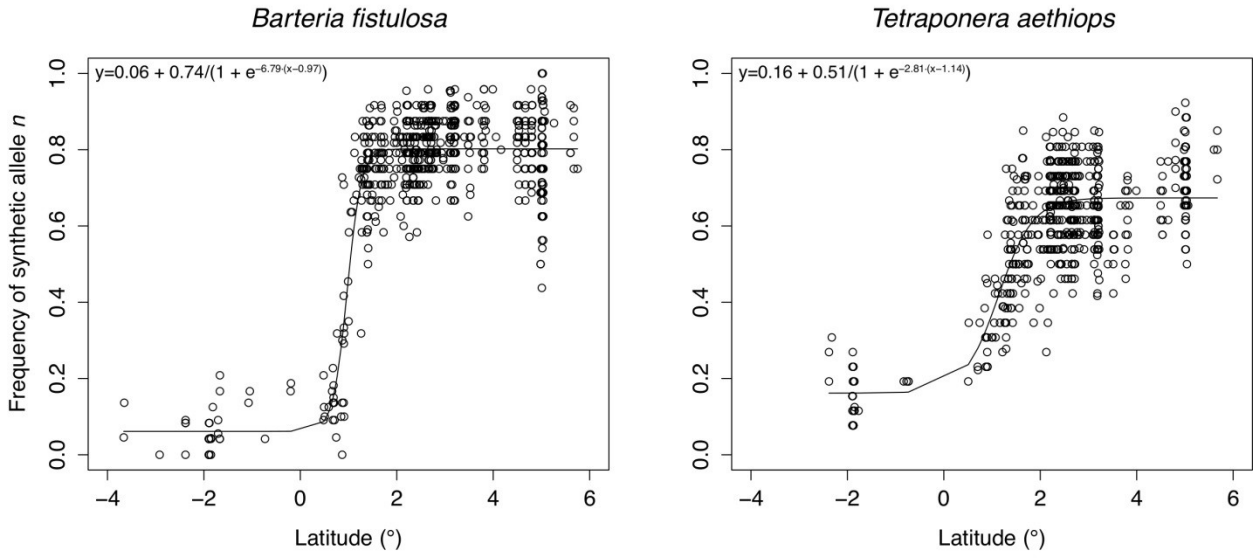
641 Figure 1



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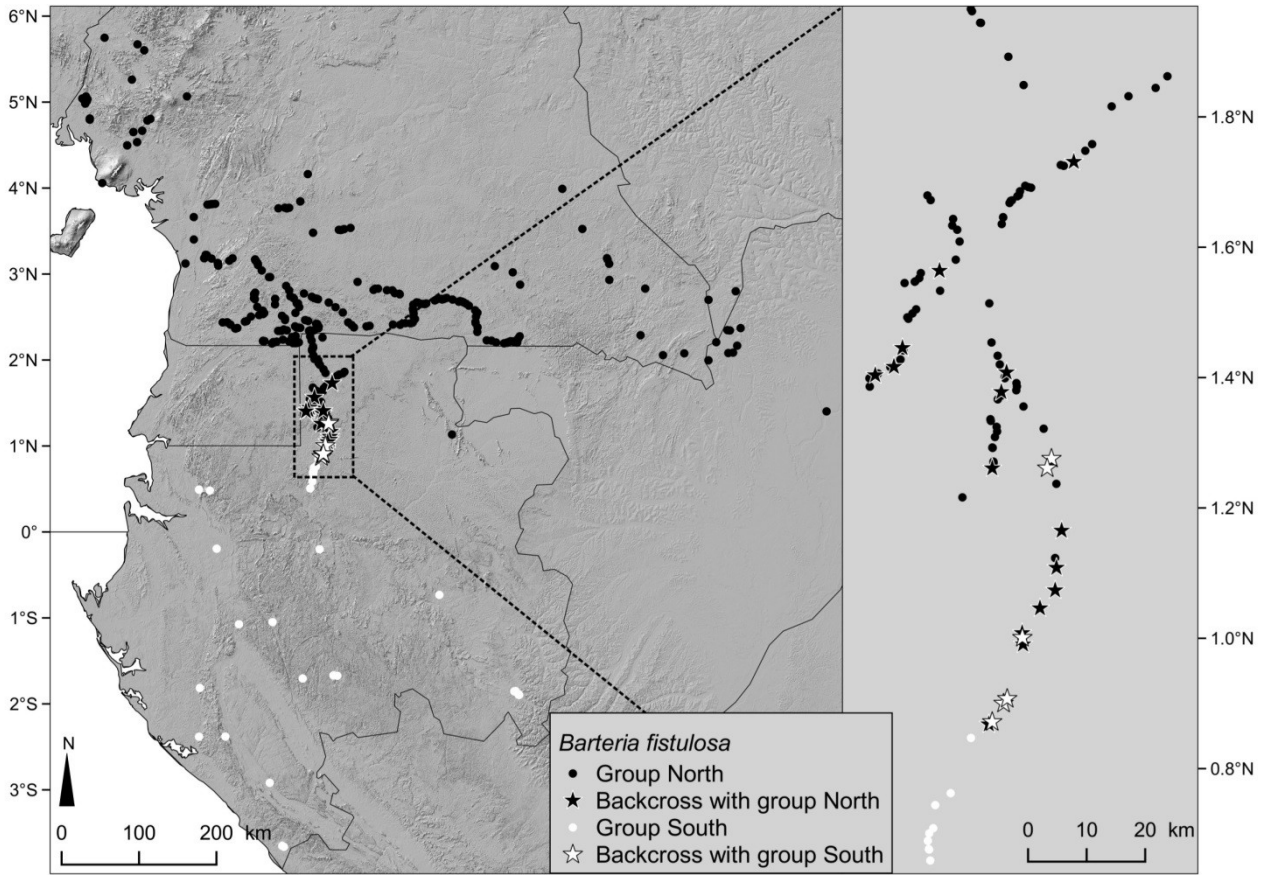
644 Figure 2



