1 **Biological invasions in rodent communities: from ecological interactions to zoonotic**

2 **bacterial infection issues.**

3 Christophe Diagne[1,2,3], M. Galan[4], Lucie Tamisier[4], Jonathan d'Ambrosio[4], Ambroise

4 Dalecky[5], Khalilou Bâ[2], Mamadou Kane[2], Youssoupha Niang[2], Mamoudou Diallo[2], Aliou

5 Sow[2], C. Tatard[4], A. Loiseau[4], O. Fossati-Gaschignard[1], Mbacké Sembène[2,3], Jean-François

6 Cosson[6], Nathalie Charbonnel[4] & Carine Brouat[1]

7 [1] IRD, CBGP (UMR INRA / IRD / Cirad / Montpellier SupAgro), Campus International de

8 Baillarguet, Montferrier sur Lez, France

9 [2] IRD, CBGP (UMR INRA / IRD / Cirad / Montpellier SupAgro), Campus ISRA/IRD de Bel

10 Air, Dakar, Senegal

11 [3] Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh

12 Anta Diop (UCAD), BP 5005 Fann, Dakar, Senegal

13 [4] INRA, CBGP (UMR INRA / IRD / Cirad / Montpellier SupAgro), Campus International de

14 Baillarguet, Montferrier sur Lez, France.

15 [5] IRD, LPED (UMR AMU / IRD), Marseille, France.

16 [6] INRA, Anses, USC Bipar, Bartonella et Tiques, 23 Avenue du Général de Gaulle, 94700

17 Maisons-Alfort, France

18

19 Corresponding author: Christophe Diagne. E-mail: chrisdiagne89@hotmail.fr

20

21 **Abstract**

22 Several hypotheses (such as 'enemy release', 'novel weapon', 'spillback' and

23 'dilution/density effect') suggest changes in host-parasite ecological interactions during

24 biological invasion events. Such changes can impact both invasion process outcome and the

25 dynamics of exotic and/or endemic zoonotic diseases. To evaluate these predictions, we

26 investigated the ongoing invasions of the house mouse *Mus musculus domesticus*, and the

27 black rat, *Rattus rattus*, in Senegal (West Africa). We focused on zoonotic bacterial

28 communities depicted using 16S rRNA amplicon sequencing approach in both invasive and

29 native rodents sampled along two well-defined invasion routes. Overall, this study provided

30 new ecological evidence connecting parasitism and rodent invasion process, with diverse

31 potential roles of zoonotic bacteria in the invasion success. Our results also highlighted the

32 main factors that lie behind bacterial community structure in commensal rodents. Further

33 experimental studies as well as comparative spatio-temporal surveys are necessary to decipher

34 the actual role of zoonotic bacteria in these invasions. Our data also gave new support for the

35 difficulty to predict the direction in which the relationship between biodiversity changes and

36 disease risk could go. These results should be used as a basis for public health prevention

37 services to design reservoir monitoring strategies based on multiple pathogen surveillance.

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39

40 **Keywords:** Biological invasions; *Mus musculus domesticus*; *Rattus rattus*; bacterial

41 communities; host-parasite interactions; zoonoses.

42

43 **Introduction**

44   There is a close link between biological invasions (the establishment and spread of a non-

45 native species in a new range) and parasites (here referred collectively to macroparasites and

46 microparasites), with the potential for each to feed back on the other (Dunn and Hatcher

47 2015). First, parasites may alter the outcome of biological invasions by impacting host fitness

48 and/or changing the strength of interactions between introduced and native hosts (Prenter, et

49 al. 2004). As such, native parasites may prevent new host invasion if they infect introduced

50 hosts and induce detrimental (direct and/or indirect) effects on their fitness (Strauss, et al.

51 2012). Also, native parasite pressure in the resident hosts may be reduced by introduced hosts

52 through dilution or density effects (Keesing, et al. 2010), and indirectly contribute to increase

53 the competitive ability of native hosts against invaders. Conversely, parasites may favour

54 invasion success when introduction results in the loss of (a subset of) parasites by the exotic

55 host (enemy release hypothesis; (Colautti, et al. 2004). Indeed the low number of introduced

56 individuals may not carry the complete range of parasites found in source sites (founder

57 effect) or introduced parasites may fail to thrive in the new environment (MacLeod, et al.

58 2010). Such parasite reduction is predicted to enhance the fitness and competitive ability of

59 the introduced species in the new range. Moreover, parasites shared by both native and

60 introduced hosts may also favour invasion success by inducing adverse effects on the fitness

61 and/or survival of the native competitors. Invaders may introduce and transmit novel parasites

62 to native hosts ("novel weapon" hypothesis; (Strauss, et al. 2012) or they may acquire and

63 better amplify endemic parasites, resulting in higher prevalence/abundance in native hosts

64 than expected in the absence of the new hosts (spillback hypothesis; (Kelly, et al. 2009).

65   Second, invasion related changes in host community structure and diversity may *in fine*

66 influence the dynamics of diseases potentially affecting wildlife, livestock and/or human

67 health (Young, et al. 2017). Disease emergence events associated with non-native pathogens

68   brought by animal invaders have already been observed (Dunn and Hatcher 2015). Despite a

69   growing interest these last decades, there are still gaps in the basic studies devoted to

70   relationships between parasite ecology, disease dynamics and invasion process (Young, et al.

71   2017). Notably, few studies have examined the concomitant effects of both native and

72   invasive host communities on infectious disease risks in invaded ecosystems.

73   In this paper, we examined ecological interactions between native and invasive host

74   rodents and their bacterial communities in the context of well-studied ongoing invasions in

75   Senegal (West Africa). They concern two major invasive species (Global Invasive Species

76   Database - http://www.issg.org/database/): the house mouse *Mus musculus domesticus*

77   (Dalecky, et al. 2015) Lippens et al. unpublished data) and the black rat *Rattus rattus*

78   (Konecny, et al. 2013). Historical records and molecular analyses have shown that these

79   rodents were first brought to Senegalese coasts by Europeans at colonial times, and remained

80   in coastal villages and towns until the beginning of the 20th century. Both taxa have spread

81   further inland during the last century via well-defined invasion routes, due to the increase of

82   human activities and the improvement transport infrastructures in mainland Senegal. The

83   distribution of the house mouse covers now most of the northern and central part of Senegal,

84   while the black rat is distributed in the southern part of the country. Along their invasion

85   routes, the invasive species have colonised the human settlements (either towns or small

86   villages) from which they have progressively weed out the commensal native rodent species,

87   the Guinea multimammate mouse *Mastomys erythroleucus* and the natal multimammate

88   mouse *Mastomys natalensis*. At invasion fronts, both invasive and native species cohabit

89   (Dalecky, et al. 2015; Granjon and Duplantier 2009). The role of parasites in successful

90   colonization events of the house mouse and black rat has previously been documented, mainly

91   with regard to insular ecosystems (Harris 2009; Wyatt, et al. 2008). More recently, (Diagne, et

92   al. 2016) have revealed an association between the loss of gastrointestinal helminths and the

93   range expansion of these rodents in Senegal. Because invasive and African rodent species

94   studied here are known to carry a large array of zoonotic agents (Meerburg, et al. 2009), it is

95   also likely that the risks of zoonotic diseases for human may change along the mouse and rat

96   invasion routes.

97   Here, we focused on the zoonotic bacteria detected in rodents using the 16S rRNA

98   amplicon sequencing approach described in (Galan, et al. 2016). This community comprises

99   several pathogens that may be transmitted to other mammal species including humans (Han,

100   et al. 2015; Meerburg, et al. 2009). We first analysed the relationships between rodent-

101   bacteria interactions and ongoing invasions by testing for patterns expected under the

102   ecological processes described above: (i) a decrease of bacterial species richness and

103   prevalence levels in invasive rodents at their invasion fronts compared to their original coastal

104   sites, as expected under the enemy release hypothesis, (ii) changes in bacterial species

105   richness and prevalence levels in native rodents from invaded areas compared to non-invaded

106   areas as expected under the novel weapon/spillback (increase expected for bacteria shared by

107   native and invasive rodents at invasion fronts) or dilution/density effect (decrease expected)

108   hypotheses. We next discussed the importance of these variations in bacterial communities

109   observed along rodent invasion routes in terms of public health.

110

111   **Results**

112   A total of 985 rodents were sampled in 24 sites. Trapping sessions showed variations in

113   rodent communities along both invasion routes, from western coastal sites of long-established

114   invasion to inland invasion fronts and non-invaded sites (Table 1; Fig. 1).

115   Along the mouse invasion route (Table 1a), only invasive *M. m. domesticus* were captured in

116   sites of long-established invasion. At the invasion front, native *Ma. erythroleucus* co-occurred

117   generally   with *M. m. domesticus*, although being nearly systematically less abundant. The

118   only exception was the site of Croisement Boubé, where *Ma. erythroleucus* was not detected

119   despite the recent introduction of *M. m. domesticus* in this area (Dalecky et al. 2015). As

120   expected, only native rodents were sampled in non-invaded sites.

121   Along the rat invasion route (Table 1b), native rodent communities belonged to two sister

122   species, *Ma. erythroleucus* and *Ma. natalensis*. Sites of long-established invasion were highly

123   dominated by invasive *R. rattus*. At the invasion front, invasive *R. rattus* co-occurred with

124   *Ma. erythroleucus* in three of the four sites, and with *Ma. natalensis* in the southernmost site

125   (Kedougou). Only native rodents were sampled in non-invaded sites, and they mainly

126   belonged to *Ma. natalensis*. Some sparse *Ma. erythroleucus* individuals were sampled in one

127   non-invaded site (Bransan) and in sites of long-established invasion (cf. Table 1), probably

128   coming from the surrounding fields where this rodent species is generally abundant (Granjon

129   and Duplantier 2009). They were not included in further statistical analyses due to low sample

130   sizes.

131   Bacteria were screened in 679 rodents (Table 1). Twelve zoonotic bacterial Operational

132   Taxonomic Units (OTUs) were described (Galan et al. 2016): *Bartonella*, *Borrelia*, *Ehrlichia*,

133   *Mycoplasma_1*,   *Mycoplasma_2*,   *Mycoplasma_3*,   *Mycoplasma_4,*   *Mycoplasma_5,*

134   *Mycoplasma_6, Orientia, Rickettsia* and *Streptobacillus*.

