Analyses of symbiotic bacterial communities in the plant pest *Bemisia tabaci* reveal high prevalence of ~~*Hemipteriphilus*~~*Candidatus* Hemipteriphilus asiaticus ~~*asiaticus*~~ on the African continent

Mis en forme : Police :Non Italique

Laurence Mouton, [a]# Hélène Henri, [a] Rahim Romba, [b] Zainab Belgaidi, [a] Olivier Gnankiné, [b] Fabrice Vavre [a]

[a]Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR5558, F-69622 Villeurbanne, France.

[b]Laboratoire d'Entomologie Fondamentale et Appliquée, Unité de Formation et de Recherche en Sciences de la Vie et de la Terre (UFR-SVT), Université Ouaga I Pr Joseph Ki Zerbo, Ouagadougou, Burkina Faso.

Running Head: ~~*Hemipteriphilus*~~*Candidatus* Hemipteriphilus asiaticus ~~*asiaticus*~~ in African whiteflies

Mis en forme : Police :Non Italique

#Address correspondence to Laurence Mouton, laurence.mouton@univ-lyon1.fr.

Abstract

Microbial symbionts are widespread in insects and some of them have been associated to adaptive changes. Primary symbionts (P-symbionts) have a nutritional role that allows their hosts to feed on unbalanced diets (plant sap, wood, blood). Most of them have undergone genome reduction, but their genomes still retain genes involved in pathways that are necessary to synthesize the nutrients that their hosts need. However, in some P-symbionts, essential pathways are incomplete and secondary symbionts (S-symbionts) are required to complete parts of their degenerated functions. The P-symbiont of the phloem sap-feeder *Bemisia tabaci*, *Candidatus* Portiera aleyrodidarium, lacks genes involved in the synthesis of vitamins, cofactors, and also of some essential amino-acids. Seven S-symbionts have been detected in the *B.* tabaci species complex. Phenotypic and genomic analyses have revealed various effects, from reproductive manipulation to fitness benefits, notably some of them have complementary metabolic capabilities to *Candidatus* Portiera aleyrodidarium~~*Portiera*~~, suggesting that their presence may be obligatory. In order to get the full picture of the symbiotic community of this pest, we investigated, through metabarcoding approaches, the symbiont content of individuals from Burkina Faso, a West African country where *B. tabaci* induces severe crop damage. While no new putative *B. tabaci* S-symbiont was identified, ~~***Hemipteriphilus***~~*Candidatus* Hemipteriphilus asiaticus, a symbiont only described in *B. tabaci* populations from Asia, was detected for the first time on this continent. Phylogenetic analyses however reveal that it is a different strain than the reference found in Asia. Specific diagnostic PCRs showed a high prevalence of these S-symbionts and especially of *Candidatus* Hemipteriphilus asiaticus ~~***Hemipteriphilus***~~ in different genetic groups. These results suggest that *Candidatus* Hemipteriphilus asiaticus ~~***Hemipteriphilus***~~ may affect the biology of *B. tabaci* and provide fitness advantage in some *B. tabaci* populations.

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

Keywords: *Bemisia tabaci, Candidatus* Hemipteriphilus asiaticus~~*Hemipteriphilus asiaticus*~~, secondary symbionts

Introduction

Many insects feed on nutrient-imbalanced diet (plant sap, wood, blood), and there is strong experimental evidence that microbial symbionts can promote utilization of these resources by the synthesis of essential nutrients, like amino-acids and vitamins (for review see 1). In hemipterans, t~~T~~hese obligatory symbionts, also called primary symbionts (P-symbionts), are intracellular, strictly maternally inherited and, for most of them, have evolved with their hosts for millions of years (review in 2). They are often housed inside specialized cells, bacteriocytes, within a dedicated organ, the bacteriome, localized in the host's abdomen, which constitutes a stable environment for the symbionts and facilitates their transmission to offspring (3, 4). As a consequence of this lifestyle, their genomes are extremely reduced (5, 6). Their minimal genomes retain genes involved in pathways that complement essential nutrients lacking in their host' diets (7). However, in some bacterial symbionts, gene repertoire seems insufficient to meet the metabolic demand of their hosts (review in 8). For example, in aphids, in the subfamily Lachninae, the P-Symbiont *Buchnera aphidicola* has lost its ability to synthesize tryptophan and riboflavin (9).