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647 Figure 3

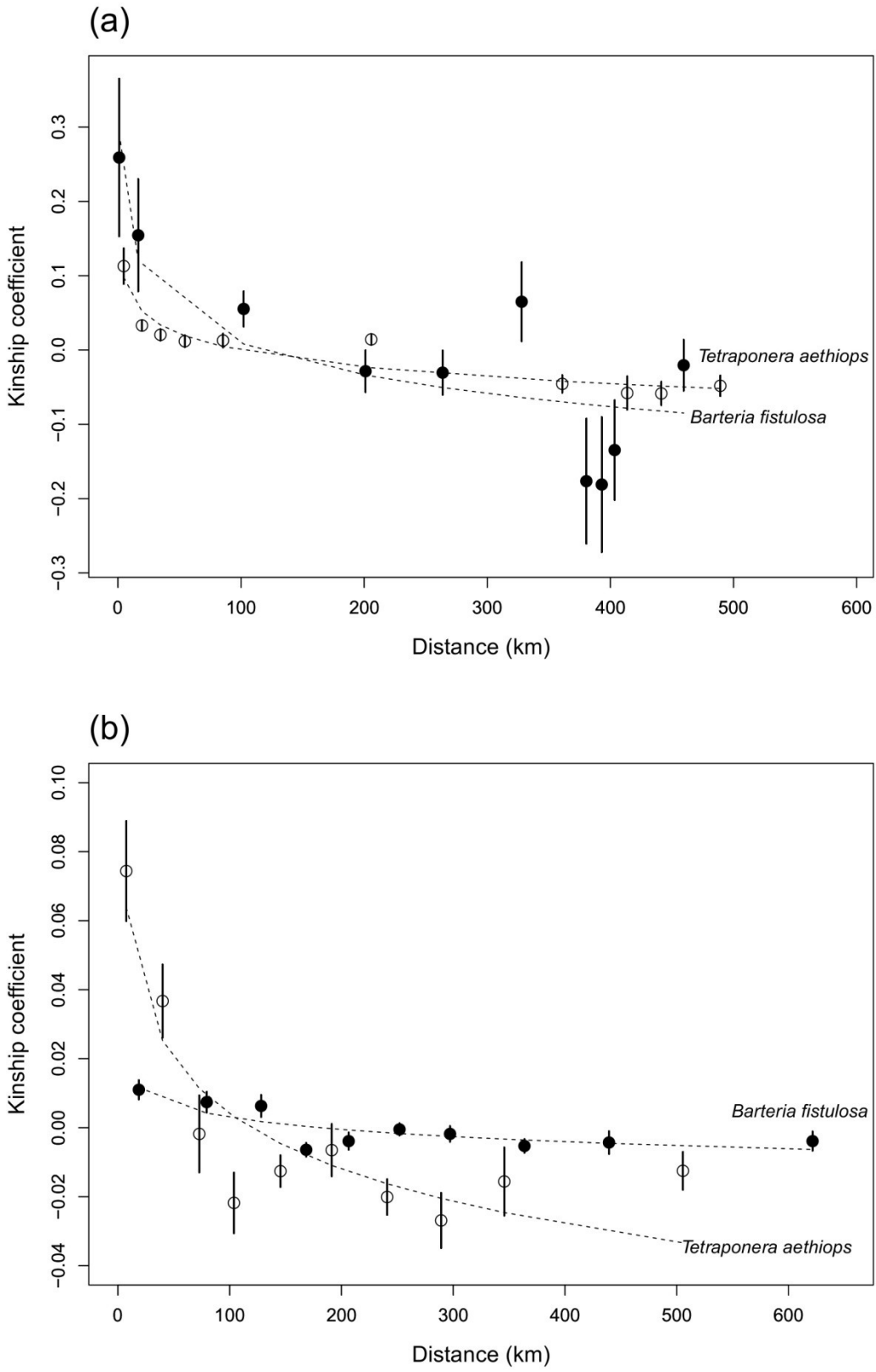