135

136   **Distribution of bacterial communities along invasion routes.** Along the mouse invasion

137   route, six zoonotic OTUs were found (Table 2): *Bartonella*, *Borrelia*, *Ehrlichia*,

138   *Mycoplasma_1*, *Mycoplasma_3* and *Orientia*. *Orientia* was found exclusively in *M. m.*

139   *domesticus*. Otherwise, all the OTUs were found in both *Ma. erythroleucus* and *M. m.*

140   *domesticus*. *Mycoplasma_1*, *Ehrlichia* and *Borrelia* were found in all categories of sites (sites

141   of long-established invasion, invasion front and non invaded sites). *Bartonella* and

142   *Mycoplasma_3* were detected in *M. m. domesticus* in only one site of long-established

143   invasion, while these OTUs were found in *Ma. erythroleucus* in both invasion front and non-

144   invaded sites.

145   Along the rat invasion route, twelve pathogenic OTUs were recorded (Table 3): *Bartonella*,

146   *Borrelia*, *Ehrlichia*, *Mycoplasma_1*, *Mycoplasma_2*, *Mycoplasma_3*, *Mycoplasma_4,*

147   *Mycoplasma_5, Mycoplasma_6, Orientia, Rickettsia* and *Streptobacillus*. Four OTUs showed

148   host specificity: *Mycoplasma_2*, *Mycoplasma_4*, *Rickettsia* in *R. rattus*, *Orientia* in *Ma.*

149   *natalensis*, *Mycoplasma_5* and *Mycoplasma_6* in *Mastomys* spp. The six OTUs detected in

150   both native and invasive rodents exhibited different distribution patterns. *Bartonella* was

151   found in all categories of sites. *Borrelia* and *Ehrlichia* were restricted to one site at the

152   invasion front. *Mycoplasma_1* and *Mycoplasma_3* were detected in *R. rattus* in one site only

153   at the invasion front and in *Mastomys* spp. in both invasion front and non-invaded sites.

154   For both invasion routes, hierarchical clustering and permutational multivariate analysis of

155   variance (permanova) carried out on Bray-Curtis (BC) dissimilarity index-based matrices

156   revealed that the bacterial communities differed significantly between invasive and native

157   host species (mouse invasion route: BC percentage of total variance explained $R2 = 0.29$; $F =$

158   5.77, *p = 0.002*, Fig. 2; rat invasion route: BC $R2 = 0.52$; $F = 14.35$, *p < 0.001*, Fig. 3), but

159   not between invasion-related categories of sites at intraspecific level (mouse invasion route:

160   BC $R2 = 0.05$; $F = 0.53$, *p = 0.817*, Fig. 2; rat invasion route: BC $R2 = 0.08$; $F = 1.13$, *p =*

161   *0.334*, Fig. 3). The same analyses performed using the presence/absence data-based Jaccard

162   dissimilarity index showed similar bacterial community structure patterns (Supplementary

163   materials, Fig. 1, Fig. 2).

164

165   **Factors shaping bacterial communities.** Generalized Linear Mixed Models (GLMMs) were

166   used to decipher the effects of the site invasion status (e.g., site of long-established invasion,

167   invasion front or non-invaded site) and of individual factors (gender, body mass of the rodent)

168   on individual OTU species richness (number of OTUs found in a single host individual) or

169   OTUs prevalence (proportion of hosts infected with a given OTU).

170   ***Bacterial OTUs richness.*** Considering the mouse invasion route, a significant effect of

171   *invasion status* ($p = 0.024$) indicated less bacterial OTUs in *M. m. domesticus* than in *Ma.*

172   *erythroleucus* (pairwise Wilcoxon tests with Holm's correction (WH tests), $p < 0.05$). There

173   was no difference of species richness between *M. m. domesticus* from invasion front and from

174   long-established invasion sites (WH test, $p = 0.99$), or between *Ma. erythroleucus* from

175   invasion front and from non-invaded sites (WH test, $p = 0.39$). Species richness was higher in

176   males than in females (*gender* effect: $p = 0.001$).

177   Considering the rat invasion route, *invasion status* ($p < 0.001$) had also a significant effect on

178   species richness. Invasive *R. rattus* had less bacterial OTUs than native *Mastomys* rodents

179   (WH tests, $p < 0.05$), and *Mastomys* rodents had less bacterial OTUs at the invasion front

180   than in non-invaded sites (WH test, $p = 0.008$). No significant difference was detected

181   between *R. rattus* populations from invasion front and long-established invasion sites (WH

182   test, $p = 0.580$). Finally, male and heavier rodents harboured more OTUs than female and

183   lighter ones ($p < 0.001$ for *gender* and *body mass* effects).

184   ***Bacterial OTUs prevalence.*** Considering the mouse invasion route, *invasion status* ($p <$

185   0.001) was significant for *Ehrlichia* only, indicating higher infection levels in *Ma*

186   *erythroleucus* at the invasion front (WH tests, $p < 0.005$). Besides, males were more infected

187   by *Mycoplasma_1* ($p < 0.001$), *Ehrlichia* ($p = 0.011$) and *Borrelia* ($p = 0.002$) than females.

188   A significant interaction was found between *gender* and *invasion status* ($p = 0.014$) for

189   *Mycoplasma_3*, suggesting that males were more infected in non-invaded sites than at

190   invasion front, and the opposite trend for females. Finally, heavier rodents were less infected

191   by *Ehrlichia* than lighter ones ($p = 0.039$).

192   Considering the rat invasion route, *invasion status* was significant for *Bartonella* (*p < 0.001*)

193   and *Mycoplasma_6* (*p = 0.006*). At invasion front, *R. rattus* were less infected by *Bartonella*

194   than *Mastomys* rodents (WH test, *p < 0.001*). *Mastomys* rodents were also less infected by

195   *Bartonella* (WH test, *p = 0.001*) and *Mycoplasma_6* (WH test, *p = 0.004*) at the invasion

196   front than in non-invaded sites. The factor *gender* was significant for *Bartonella* (*p = 0.025*),

197   *Mycoplasma_1* (*p = 0.004*), *Mycoplasma_4* (*p = 0.02*), and *Mycoplasma_6* (*p = 0.003*) with

198   higher infection levels observed in males than in females. The factor *body mass* significantly

199   explained the prevalence of *Mycoplasma 1* (*p < 0.001*), *Mycoplasma_2* (*p = 0.001*),

200   *Mycoplasma 4* (*p = 0.003*) and *Mycoplasma_5* (*p = 0.036*), with heavier rodents being more

201   infected than lighter ones.

202

203   **Discussion**

204   In this study, we examined variations of bacterial communities in invasive and native

205   rodents sampled along two invasion routes in Senegal. Our results have several implications

206   regarding (i) the factors that lie behind bacterial community structure, (ii) the ecological

207   hypotheses relating parasitism to invasion outcome in commensal rodents, and (iii) the

208   putative relationships between host biodiversity and disease risks.

209   Our study first confirmed that host individual factors such as body mass (Moore and

210   Wilson 2002) and gender (Zuk and Stoehr 2010) are important drivers of bacterial infections

211   in small mammals. In accordance with previous works (but see (Kiffner, et al. 2013)), we

212   found that larger and male rodents were significantly more infected than lighter and female

213   ones. Male-biased parasitism in rodents could be due to profound sexual differences in

214   anatomy, physiology, behaviour, and evolutionary roles that could increase probabilities of

215   both encountering and acquiring parasites (reviewed in (Krasnov, et al. 2012). Larger

216   individuals may have greater home range, what may increase contact rates with parasites

217 (Boyer, et al. 2010). Besides, as body mass can be considered as a proxy of host age, the

218 positive correlation that is generally found between infection and body mass could correspond

219 to higher levels of infection in older individuals. This pattern is likely to result from a longer

220 time of exposure in older than younger rodents. Alternatively, the negative relationship found

221 between *Ehrlichia* infection and body mass on the mouse invasion route may suggest that

222 older individuals would be less susceptible to the infection, or that infected individuals died

223 rapidly.

224 Establishing the origin of parasites (either native or exotic) is a key element in the context

225 of the hypotheses relating parasitism and invasion success. The distribution of bacterial OTUs

226 among native and invasive rodents may give useful insights when investigating their origin.

227 As already found in rodents (Badenhorst, et al. 2012), some of the OTUs recorded here

228 appeared to be relatively host specific. Indeed, three OTUs (*Mycoplasma_3*, *Mycoplasma_5*,

229 *Mycoplasma_6*) were mainly found in *Mastomys* rodents, and are thus probably native. This

230 could also be the case for *Ehrlichia,* which was detected at high prevalence in native rodents,

231 and almost exclusively at invasion fronts in invasive rodents. The *Borrelia* OTU found in

232 native and invasive rodents is also probably native, as large surveys have detected only one

233 local species (e.g., *Borrelia crocidurae*) in small mammal communities of West Africa

234 (Trape, et al. 2013; Vial, et al. 2006). Conversely, two OTUs (*Mycoplasma_2*, *Mycoplasma_4*

235 along the rat invasion route) were found repeatedly and exclusively in invasive *R. rattus*. The

236 distribution of these bacteria suggested that they have been introduced in Senegal by exotic *R.*

237 *rattus*. A high susceptibility of *Mastomys* species to these OTUs may prevent the detection of

238 infected native rodents. The detection of *Streptobacillus* in *R. rattus* at long-established

239 invasion sites and in native rodents only at the invasion front of *R. rattus*, also indicated an

240 exotic origin for this OTU. In a previous work, we suggested an exotic origin for *Orientia*,

241 which is found exclusively in *M. m. domesticus* along the mouse invasion route (Cosson, et al.

242   2015). However, the detection of identical sequences of this OTU in some native *Ma.*

243   *natalensis* samples from outside the range of *M. m. domesticus* on the otherside of Senegal

244   raises questions about the exotic origin of this bacterial OTU or the involvement of host

245   species other than exotic rodents in its introduction (e.g., migratory birds: (Varma 1964)).

246   As expected, bacterial communities varied along invasion routes and between invasive and

247   native rodents. A main result was that bacterial species richness was globally higher in native

248   rodents than in invasive ones. This pattern may reflect a loss of zoonotic bacteria experienced

249   by *M. m. domesticus* and *R. rattus* at the time of their introduction in Senegal. Signatures of

250   bacterial loss were however not observed for potentially exotic OTUs specifically carried by

251   *M. m. domesticus* (i.e., *Orientia*) and *R. rattus* (i.e., *Mycoplasma_2* and *Mycoplasma_4*) along

252   their respective invasion routes. In contrast to previous surveys of gastrointestinal (Diagne, et

253   al. 2016), there was thus no support for a bacterial release hypothesis favouring the spread of

254   *M. m. domesticus* and *R. rattus* in Senegal. Possible explanations could rely on the mode of

255   transmission and/or vector specificity of the introduced bacteria. All bacterial OTUs

256   mentioned here (except *Mycoplasma* and *Streptobacillus*) are vectored by arthropods (e.g.,

257   fleas, ticks, see (Galan, et al. 2016) for a synthesis). For instance, trombiculid mites involved

258   in the transmission of *Orientia* (Paris, et al. 2013) are known for their very large host

259   spectrum and low host specificity (Shatrov and Kudryashova 2006), which may have

260   facilitated the introduction of the bacteria into new environments. The direct cycle of non-

261   vectored bacteria such as *Mycoplasma* may also enhance their establishment probabilities.