These deficiencies can be compensated by the acquisition of alternative symbionts that can mediate equivalent functions. Indeed, in addition to their P-symbiont, insects often harbour ~~facultative symbionts, also termed~~ secondary symbionts (S-symbionts). These ~~“non-essential”~~ symbionts are predominantly vertically transmitted but can also be horizontally transmitted, and have a wide range of effects from mutualism to parasitism (review in 10). Several studies have shown that these co-resident S-symbionts can complement the metabolic network of the P-symbionts leading to an inter-dependency between the symbiotic partners. Thus, all the members of the aphid subfamily Lachninae depend on a second co-obligate symbiont to complement specific gene losses of the P-symbiont *Buchnera* (9, 11; review in 12). These co-symbionts are numerous with eleven identified up to now: ten γ-proteobacteria and one α-

75 proteobacteria. This inter-dependency between the symbiotic partners is not restricted to aphids
76 and *Buchnera*, it has been described in other hemiptera like in Cicadas where the P-symbiont
77 *Sulcia* has almost always been detected with one or more co-obligate symbionts (12).
78 Like other phloem-sap feeders, the whitefly *Bemisia tabaci* harbours a P-symbiont, *Candidatus*
79 Portiera aleyrodidarum (13), that synthetizes essential nutrients. However, *Ca.* Portiera
80 aleyrodidarum ~~*Portiera*~~ has a tiny genome, around 355kb (14-17) and, while this γ-
81 proteobacterium has the capacity to synthesize carotenoids and most essential amino-acids (14),
82 it lacks almost all the genes involved in the synthesis of vitamins and cofactors. Moreover,
83 pathways involved in the synthesis of some essential amino acids are incomplete (18). Bacteria
84 belonging to s~~S~~even genera of S-symbionts have been identified in the cryptic species complex
85 of *B. tabaci*: *Hamiltonella* ~~*defensa*~~, *Arsenophonus* ~~sp.~~, *Cardinium* ~~*hertigii*~~, *Rickettsia* ~~sp.~~,
86 *Wolbachia* ~~*pipientis*~~, *Fritschea* ~~*bemisiae*~~ and *Hemipteriphilus* ~~*Hemipteriphilus asiaticus*~~ (19-
87 22). They are all localized in the same bacteriocytes as the P-symbiont but some can also be
88 found in the hemolymph (23). Their roles remain poorly understood but range from
89 reproductive parasitism (24) to fitness benefits such as thermal tolerance (25, 26). Moreover,
90 the analysis of their genomes suggests that some of them could play a nutritional role. For
91 example, *Hamiltonella* can provide vitamins and cofactors, and could also complete the missing
92 steps of the lysine pathway of *Ca.* Portiera aleyrodidarum~~*Portiera*~~ (18). Because of these
93 complementations, presence of S-symbionts is expected in all whitefly individuals. In a
94 sampling performed in West Africa in 2007 and 2009, *B. tabaci* individuals were indeed
95 predominantly found infected with S-symbionts, but in some populations no S-symbiont were
96 recorded (27). In this former study, prevalence of S-symbionts was determined with an *a priori*
97 method, *i. e.* through PCRs using specific primers targeting the six symbionts identified in *B.*
98 *tabaci* at the time, thus leaving the possibility that other, undescribed endosymbionts were
99 present. Since, a seventh S-symbiont, *Candidatus* Hemipteriphilus asiaticus~~Hemipteriphilus~~

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

Mis en forme : Police :Non Italique

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

100 ~~asiaticus~~ (hereafter "*Ca.* ~~Hemipteriphilus~~Hemipteriphilus asiaticus"), has been described in *B.*
101 *tabaci* (19-20). This makes it possible that *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~, as
102 well as other bacterial symbionts, are in fact present in the S-symbiont free *B. tabaci*
103 individuals.