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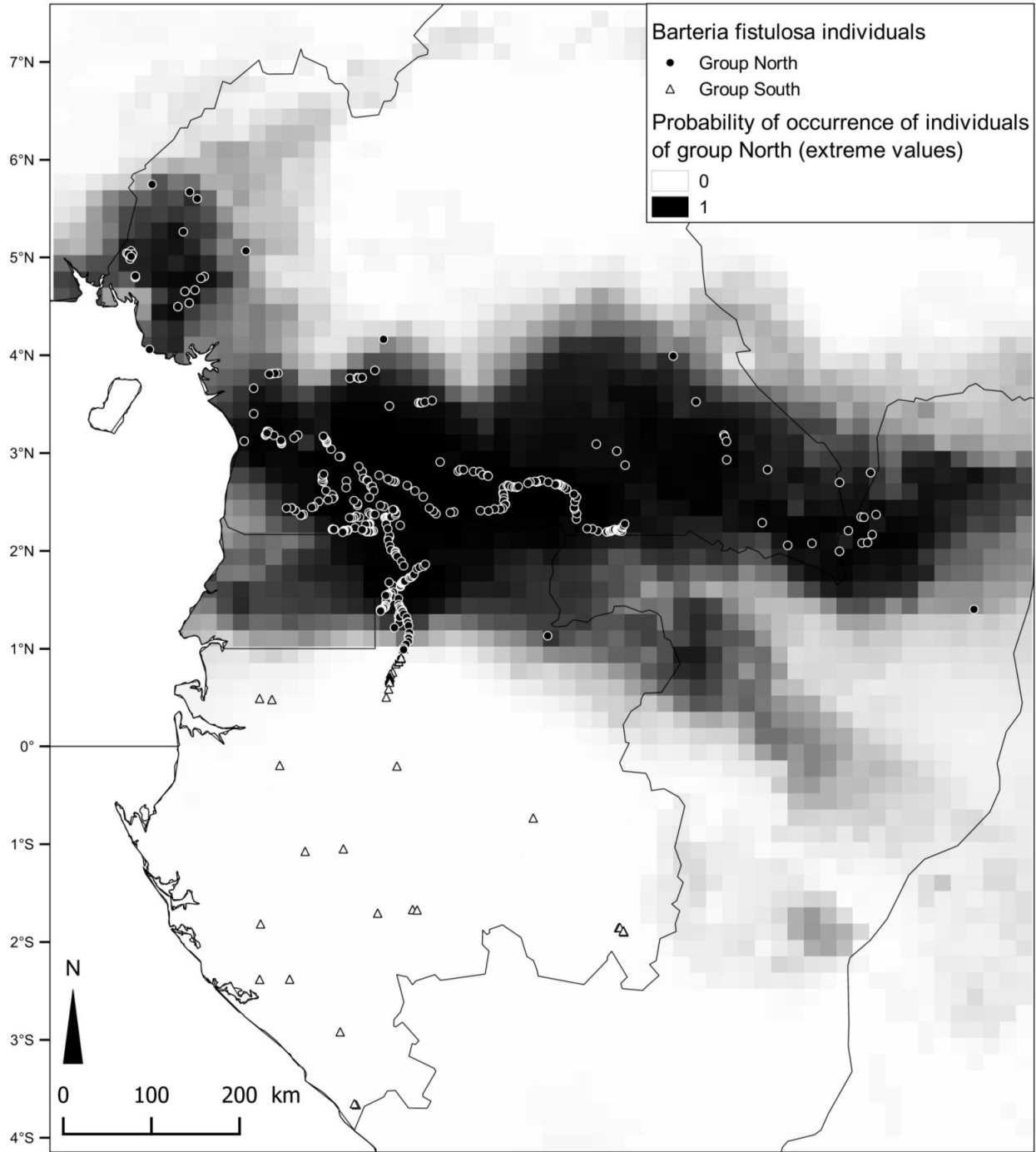
650 Figure 4