262   Data on native rodents provided evidence in favour of ecological processes connecting

263   parasitism and invasion success. The higher prevalence of *Ehrlichia* in native rodents at the

264   mouse invasion front compared to non-invaded sites could result from a spillback process.

265   The 'novel weapon' hypothesis might explain the relatively high prevalence of

266   *Streptobacillus* in native *Mastomys* spp. at the invasion front of *R. rattus*. Indeed, this OTU is

267 known to be common and commensal in rats, but putatively pathogenic in other rodents

268 (Elliott 2007). Finally, our data also revealed patterns that are consistent with the

269 dilution/density hypotheses on the rat invasion route. Indeed, species richness as well as

270 *Bartonella* and *Mycoplasma_6* prevalence in native rodents were shown to be lower at

271 invasion front than in non-invaded areas. The non-detection of *Mycoplasma_6* in *R. rattus* did

272 not support an involvement of the exotic rodent as a sink reservoir, and may thus suggest a

273 host density (i.e., decrease of native host densities due to the presence of invaders (Keesing, et

274 al. 2010)) rather than a host dilution effect. In contrast, the occurrence *Bartonella* in *R. rattus*

275 may suggest a dilution effect by the exotic rodent. Dilution effects were already shown by

276 longitudinal surveys for *Bartonella* infections in native woodmouse *Apodemus sylvaticus*

277 communities invaded by the bank vole *Myodes glareolus* in the United Kingdom (Telfer, et

278 al. 2007).

279 Overall, this study evidenced diverse potential roles of zoonotic bacteria in the invasion

280 success of rodents in Senegal. Because the ecological patterns described here did not enable to

281 measure the impact of zoonotic bacteria at both host individual and population levels,

282 experimental studies are necessary to decipher the role of zoonotic bacteria in the invasion

283 process. Besides, developing comparative spatio-temporal surveys of various invasion routes

284 is a further critical step to understand if it is possible to generalise on the relationships

285 between parasitism and invasion success, despite environmental and demographic

286 stochasticity (Colautti and Lau 2015).

287 Research on bacterial communities infecting wild rodents remains scarce in continental

288 Africa. To our knowledge, it mainly concerned *Yersinia pestis* (e.g., (Moore, et al. 2015))*,*

289 *Borrelia* sp. (Trape, et al. 2013), *Bartonella* sp. (Hayman, et al. 2013), and *Leptospira* sp.

290 (Dobigny, et al. 2015). Our work allowed to identify four other genera of zoonotic bacteria

291 (e.g., *Ehrlichia*, *Orientia*, *Rickettsia*, *Streptobacillus*) in invasive and/or native commensal

292   rodents from Senegal, which may cause severe human diseases still underdiagnosed in Africa

293   (WHO 2009). By doing so, this study corroborated previous works highlighting the major role

294   of exotic rodents in public health issues (e.g., (Himsworth, et al. 2013). Indeed, we showed

295   that invasive rodents have probably brought exotic bacteria at invasion fronts (e.g. *Orientia* in

296   North Senegal by *M. m. domesticus*; *Streptobacillus* or *Rickettsia* in South-East Senegal by *R.*

297   *rattus*). We also showed that the introduction of invasive rodents may change the prevalence

298   of some bacteria carried by native rodents (e.g., increase of infections related to *Ehrlichia*;

299   decrease of infections related to *Bartonella*), or lead to the replacement of local lineages or

300   species of *Mycoplasma* by exotic ones. These results advocated for an increased vigilance

301   along the invasion roads of these exotic rodents with regard to the emergence of rodent borne

302   diseases.

303

304   In conclusion, our focus on invasion and parasitism provided a larger support to the

305   assumption still strongly debated (Salkeld, et al. 2015) that the relationship between

306   biodiversity and disease risk is difficult to predict, especially when tracking multiple diseases

307   (Wood, et al. 2014). We advocate for public health prevention strategies based on multiple

308   pathogen surveillance, especially in the case of invasive reservoir/vector species monitoring

309   (Caffrey, et al. 2014). This is particularly true for underdeveloped regions, where many

310   infectious diseases originating from wildlife are underreported.

311

312   **Methods**

313   **Sample collection.** We used data from historical records and population genetics studies

314   (Dalecky, et al. 2015; Konecny, et al. 2013); Lippens et al. unpublished data) as well as

315   longitudinal sampling surveys carried out in rodent communities of Senegal since the 1980s

316   (http://www.bdrss.ird.fr/bdrsspub_form.php (Granjon and Duplantier 2009) to select sampling

317 sites (villages or towns) into three categories of '*invasion status*' for each invasion route: (i)

318 coastal sites of long-established invasion, where rats/mice have settled in large and permanent

319 populations since at least the 18th-19th century and have excluded native rodents, (ii) sites at

320 invasion front where invasive rodents have recently arrived (10-30 years ago) and currently

321 co-occur with native ones, and (iii) non-invaded sites where only native species are known to

322 occur. Three to six sites were systematically sampled for each category (Fig. 1). Details on

323 rodent trapping and identification as well as autopsy procedures were provided elsewhere

324 (Dalecky, et al. 2015; Diagne, et al. 2016). For bacterial 16S rRNA amplicon sequencing,

325 spleens were carefully removed, placed in RNAlater storage solution, stored at 4°C one night

326 and then at -20°C until further analyses.

327

328 **Bacterial 16S rRNA amplicon sequencing.** Data on bacterial communities considered in this

329 study were retrieved from a 16S rRNA amplicon sequencing approach described in (Galan, et

330 al. 2016). It enabled to get an almost complete inventory of the bacterial community infecting

331 the rodents sampled in this study. Briefly, genomic DNA was extracted from the spleen using

332 DNeasy 96 Tissue Kit (Qiagen) and screened via Illumina MiSeq sequencer after a PCR

333 amplification using a slightly modified version of the universal primers of (Kozich, et al.

334 2013) to amplify a 251bp portion of the 16S rRNA V4 region (V4F:

335 GTGCCAGCMGCCGCGGTAA; V4R: GGACTACHVGGGTWTCTAATCC). This hyper

336 variable region of the 16S rRNA gene allows an accurate taxonomic assignation of bacteria to

337 the genus level (Claesson, et al. 2010) for a wide range of bacteria. The technical procedures

338 and bioinformatics analyses employed to get data considered in this work are presented in

339 detail elsewhere (Galan, et al. 2016). Briefly, the MiSeq data was processed with the program

340 MOTHUR v1.34, and taxonomic assignation was performed using the SILVA SSU Ref

341 database v119 as a reference and refine using phylogenetic analyses and blast in GenBank.

342   The reads generated by the Illumina MiSeq sequencing were clustered into Operational

343   Taxonomic Units (OTUs) with a 3% divergence threshold. Finally, the data were filtered to

344   eliminate false positive and false negative results for each bacterial OTU. The presence of a

345   given OTU within a given rodent was next validated using systematic technical replicates.

346   Results were then summarized in a presence/absence dataset with only bacterial OTUs known

347   or suspected to be pathogenic for human or wildlife kept for further investigation.

348

349   **Data analyses.** Each invasion route was investigated separately. In order to avoid any

350   confounding effects in the data analyses, we considered the factor '*invasion status*' that

351   combines the host species and site category on the invasion route. It included four categories:

352   A) invasive species alone in sites of long-established invasion; B) invasive species at the

353   invasion front; C) native species at the invasion front and D) native species at non-invaded

354   sites.

355   To elucidate whether the composition of the bacterial communities was structured at site level

356   by the host species and/or the invasion status, we constructed dissimilarity matrices based on

357   Bray-Curtis (BC) β-diversity index obtained using data counts of infected individuals in each

358   host population. The structuration of the bacterial communities was then visualised with a

359   hierarchical clustering approach on BC dissimilarity matrices using a Ward linkage function

360   (Murtagh and Legendre 2014). The effects of host species and invasion status were tested

361   using a permutational multivariate analysis of variance (permanova) performed on distance

362   matrices. These analyses were carried out with vegan v2.4.2 (Oksanen, et al.) and phyloseq

363   v1.19.1 (McMurdie and Holmes 2013) R packages.

364   We next applied individual-level generalized linear mixed models (GLMMs) to test for

365   patterns of enemy release, novel weapon, spillback, and dilution/density hypotheses. We used

366   individual species richness (number of OTUs recorded in a single individual host) and OTUs

367   prevalence (infection by a specific OTU for which prevalence in the dataset reached at least

368   5%) as response variables. When an OTU was found exclusively or mainly (more than 95%

369   of infected hosts) in only one host species, variations in specific prevalence were tested using

370   a dataset restricted to individuals of this host species only. We assumed a binomial

371   distribution (quasibinomial if overdispersion) for incidence data and a Poisson distribution

372   (negative binomial if overdispersion) for species richness data. The full model included

373   individual host factors (*gender* and *body mass*), *invasion status* (A, B, C and D) and pairwise

374   interactions between host factors and specific invasion status. *Sampling site* was considered as

375   a random factor. The best-fitting model was selected based on a goodness-of-fit criterion, the

376   Akaike's Information Criterion with correction for samples of finite size (AICc). We then

377   chose the most parsimonious model among those selected within two AIC units of the best

378   model. *P*-values were obtained by stepwise model simplification using likelihood-ratio tests

379   (Crawley 2007). For each final model, linear regression residuals were checked to ensure that

380   regression assumptions regarding normality, independence and homogeneity of variance were

381   met (Crawley 2007). Multiple pairwise comparisons were performed via Wilcoxon test with

382   Holm's method correction for *p-value* adjustment. All model analyses were performed using

383   lme4 v1.1-8 (Bates, et al. 2015) and MuMIn v1.15.1 (Bartoń 2013) R packages.

384

385   **Ethical statements.** Trapping campaigns inside villages and private lands were conducted

386   with permission given by the adequate institutional and familial authorities. None of the

387   rodent species investigated in the present study has protected status (see list of the

388   International Union for Conservation of Nature). All procedures were designed to minimize

389   animal suffering and were approved by the official guidelines of the American Society of

390   Mammalogists (Sikes, et al. 2011). The spleen samples were sent in dry ice to Montpellier

391   (France) to be conserved in experimental animal room from CBGP (34-169-003). All transfer

392 and conservation procedures were carried out paying attention to comply with current

393 international legislations.