104 In order to get a full picture of the symbiont diversity, we investigated the symbiont content of
105 individuals sampled in Burkina Faso (West Africa) using a metabarcoding~~genomic~~ approach.
106 In this country, *B. tabaci* is a pest of primary importance, with a severe impact on economic
107 activity (28). Indeed, *B. tabaci* is a cryptic species complex (42 species reported till now based
108 on a 657bp portion of the mitochondrial cytochrome oxidase 1 (*mtCOI*) DNA sequence: 29-
109 33), and in Burkina Faso individuals belong either to SSA (Sub-Saharan Africa), ASL (Africa
110 Silver-Leafing) or MED (Meditteranean) species (34). Using universal bacterial primer sets we
111 detected few bacterial species and, more importantly, no new putative S-symbiont. However,
112 the S-symbionts known in *B. tabaci* were found, except *Fritschea*, and notably *Ca.*
113 Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ that is described for the first time in Africa.
114 Interestingly, phylogenetic analyses revealed that this *Ca.* Hemipteriphilus
115 asiaticus~~*Hemipteriphilus*~~ differs from the reference strain identified in Asia (20). In addition,
116 diagnostic PCRs revealed high prevalence of S-symbionts in these populations, and notably *Ca.*
117 Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ in ASL and MED individuals, which questions its
118 possible role in the biology of *B. tabaci*.

Mis en forme : Police :Italique

Mis en forme : Police :Non Italique

Mis en forme : Police :Non Italique

Results

Among the 630 individuals sampled in Burkina Faso in 2015 and 2016, the majority, almost 84%, belonged to the MED species, more especially to the MED-Q1 genetic group (81% ; 3% belonged to MED-Q3). ASL and SSA (more precisely SSA2) genetic groups represented 10.5% and 3% of individuals respectively (34).

Bacterial community characterization

The bacterial community of *B. tabaci* collected in Burkina Faso was characterized on 72 individuals by a metabarcoding~~genomic~~ approach, without *a priori* assumptions, using universal bacterial primers targeting the ~~*16SrDNA*~~ 16S rRNA gene and an Illumina sequencing technology. Between 107,000 to 927,000 reads were obtained per sample (average : 215~~5~~,000 reads ± 9,420). The majority of the reads belonged to the known *B. tabaci* P- and S-symbionts and, in most field individuals, sequences from *Ca.* Portiera aleyrodidarum~~*Portiera*~~ constituted the majority of reads (up to 98.1~~6~~%; 71~~1~~.2~~8~~% on average ± 2.9%; see "table_level6" in Dryad, https://doi.org/10.5061/dryad.547d7wm91, and Figure 1). We detected bacterial taxa belonging to the genera ~~*Alkanindiges*~~*Acinectobacter* in few samples at ~~extremely~~ low abundances since they represented between 0 and 7,4~~6~~% of the sequences obtained per individual, both in field and lab samples. They may represent gut bacteria or contaminations. Overall, no new S-symbiont has been detected.

All the known *B. tabaci* S-symbionts have been found except *Fritschea*. However, it is not surprising since this S-symbiont seems to be scarce worldwide in *B. tabaci* (35). Moreover, in a previous survey done in West Africa using specific primers *Fritschea* was not found (27). As already shown, there is a link between symbiotic bacterial communities and genetic groups: *Hamiltonella* was found to be the most common S-symbiont in MED-Q1, and *Arsenophonus*

Mis en forme : Non souligné
Mis en forme : Police :Non Italique
Mis en forme : Non Surlignage
Mis en forme : Non Surlignage
Mis en forme : Anglais (États-Unis), Non Surlignage
Code de champ modifié
Mis en forme : Anglais (États-Unis)
Mis en forme : Anglais (États-Unis)

144 in MED-Q3 and SSA2 individuals. *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ is the only S-

145 symbiont presents in all genetic groups except SSA2 (*i.e.* in MED-Q1, MED-Q3 and ASL). It

146 was not detected in individuals of the laboratory lines.

147

148 **Phylogenetic analysis of ~~*Hemipteriphilus*~~*Ca.* Hemipteriphilus asiaticus ~~*asiaticus*~~**

149 Twenty individuals positive for *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~, belonging to

150 MED-Q1, MED-Q3 and ASL genetic groups, were used for phylogenetic analysis. They

151 originated from eight localities and, when possible, in each locality, the three genetic groups

152 were represented (Table 1). The 16S rRNA~~*16SrRNA*~~ sequences (483bp) obtained with the new

153 primers designed in the present study (Table 2) were 100% identical in all the individuals (*i. e.*

154 8 MED-Q1, 7 MED-Q3 and 5 ASL). This sequence showed ~~the highest~~100% similarity with

155 the ones of *Ca.* Hemipteriphilus asiaticus ~~*Candidatus* Hemipteriphilus asiaticus~~ endosymbiont

156 and *Rickettsia* of *B. tabaci* available in the genbank database (Blast done in December 2021).