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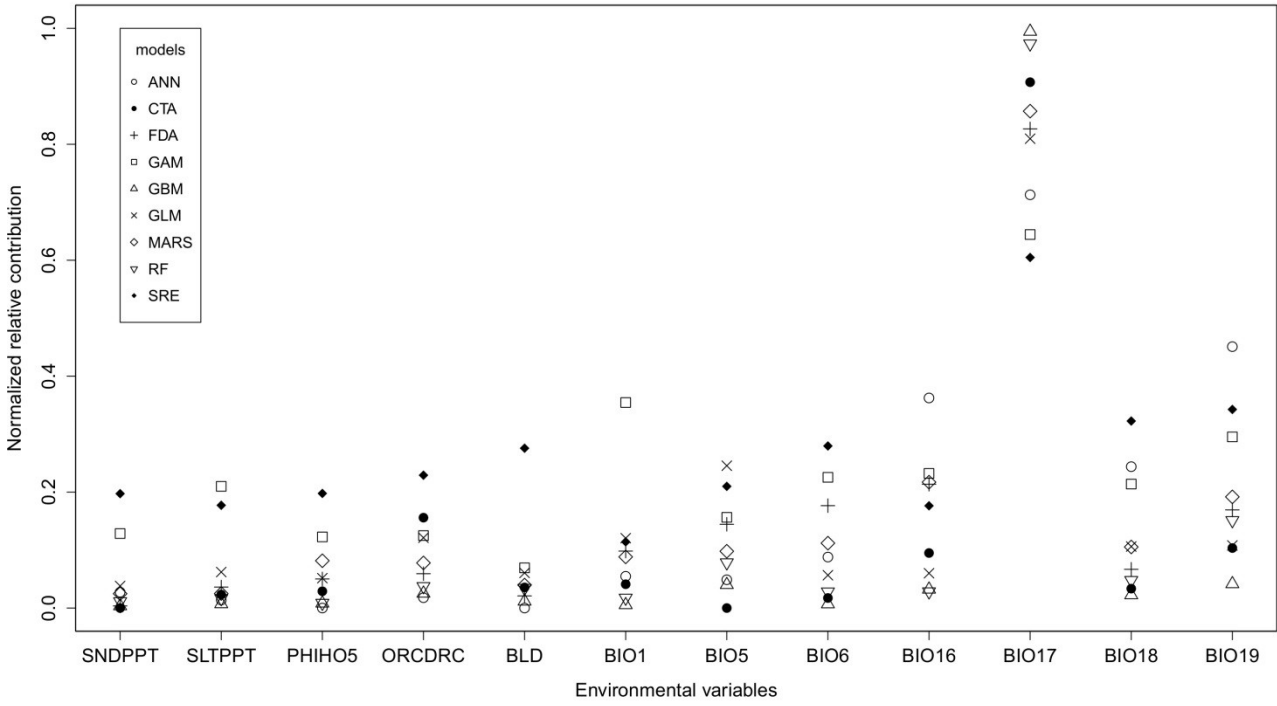
653 Figure 5



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656 Figure 6



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