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BIBLIOGRAPHY

405
406
407 Badenhorst, Daleen, et al. 2012. Host cell/Orientia tsutsugamushi interactions: Evolution

408 and expression of syndecan-4 in Asian rodents (Rodentia, Muridae). Infection, Genetics and

409 Evolution 12(5):1136-1146.

410 Bartoń, Kamil. 2013. MuMIn: multi-model inference. R package version 1(5).

411 Bates, Douglas, et al. 2015. Fitting linear mixed-effects models using lme4; 2014. Preprint.

412 Available.

413 Boyer, Nelly, et al. 2010. Personality, space use and tick load in an introduced population

414 of Siberian chipmunks Tamias sibiricus. Journal of Animal Ecology 79(3):538-547.

415 Caffrey, Joe M, et al. 2014. Tackling invasive alien species in Europe: the top 20 issues.

416 Management of Biological Invasions 5(1):1-20.

417   Claesson, Marcus J., et al. 2010. Comparison of two next-generation sequencing

418   technologies for resolving highly complex microbiota composition using tandem variable 16S

419   rRNA gene regions. Nucleic Acids Research 38(22).

420   Colautti, R.I., et al. 2004. Is invasion success explained by the enemy release hypothesis?

421   Ecology letters 7(8):721-733.

422   Colautti, Robert I, and Jennifer A Lau. 2015. Contemporary evolution during invasion:

423   evidence for differentiation, natural selection, and local adaptation. Molecular Ecology

424   24(9):1999-2017.

425   Cosson, Jean François, et al. 2015. Detection of Orientia sp. DNA in rodents from Asia,

426   West Africa and Europe. Parasites & Vectors 8(1):172.

427   Crawley, Michael J. 2007. The R book. Chichester. UK: Wiley.

428   Dalecky, Ambroise, et al. 2015. Range expansion of the invasive house mouse Mus

429   musculus domesticus in Senegal, West Africa: a synthesis of trapping data over three decades,

430   1983–2014. Mammal Review 45(3):176-190.

431   Diagne, Christophe, et al. 2016. Parasites and invasions: changes in gastrointestinal

432   helminth assemblages in invasive and native rode nts in Senegal. International Journal for

433   Parasitology:053082.

434   Dobigny, Gauthier, et al. 2015. Urban Market Gardening and Rodent-Borne Pathogenic

435   Leptospira in Arid Zones: A Case Study in Niamey, Niger. PLoS Negl Trop Dis

436   9(10):e0004097.

437   Dunn, Alison M, and Melanie J Hatcher. 2015. Parasites and biological invasions:

438   parallels, interactions, and control. TRENDS in Parasitology 31(5):189-199.

439   Elliott, Sean P. 2007. Rat bite fever and Streptobacillus moniliformis. Clinical

440   microbiology reviews 20(1):13-22.

441   Galan, Maxime, et al. 2016. 16S rRNA amplicon sequencing for epidemiological surveys

442   of bacteria in wildlife: the importance of cleaning post-sequencing data before estimating

443   positivity, prevalence and co-infection. bioRxiv:039826.

444   Granjon, L., and J.M. Duplantier. 2009. Les rongeurs de l'Afrique sahélo-soudanienne.

445   Marseille: Publications scientifiques du Muséum.

446   Han, Barbara A, et al. 2015. Rodent reservoirs of future zoonotic diseases. Proceedings of

447   the National Academy of Sciences 112(22):7039-7044.

448   Harris, D.B. 2009. Review of negative impacts of introduced rodents on small mammals

449   on islands. Biological Invasions 11:1611-1630.

450   Hayman, David TS, Katherine D McDonald, and Michael Y Kosoy. 2013. Evolutionary

451   history of rat-borne Bartonella: the importance of commensal rats in the dissemination of

452   bacterial infections globally. Ecology and evolution 3(10):3195-3203.

453   Himsworth, C.G., et al. 2013. Rats, Cities, People, and Pathogens: A Systematic Review

454   and Narrative Synthesis of Literature Regarding the Ecology of Rat-Associated Zoonoses in

455   Urban Centers. Vector-Borne and Zoonotic Diseases 13(6):349-359.

456   Keesing, F., et al. 2010. Impacts of biodiversity on the emergence and transmission of

457   infectious diseases. Nature 468:647-652.

458   Kelly, D. W., et al. 2009. Parasite spillback: A neglected concept in invasion ecology?

459   Ecology 90(8):2047-2056.

460   Kiffner, Christian, et al. 2013. Sex-biased parasitism is not universal: evidence from

461   rodent–flea associations from three biomes. Oecologia 173(3):1009-1022.

462   Konecny, A., et al. 2013. Invasion genetics of the introduced black rat (Rattus rattus) in

463   Senegal, West Africa. Molecular Ecology 22(2):286-300.

464   Kozich, James J., et al. 2013. Development of a Dual-Index Sequencing Strategy and

465   Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing

466   Platform. Applied and Environmental Microbiology 79(17):5112-5120.

467   Krasnov, Boris R, et al. 2012. Gender-biased parasitism in small mammals: patterns,

468   mechanisms, consequences. Mammalia 76(1):1-13.

469   MacLeod, C.J., et al. 2010. Parasites lost – do invaders miss the boat or drown on arrival?

470   Ecology Letters 13:516–527.

471   McMurdie, Paul J, and Susan Holmes. 2013. phyloseq: an R package for reproducible

472   interactive analysis and graphics of microbiome census data. PloS one 8(4):e61217.

473   Meerburg, B. G., G. R. Singleton, and A. Kijlstra. 2009. Rodent-borne diseases and their

474   risks for public health. Critical reviews in microbiology 35(3):221-70.

475   Moore, Sarah L, and Kenneth Wilson. 2002. Parasites as a viability cost of sexual selection

476   in natural populations of mammals. Science 297(5589):2015-2018.

477   Moore, Sean M, et al. 2015. Seasonal fluctuations of small mammal and flea communities

478   in a Ugandan plague focus: evidence to implicate Arvicanthis niloticus and Crocidura spp. as

479   key hosts in Yersinia pestis transmission. Parasites & vectors 8(1):11.

480   Murtagh, Fionn, and Pierre Legendre. 2014. Ward's hierarchical agglomerative clustering

481   method: which algorithms implement ward's criterion? Journal of Classification 31(3):274-

482   295.

483   Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B.,

484   Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. 2016. vegan version 2.3-5:

485   Community Ecology Package. CRAN-The Comprehensive R Archive Network.

486   Organization, World Health. 2009. Integrated control of neglected zoonotic diseases in

487   Africa: applying the one health concept, report of a joint WH.

488   Paris, Daniel H, et al. 2013. Unresolved problems related to scrub typhus: a seriously

489   neglected life-threatening disease. The American journal of tropical medicine and hygiene

490   89(2):301-307.

491   Prenter, John, et al. 2004. Roles of parasites in animal invasions. Trends in Ecology &

492   Evolution 19(7):385-390.

493   Salkeld, Daniel J, et al. 2015. Public health perspective on patterns of biodiversity and

494   zoonotic disease. Proceedings of the National Academy of Sciences 112(46):E6261-E6261.

495   Shatrov, Andrey B, and Naina I Kudryashova. 2006. Taxonomy, life cycles and the origin

496   of parasitism in trombiculid mites. *In* Micromammals and macroparasites. Pp. 119-140:

497   Springer.

498   Sikes, R. S., W. L. Gannon, and Mammalogists Amer Soc. 2011. Guidelines of the

499   American Society of Mammalogists for the use of wild mammals in research. Journal of

500   Mammalogy 92(1):235-253.

501   Strauss, A., A. White, and M. Boots. 2012. Invading with biological weapons: the

502   importance of disease-mediated invasions. Functional Ecology 26(6):1249-1261.

503   Telfer, S, et al. 2007. Contrasting dynamics of Bartonella spp. in cyclic field vole

504   populations: the impact of vector and host dynamics. Parasitology 134(03):413-425.

505   Trape, J. F., et al. 2013. The epidemiology and geographic distribution of relapsing fever

506   borreliosis in West and North Africa, with a review of the Ornithodoros erraticus complex

507   (Acari: Ixodida). PLoS One 8(11):e78473.

508   Varma, MGR. 1964. Mites (family Trombiculidae) parasitizing birds migrating from Africa

509   to Europe. Bulletin of the World Health Organization 31(3):411.

510   Vial, Laurence, et al. 2006. Incidence of tick-borne relapsing fever in west Africa:

511   longitudinal study. The Lancet 368(9529):37-43.

512   Wood, Chelsea L, et al. 2014. Does biodiversity protect humans against infectious disease?

513   Ecology 95(4):817-832.

514   Wyatt, K.B., et al. 2008. Historical Mammal Extinction on Christmas Island (Indian

515   Ocean) Correlates with Introduced Infectious Disease. PLoS ONE 3:e3602.

516   Young, Hillary S, et al. 2017. Introduced Species, Disease Ecology, and Biodiversity–

517   Disease Relationships. Trends in Ecology & Evolution 32(1):41-54.

518   Zuk, Marlene, and Andrew M Stoehr. 2010. Sex differences in susceptibility to infection:

519   an evolutionary perspective. *In* Sex hormones and immunity to infection. Pp. 1-17: Springer.

520 **Table 1**: Sampling sites and their invasion status as well as rodent sample sizes and
521 proportion of infected hosts in each site on a) the mouse invasion route and b) the rat invasion
522 route. Legend: LI: sites of long-established invasion; IF: recently invaded sites (invasion
523 front); NI: non-invaded sites; Lat: latitude of sites; Long: longitude of sites; N: total number
524 of rodents sampled; M: males; F: females; n: number of hosts screened for bacteria; P:
525 prevalence (number of infected hosts / number of screened hosts) in %. '-' indicates no data
526 (no rodent sampled or screened).