157 16S rRNA~~*16SrRNA*~~ gene is highly conserved (36) we thus ~~we~~ designed new primers on two

158 other loci, *GltA* and *GroEL*, using the sequences of the *Ca.* Hemipteriphilus asiaticus

159 ~~*Hemipteriphilus*~~ isolate YH-ZHJ available in Genbank (20). The 190bp sequences of *GltA* were

160 identical in all our 20 samples, but one substitution was found in the *GroEL* sequences (269bp)

161 between MED individuals (Q1 and Q3) and ASL individuals, whatever their sampling locality.

162 Analyses of the concatenated sequences obtained for the three loci (942bp in total) revealed

163 ~97% identity between the *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ strains identified in

164 Burkina Faso and the YH-ZHJ reference isolate from the China *B. tabaci* species (between 1 to

165 13 different bases according to the gene). Topologies of the trees for *GroEL* and *GltA* were

166 similar to the one of the concatenated tree (*16s rRNA* is not informative since the 483bp

167 sequence of *Ca.* Hemipteriphilus asiaticus is 100% similar to *Rickettsia*).

Mis en forme : Police :Non Italique

Mis en forme : Police :Non Italique

Mis en forme : Interligne : Double

Mis en forme : Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Police :Italique, Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Police :Italique, Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Police :Italique, Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Couleur de police : Automatique

Mis en forme : Couleur de police : Automatique, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

168 We also analysed the phylogenetic relationships of *Ca.* Hemipteriphilus asiaticus
169 ~~*Hemipteriphilus*~~ and other close Rickettsiales, *Rickettsia*~~,~~ ~~*Orientia tsutsumagushi*~~ and *Sitobion*
170 *miscanthi L Type Symbiont* SMLS. The trees constructed with maximum-likelihood and
171 Bayesian inference methods are identical and showed that *Ca.* Hemipteriphilus asiaticus is
172 closer to SMLS than to *Rickettsia* (Figure 2), which is concordant with analyses of Bing *et al.*
173 (20) and Li *et al.* (37). ~~(Figure 1), and showed that *Hemipteriphilus* is closer to SMLS than to~~
174 ~~*Orientia tsutsumagushi* and *Rickettsia*, which is concordant with analyses of Bing *et al.* (20)~~
175 ~~and Li *et al.* (37).~~
176 This phylogenetic analysis thus confirms the presence of *Ca.* Hemipteriphilus
177 asiaticus~~*Hemipteriphilus*~~ in MED-Q1, MED-Q3 and ASL genetic groups in Burkina Faso. It
178 also reveals that they represent different strains than the one found in Asia, and that the strain
179 found in ASL differs slightly from the strains in MED-Q1 and MED-Q3.
180

181 **Distribution and prevalence of bacterial endosymbionts in field populations**

182 The presence of the P- and S-symbionts was checked in 334 individuals from nine localities in
183 Burkina Faso (Figure 3~~2~~) and several host plants (vegetables, ornamental plants and weeds) by
184 specific diagnostic qPCRs. All the S-symbionts described so far in *B. tabaci* were targeted
185 except *Fritschea bemisiae* because this bacterium was not found in the 16S rRNA ~~*16SrRNA*~~
186 ~~metagenomic~~ metabarcoding analysis. The infection status of individuals for the P-symbiont as
187 well as the S-symbionts (*Hamiltonella*, *Arsenophonus*, *Cardinium*, *Rickettsia*, *Wolbachia, Ca.*
188 Hemipteriphilus asiaticus~~*Hemipteriphilus*~~) are presented in Figure 4~~3~~. *Ca.* Portiera
189 aleyrodidarum~~*Portiera*~~ was found in all but five individuals from different sampling sites. PCRs
190 done on the actine host gene as well as the detection of S-symbionts ensured the extraction
191 quality, but we cannot exclude that the quantity of *Ca.* Portiera aleyrodidarum~~*Portiera*~~ was
192 under the real-time PCR detection threshold in these samples. The fact that these five

individuals all belong to MED-Q1 can be explained by the high prevalence of this genetic group (262/334).