527 a)

Sites (Invasion status)	Lat (North)	Long (West)	*Mus musculus domesticus*		*Mastomys erythroleucus*	
			N (M/F)	n (P)	N (M/F)	n (P)
Dagathie (LI)	15.62	-16.25	30 (18/12)	24 (66.7)	-	-
Mbakhana (LI)	16.08	-16.36	30 (14/16)	26 (19.2)	-	-
Ndombo (LI)	16.43	-15.70	23 (10/13)	21 (38.4)	-	-
Thilene (LI)	16.26	-16.18	26 (9/17)	19 (47.4)	-	-
Aere Lao (IF)	16.40	-14.32	50 (22/28)	25 (60)	22 (13/9)	20 (60)
Croisement Boube (IF)	16.49	-15.00	45 (24/21)	27 (18.5)	3 (2/1)	-
Dendoudi (IF)	15.39	-13.53	19 (13/5)	15 (33.3)	28 (18/10)	22 (91)
Dodel (IF)	16.48	-14.42	24 (9/15)	20 (45)	33 (16/17)	20 (30)
Lougue (IF)	16.06	-13.91	28 (13/15)	25 (28)	25 (15/10)	20 (65)
Diomandiou Walo (NI)	16.49	-15.00	-	-	14 (10/4)	13 (84.6)
Doumnga Lao (NI)	16.33	-14.22	-	-	43 (18/25)	20 (35)
Lambango (NI)	15.46	-13.53	-	-	23 (10/13)	21 (85.7)
Sare Maounde (NI)	15.98	-13.91	-	-	9 (5/4)	9 (33.3)

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529 b)

Sites (Invasion status)	Lat (North)	Long (West)	*Rattus rattus*		*Mastomys erythroleucus*		*Mastomys natalensis*	
			N (M/F)	n (P)	N (M/F)	n (P)	N (M/F)	n (P)
Diakene-Wolof (LI)	12.45	-16.64	30 (12/18)	24 (25)	8 (5/3)	-	-	-
Diattacounda (LI)	12.57	-15.68	35 (19/16)	27 (70.4)	16 (9/7)	-	-	-
Marsassoum (LI)	12.83	-15.97	29 (13/16)	25 (76)	3 (0/3)	-	-	-
Tobor (LI)	12.66	-16.25	29 (12/17)	21 (81)	1 (1/0)	-	-	-
Badi Nieriko (IF)	13.37	-13.37	73 (35/38)	21 (62)	14 (6/8)	13 (84.6)	-	-
Boutougoufara (IF)	13.39	-12.48	40 (15/25)	28 (28.6)	21 (12/9)	21 (95.2)	-	-
Kedougou (IF)	12.55	-12.17	34 (13/21)	21 (9.5)	-	-	30 (11/19)	22 (72.7)
Soutouta (IF)	13.80	-12.71	26 (13/13)	22 (86.4)	12 (5/7)	12 (75)	-	-
Bransan (NI)	13.26	-12.10	-		6 (4/2)	-	33 (14/19)	23 (78.3)
Mako (NI)	12.85	-12.35	-		-	-	48 (28/20)	24 (95.8)
Segou (NI)	12.41	-12.28	-		-	-	26 (10/16)	24 (100)

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Host species	Sites	*Bartonella*	*Borrelia*	*Ehrlichia*	*Mycoplasma_1*	*Mycoplasma_3*	*Orientia*
	DAG (LI)				29.2 [13.9 - 50.0]		54.2 [34 - 73.3]
	MBA (LI)		3.8 [0.2 - 18.8]	7.7 [4.0 - 25.0]	11.5 [3.2 - 30.4]		
	NDB (LI)	4.8 [0.3 - 23.3]					38.1 [19.7 - 60.0]
	THL (LI)				21.1 [7.5 - 44.6]	11.5 [3.2 - 30.4]	11.5 [3.2 - 30.4]
M. m. domesticus	AEL (IF)		4.0 [0.2 - 19.6]		48.0 [29.6 - 68.3]		28.0 [13.4 - 48.0]
	CRB (IF)		3.7 [0.2 - 18.1]		3.7 [0.2 - 18.1]		11.0 [3.1 - 29.2]
	DEN (IF)			33.3 [14.2 - 60.3]			6.7 [0.4 - 30.2]
	DOD (IF)				10.0 [1.8 - 32.0]		40.0 [20.9 - 63.0]
	LOU (IF)		20.0 [8.2 - 40.0]	20.0 [8.2 - 40.0]			4.0 [0.1 - 26.5]
	AEL (IF)			20.0 [7.1 - 42.4]	5.0 [0.2 - 24.4]	45.0 [24.2 - 68]	
	DEN (IF)		13.6.0 [3.28- 33.8]	9.1 [1.6 - 29.1]	40.9 [22.2 - 61.7]	77.3 [54.7 - 90.6]	
	DOD (IF)			25.0 [10.4 - 47.5]		5.0 [0.3 - 24.4]	
Ma. erythroleucus	LOU (IF)	5.0 [0.3 - 24.4]	25.0 [10.4 - 47.5]	55.0 [32.0 - 75.6]			
	DIW (NI)	38.5 [16.6 - 65.8]			30.8 [11.3 - 58.6]	53.8 [26.1 - 77.6]	
	DOL (NI)		10.0 [1.8 - 32.0]	15.0 [4.2 - 37.2]		30.0 [14.0 - 52.5]	
	LAM (NI)	9.5 [1.7 - 30.5]	33.3 [15.9 - 55.5]	33.3 [15.9 - 55.5]	28.6 [13.3 - 50.6]	52.4 [30.5 - 72.4]	
	SAM (NI)		22.2 [4.11 - 55.8]	33.3 [9.8 - 67.7]			

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Host species	Sites	Bartonella	Borrelia	Ehrlichia	Mycoplasma_1	Mycoplasma_2	Mycoplasma_3	Mycoplasma_4	Mycoplasma_5	Mycoplasma_6	Orientia	Rickettsia	Streptobacillus
R. rattus	DIK (LI)					20.0 [8.2 - 39.8]							4.0 [0.2 - 19.6]
	DIT (LI)	11.1 [3.1 - 29.2]				51.9 [33.1 - 70.8]		33.3 [18.1 - 53.8]					11.1 [3.1 - 29.2]
	MAR (LI)					72 [52.0 - 86.6]		24.0 [11.0 - 43.9]					
	TOB (LI)	4.8 [0.3 - 23.3]				81.0 [59.7 - 93.2]						4.8 [0.3 - 23.3]	
	BAN (IF)					61.9 [40.3 - 80.3]		9.5 [1.7 - 30.5]					
	BOU (IF)	3.6 [0.2 - 17.5]			3.6 [0.2 - 17.5]	25.0 [12.0 - 44.6]	3.6 [0.2 - 17.5]						
	KED (IF)	4.8 [0.3 - 23.3]											4.8 [0.3 - 23.3]
	SOU (IF)		27.3 [12.6 - 50.0]	36.4 [18.7 - 58.2]		72.7 [50 - 87.4]		4.5 [0.1 - 22.9]					
Ma erythroleucus	BAN (IF)	53.8 [26.1 - 77.6]					53.8 [26.1 - 77.6]						23.1 [6.6 - 52]
	BOU (IF)	90.5 [69.5 - 98.3]			4.8 [0.3 - 23.3]		71.4 [49.4 - 86.8]						14.3 [4.0 - 35.4]
	SOU (IF)	8.3 [0.4 – 37.0]	16.7 [3.1 - 45.7]	41.8 [18.1 - 70.6]	16.7 [3.1 - 45.7]		58.3 [29.4 - 81.9]		8.3 [0.4 - 37.0]	8.3 [0.4 - 37.0]			16.7 [3.1 - 45.7]
Ma natalensis	KED (IF)	59.1 [38.3 - 77.8]			59.1 [38.3 - 77.8]		13.6 [3.8 - 33.8]						4.5 [0.2 - 22.2]
	BRA (NI)	73.9 [52.2 - 88.0]			43.5 [24.7 - 64.0]		8.7 [1.6 - 27.8]		8.7 [1.6 - 27.8]	21.7 [9.0 - 43.3]	4.3 [0.2 - 21.3]		
	MAK (NI)	83.3 [62.8 - 94.1]			41.7 [23.4 - 62.8]		58.3 [37.3 - 76.6]			20.8 [8.6 - 41.5]	4.2 [0.2 - 20.4]		
	SEG (NI)	95.8 [79.6 - 99.8]			37.5 [20.4 - 58.5]		87.5 [69.0 - 96.5]		8.3 [1.5 - 26.7]	12.5 [3.5 - 31.0]			

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546 **Table 4:** Factors shaping bacterial species richness and OTUs prevalence along both invasion routes.
547 Significant factors are those from the most parsimonious generalized linear mixed model obtained
548 after model selection and residuals-based validation procedures carried out for each response variable.
549 Variations in specific prevalence were tested using a dataset restricted to a single host species when at
550 least 95% of infected hosts by an OTU were from one species. AICc: Akaike's information criterion
551 with correction for finite sample size. Δ indicates the difference between the model selected and the
552 model with the lowest AICc. M: Males; F: Females. LRT: Likelihood-ratio test value. *P-value* was
553 considered significant when < 0.05.

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| Invasion route	Response variable	Dataset considered	AICc (Δ)	Significant factors	Df	LRT	*p-value*
House mouse	*Borellia*	whole	165.8 (1.95)	gender	1	10.04	0.002
	Ehrlichia	whole	241.3 (0.00)	body mass	1	4.27	0.039
				gender	1	6.54	0.011
				invasion status	3	7.46	< 0.001
	Mycoplasma 1	whole	267.0 (1.24)	gender	1	48.57	< 0.001
	Mycoplasma 3	only native	160.2 (0.14)	gender : invasion status	3	6.1	0.014
	Species richness	whole	701.9 (0.53)	gender	1	11.32	0.001
				invasion status	3	9.41	0.024
Black rat	*Bartonella*	whole	202.7 (0.08)	gender	1	5.03	0.025
	Mycoplasma 1	only native	148.6 (0.82)	body mass	1	22.6	< 0.001
	Mycoplasma 2	whole	219.0 (0.00)	body mass	1	11	0.001
	Mycoplasma 4	whole	92.6 (0.00)	body mass	1	8.98	0.003
				gender	1	5.39	0.02
	Mycoplasma 5	only native	79.3 (0.00)	Body mass	1	4.42	0.036
	Mycoplasma 6	only native	82.3 (0.00)	gender	1	8.9	0.003
				invasion status	1	7.6	0.006
	Species richness	whole	825.4 (0.00)	body mass	1	20.47	< 0.001
				gender	1	14.46	< 0.001
				invasion status	3	62.99	< 0.001

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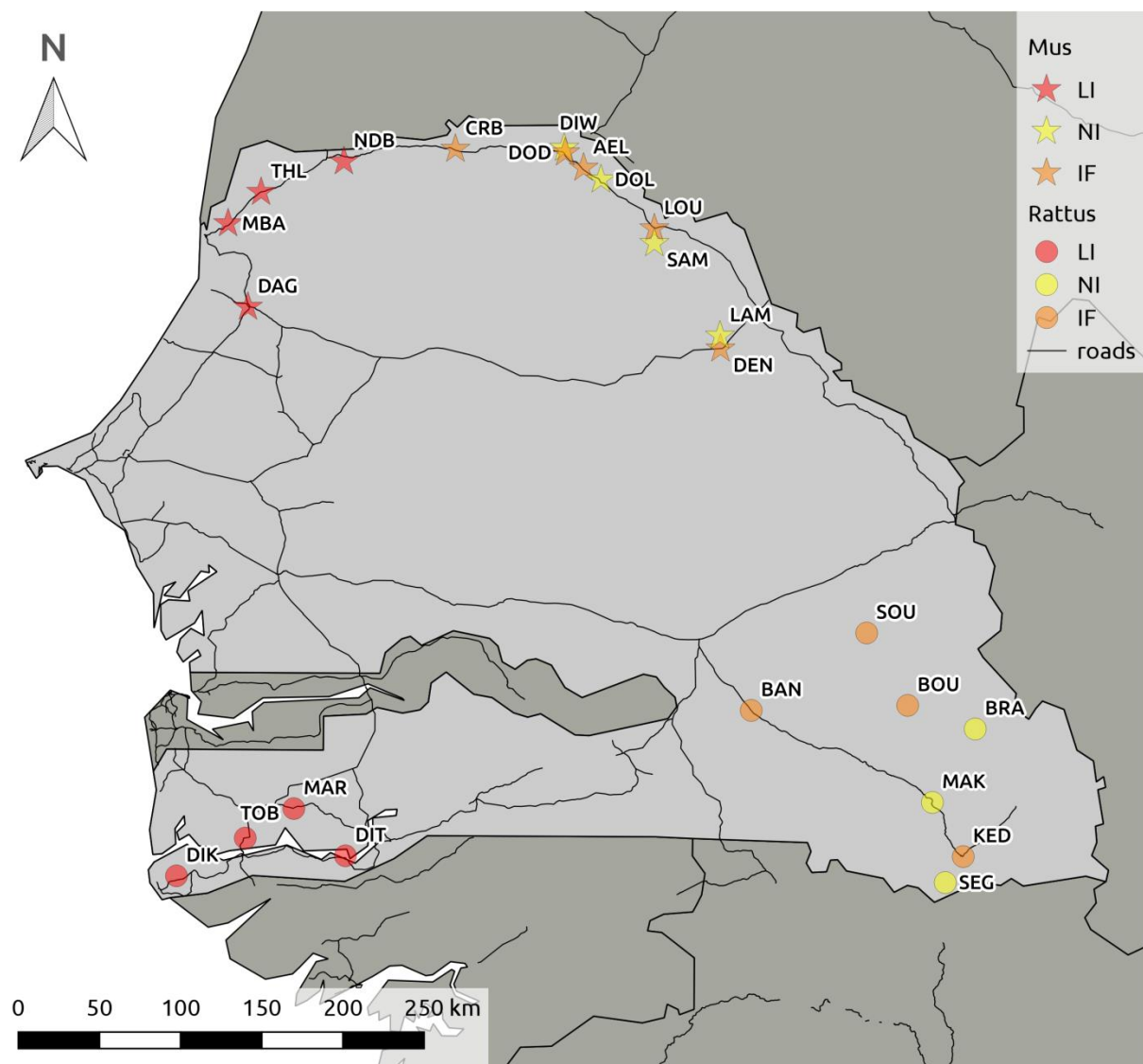
Figure 1: Rodent sampling sites on the house mouse (*Mus musculus domesticus*) (star symbols) and black rat (*Rattus rattus*) (circle symbols) invasion routes in Senegal (West Africa). Colour legend: red for sites of long-established invasion; orange for recently invaded sites; yellow for non-invaded sites. Correspondence between site codes and names are detailed in Table 1.
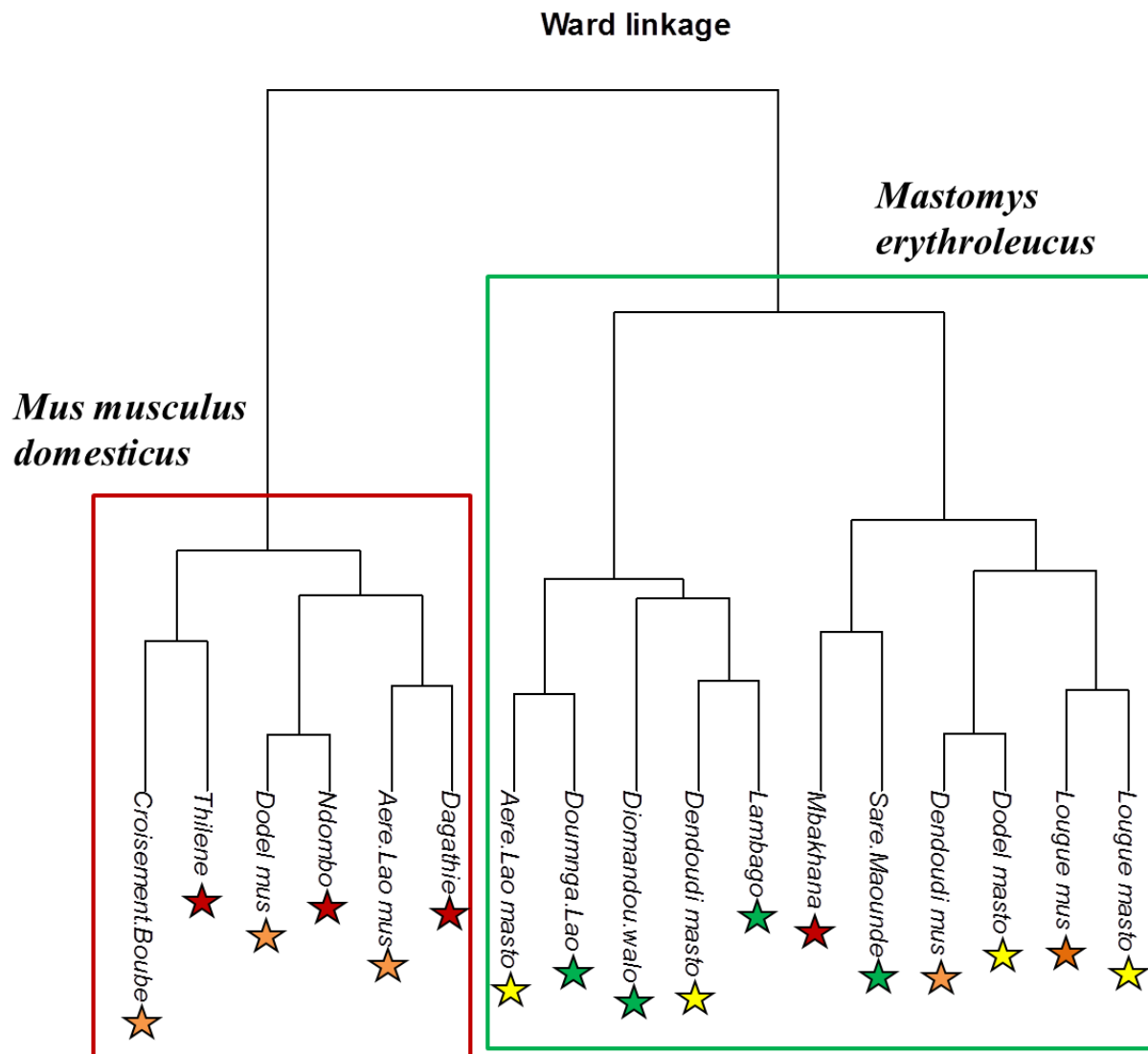
Figure 2: Bray-Curtis (BC) dissimilarity index-based Ward's hierarchical clustering of the bacterial communities described in the rodent host populations sampled along the mouse invasion route. The graph shows that the bacterial communities were clustered by the host species (permutational multivariate analyses of variance [permanova] performed on BC index-based matrices: percentage of total variance explained R2 = 0.29; F = 5.77, *p = 0.002*) but not by the invasion status of sampling sites (permanova performed on BC index-based matrices: R2 = 0.05; F = 0.53, *p = 0.817*). Legend: red stars: *Mus musculus domesticus* populations at sites of long-established invasion; orange stars: *M. m. domesticus* populations at invasion front sites; yellow stars: *Mastomys erythroleucus* populations at invasion front sites; green stars: *Ma. erythroleucus* populations at non-invaded sites. At invasion front sites where both native and invasive species co-occurred, "mus" or "masto" was added after the site name for distinguishing *M. m. domesticus* and *Ma. erythroleucus* populations, respectively.
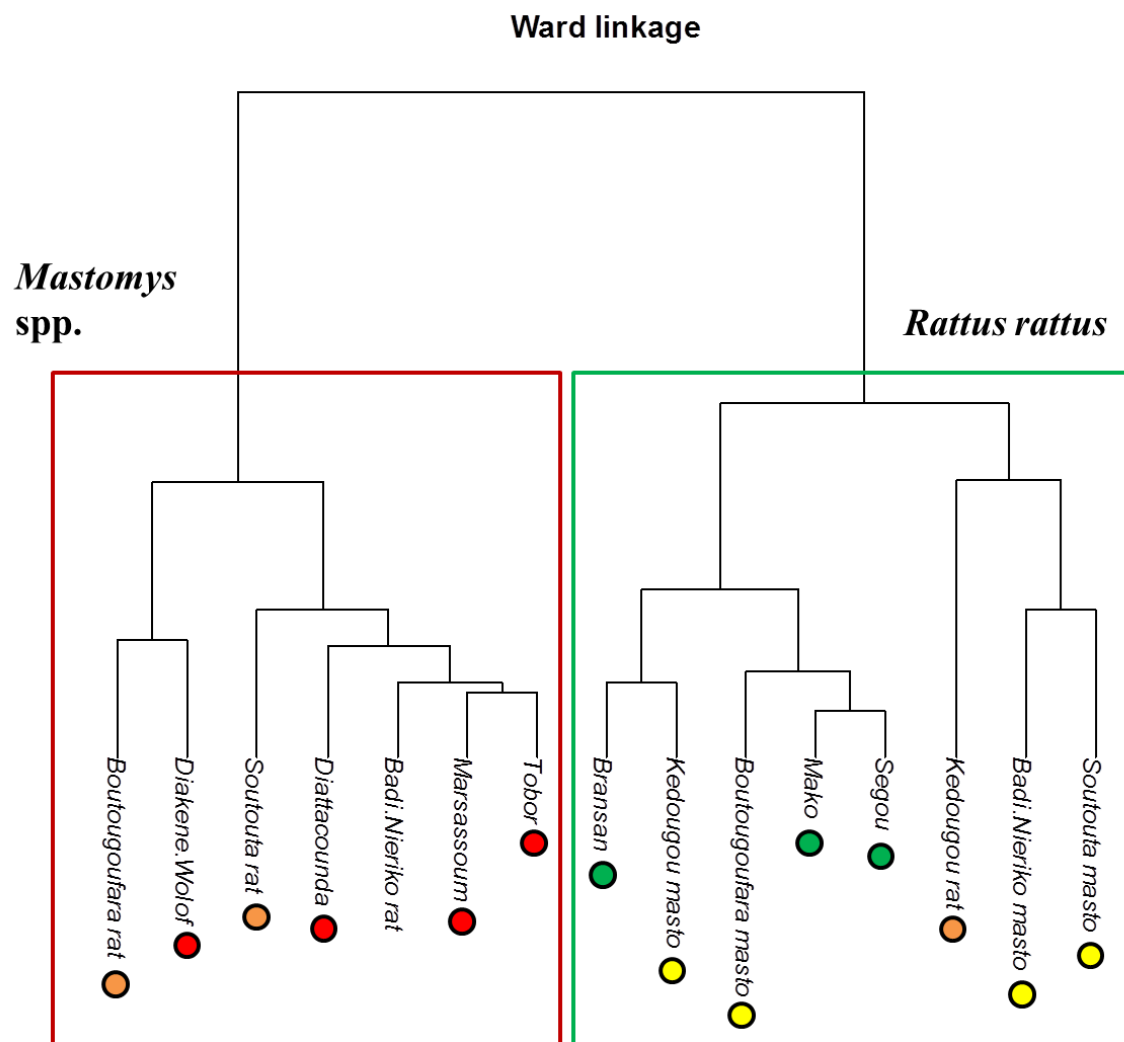
Figure 3: Bray-Curtis (BC) dissimilarity index-based Ward's hierarchical clustering of the bacterial communities described in the rodent host populations sampled along the rat invasion route. The graph shows that the bacterial communities were clustered by the host species (permutational multivariate analyses of variance [permanova] performed on BC index-based matrices: percentage of total variance explained $R2 = 0.52$; $F = 14.35$, *p < 0.001*) but not by the invasion status of sampling sites (permanova performed on BC index-based matrices: $R2 = 0.08$; $F = 1.13$, *p = 0.334*). Legend: red stars: *Rattus rattus* populations at sites of long-established invasion; orange stars: *R. rattus* populations at invasion front sites; yellow stars: *Mastomys* spp. populations at invasion front sites; green stars: *Mastomys* spp. populations at non-invaded sites. At invasion front sites where both native and invasive species co-occurred, "rat" or "masto" was added after the site name for distinguishing *R. rattus* and *Mastomys* spp. populations, respectively.
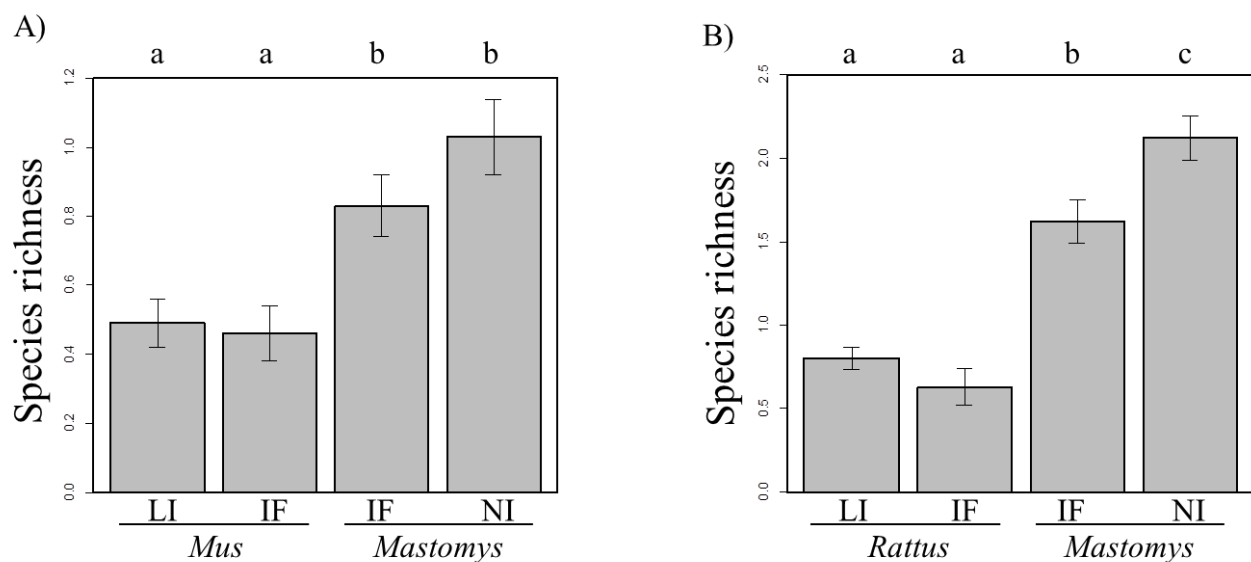
Figure 4: Difference in species richness (number of bacterial OTUs found in one host individual) between the specific invasion statuses from the A) mouse invasion route and B) rat invasion route. Error bars represent standard error for species richness data. Different letters above boxplots indicate significant difference between two categories of specific invasion status. Legend: *Mus*: *Mus musculus domesticus*; *Mastomys*: *Mastomys erythroleucus* for the mouse invasion route; *Ma. erythroleucus* and *Mastomys natalensis* in IF sites, *Ma. natalensis* in NI sites for the rat invasion route; LI: sites of long-established invasion; IF: invasion front; NI: non-invaded sites.

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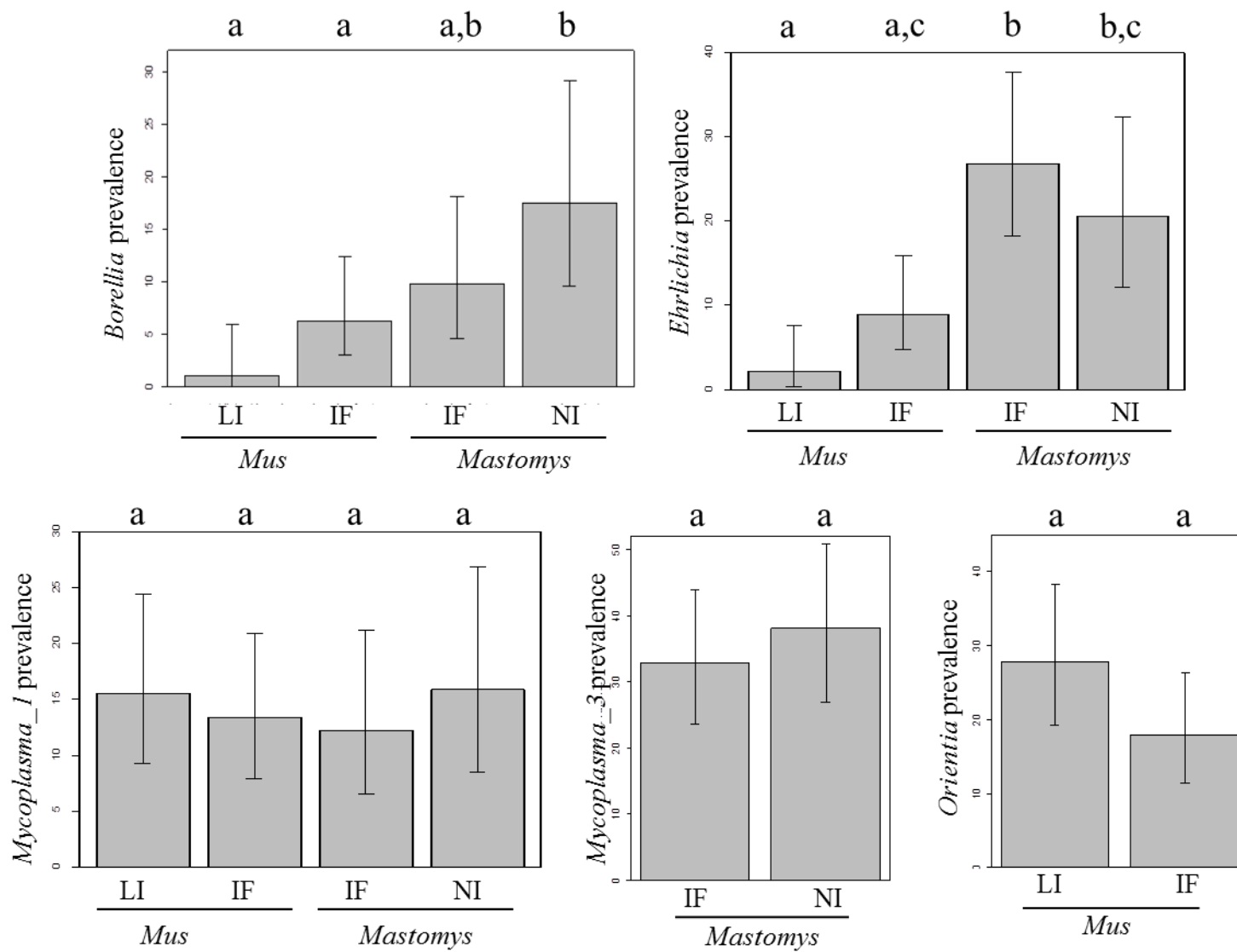
610 **<u>Figure 5</u>**: Difference in OTU prevalence between the categories of specific invasion status from the

611 mouse invasion route. Only OTUs showing overall prevalence > 5% in the dataset are showed.

612 Error bars represent 95% confidence intervals calculated with Sterne's exact method for prevalence

613 data. Different letters above boxplots indicate significant difference between two categories of

614 specific invasion status. Legend: *Mus: Mus musculus domesticus*; *Mastomys*: *Mastomys*

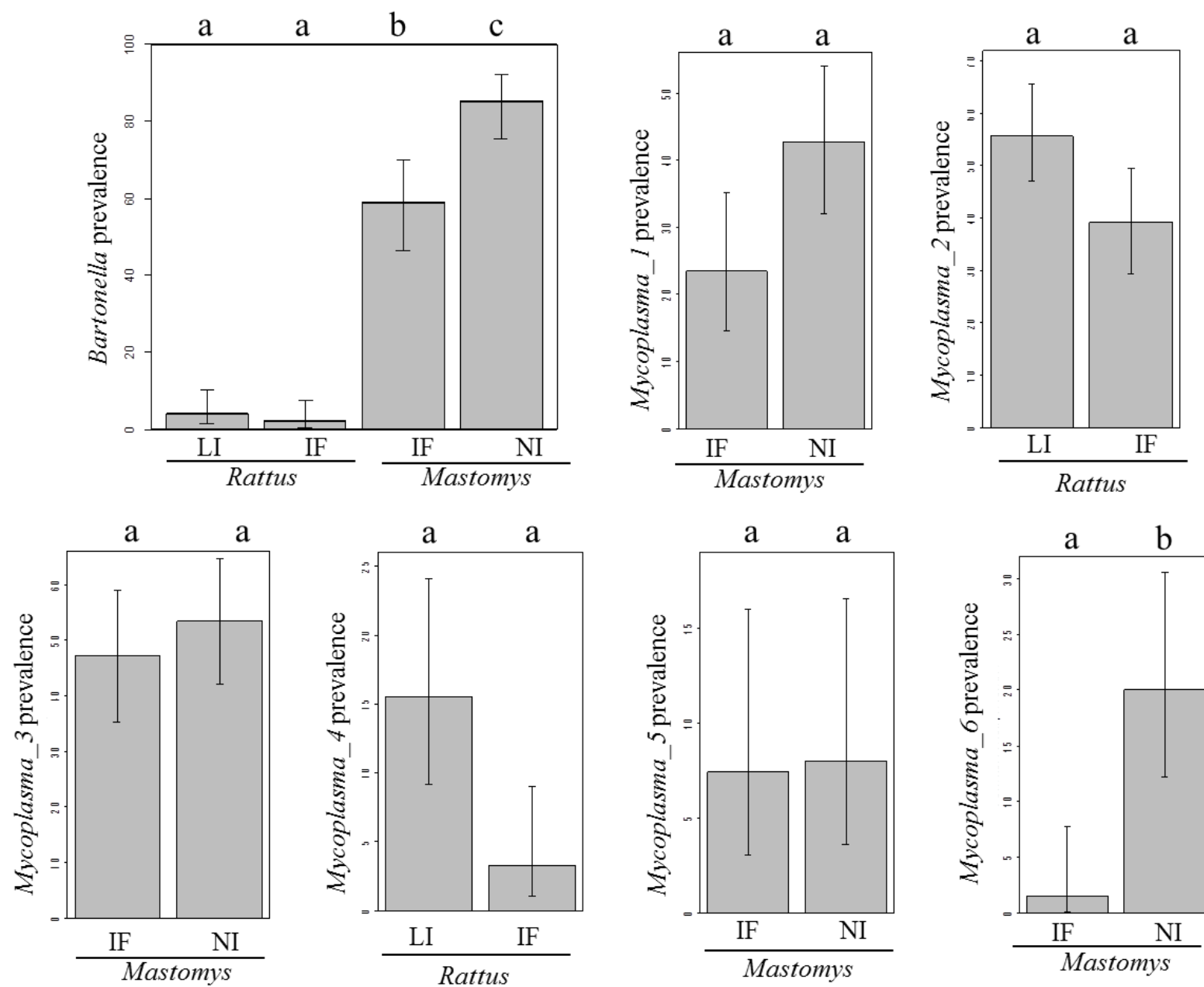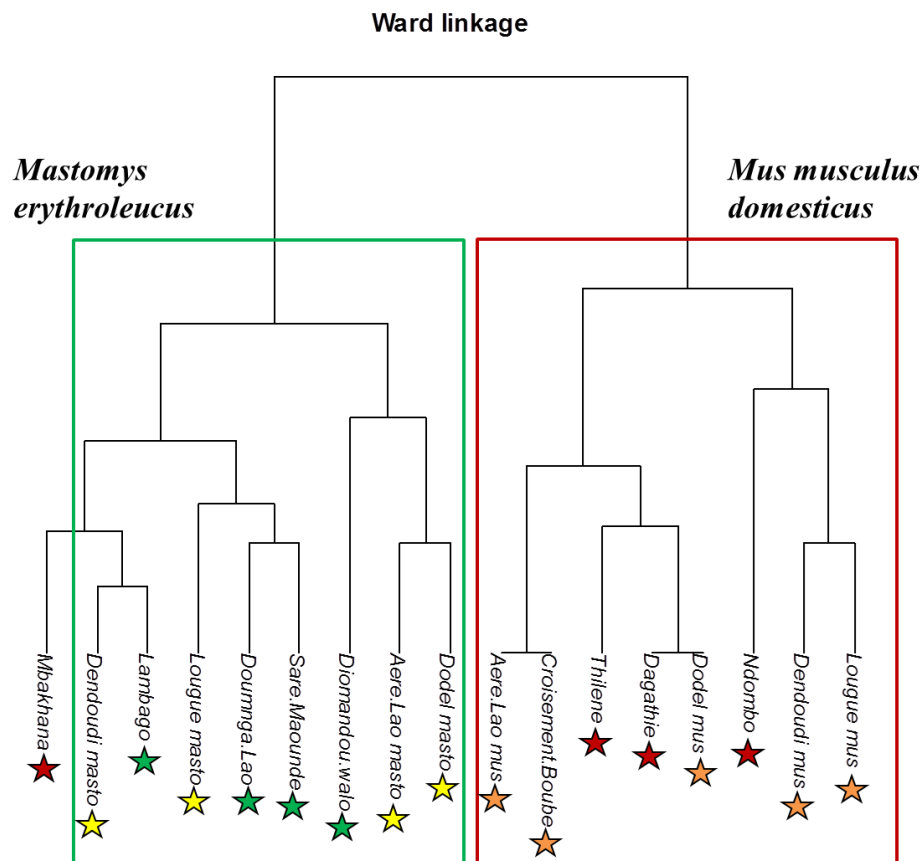615 *erythroleucus*; LI: sites of long-established invasion; IF: invasion front; NI: non-invaded sites.

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617

618   **Figure 6**: Difference in OTU prevalence between the categories of specific invasion status

619   from the rat invasion route. Error bars represent 95% confidence intervals calculated with

620   Sterne's exact method for prevalence data, and standard error for species richness data.

621   Different letters above boxplots indicate significant difference between two categories of

622   specific invasion status. Legend: *Rattus*: *Rattus rattus*; *Mastomys*: *Mastomys* spp. (*Ma.*

623   *erythroleucus* and *Ma. natalensis* in IF sites, *Ma. natalensis* in NI sites); LI: sites of long-

624   established invasion; IF: invasion front; NI: non-invaded sites.

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Ward linkage

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Supplementary figure 1: Jaccard dissimilarity index-based Ward's hierarchical clustering of the bacterial communities described in the rodent host populations sampled along the mouse invasion route. The graph shows that the bacterial communities were clustered by the host species (permutational multivariate analyses of variance [permanova] performed on Jaccard index-based matrices: percentage of total variance explained R2 = 0.33; F = 6.82, *p = 0.001*) but not by the invasion status of sampling sites (permanova performed on Jaccard index-based matrices: R2 = 0.03; F = 0.33, *p = 0.919*). Legend: red stars: *Mus musculus domesticus* populations at sites of long-established invasion; orange stars: *M. m. domesticus* populations at invasion front sites; yellow stars: *Mastomys erythroleucus* populations at invasion front sites; green stars: *Ma. erythroleucus* populations at non-invaded sites. At invasion front sites where both native and invasive species co-occurred, "mus" or "masto" was added after the site name for distinguishing *M. m. domesticus* and *Ma. erythroleucus* populations, respectively.

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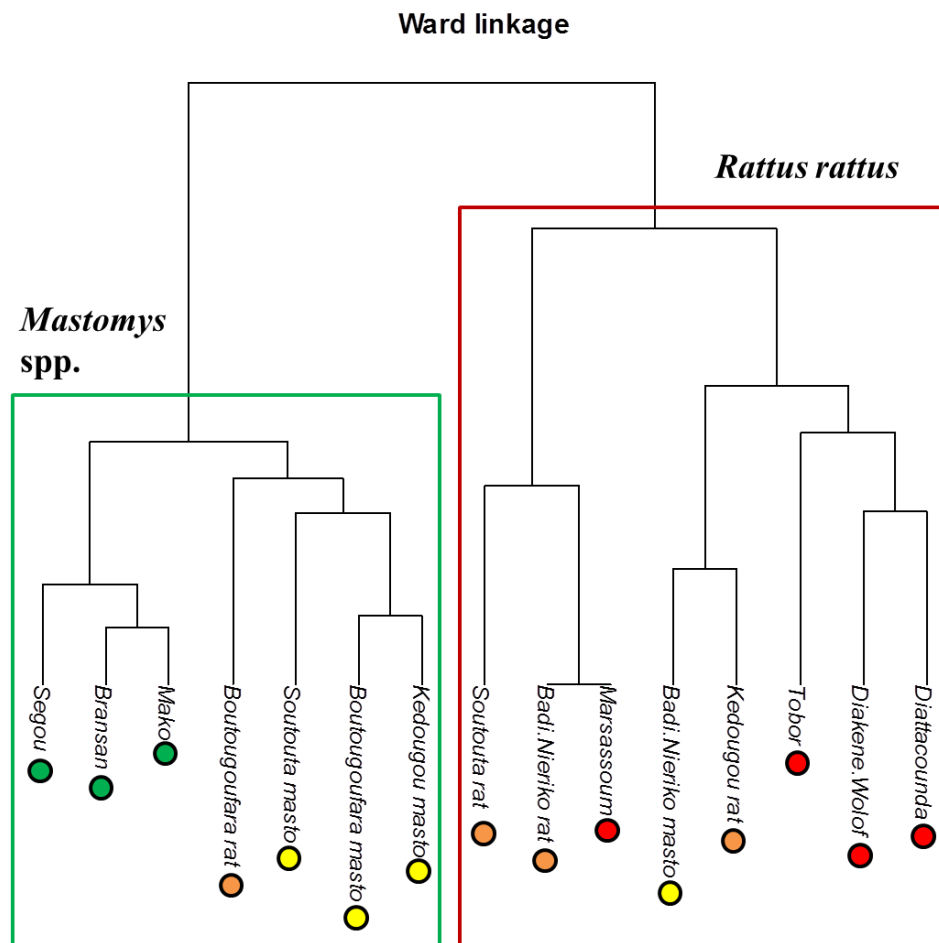
Supplementary figure 2: Jaccard dissimilarity index-based Ward's hierarchical clustering of the

bacterial communities described in the rodent host populations sampled along the rat invasion route.

The graph shows that the bacterial communities were clustered by the host species (permutational

multivariate analyses of variance [permanova] performed on Jaccard index-based matrices: percentage

of total variance explained $R2 = 0.39$; $F = 8.57$, *$p < 0.001$*) but not by the invasion status of sampling

sites (permanova performed on Jaccard index-based matrices: $R2 = 0.12$; $F = 1.31$, *$p = 0.253$*).

Legend: red stars: *Rattus rattus* populations at sites of long-established invasion; orange stars: *R.*

rattus populations at invasion front sites; yellow stars: *Mastomys* spp. populations at invasion front

sites; green stars: *Mastomys* spp. populations at non-invaded sites. At invasion front sites where both

native and invasive species co-occurred, "rat" or "masto" was added after the site name for

distinguishing *R. rattus* and *Mastomys* spp. populations, respectively.

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