More than 98% of individuals harboured at least one S-symbiont and, as expected, the prevalence of the S-symbionts genera depended on the genetic group (Fisher's Exact Test, P = 0.0005 ; see data analysis of 21). *Arsenophonus* is the most frequent symbiont in SSA2 and MED-Q3 individuals (89% and 79% respectively), while 96% of individuals belonging to MED-Q1 harbour *Hamiltonella*. Interestingly, *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ is dominant in ASL samples with 82% of individuals infected, while 50% harbour *Arsenophonus*. More generally *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ is very frequent in all biotypes, except SSA2 in which it has not been found. For the first time, this symbiont is described in MED-Q1, MED-Q3 and ASL genetic groups. In all of them, its prevalence is high: 77% in MED-Q1, 53% in MED-Q3 and 82% in ASL. Globally, the symbiotic composition in these four genetic groups corresponds to what is known in literature (meta-analysis in Zchori-Fein *et al.* (21); see Gnankiné *et al.* (27) for previous data obtained in Burkina Faso), except for *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~. The symbiotic composition is not influenced by the locality for MED-Q1 (Fisher's Exact Test, P = 0.1414), which is the only genetic group found in all the localities (Figure S1~~1~~).

Co-infection by several S-symbionts is frequent (69%), mostly double-infections which represent 90% of multiple infections. However, the presence of three or even four S-symbionts within the same host individual has also been detected (20 and 2 individuals respectively; Figure 4~~3~~). An exception is the biotype SSA2 in which only single infections have been found. Some associations of symbionts are frequent and others never found. We never found *Rickettsia*-*Cardinium*, *Rickettsia*-*Wolbachia* and *Wolbachia*-*Arsenophonus* combinations. On the other hand, in the MED-Q1 individuals *Hamiltonella* and *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ co-occur frequently: this assemblage represents 76% of bi-infections in this

218 genetic group (193/253). In MED-Q3 and ASL, *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~
219 is often associated with *Arsenophonus* (58% and 92% respectively). These results show that
220 *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ often co-infect individuals with another S-
221 symbiont, especially the two more frequent S-symbionts, that are also the ones confined to
222 bacteriocytes.

223

224 **Influence of *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ on the P-symbiont density**

Mis en forme : Police :Gras

225 As it is the first time that *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ has been detected in *B.*
226 *tabaci* individuals from the African continent, we aimed at determining whether its presence
227 has an impact on the P-symbiont. We thus compared the density of *Ca.* Portiera
228 aleyrodidarum~~*Portiera*~~ in presence and in absence of *Ca.* Hemipteriphilus asiaticus
229 ~~*Hemipteriphilus*~~ (Figure 5~~4~~). Results indicated that the presence of *Ca.* Hemipteriphilus
230 asiaticus ~~*Hemipteriphilus*~~ does not affect the density of *Ca.* Portiera aleyrodidarum~~*Portiera*~~
231 (n=282, Wilcox rank test, W=8364, P=0.0847).

232

233 **Discussion**

234 The whitefly *B. tabaci* is one of the most devastating agricultural pests worldwide. In Africa,
235 damage induced by this species complex is~~are~~ huge and results in severe impacts on the
236 economic activity of many countries. Effective control requires understanding its ability to
237 spread and determining factors involved in its important polyphagy. In this context, heritable
238 bacterial symbionts are of primary importance since they may provide their hosts with
239 important ecological traits. In the present study, we aimed at describing the symbiotic bacterial
240 communities, diversity and prevalence in *B. tabaci* populations from Burkina Faso (West
241 Africa). In this survey, several *B. tabaci* genetic groups were found: ASL (Africa Silver-
242 Leafing) and MED (Meditteranean, MED-Q1 and MED-Q3), as previously reported by
243 Gnankiné *et al.* (27), and, in addition, SSA (Sub-Saharan Africa), that was detected for the first
244 time in this country, but only in one locality (Lilboure), and only on one host plant, cassava
245 (34).

246

247 The ~~metagenomic~~ metabarcoding analysis of the bacterial symbionts did not reveal the presence
248 of symbionts not yet described in *B. tabaci*. However, *Ca.* Hemipteriphilus
249 asiaticus~~*Hemipteriphilus*~~ is described for the first time in Africa: it was detected in all the
250 genetic groups found in Burkina Faso except SSA, *i. e.* MED-Q1, MED-Q3 and ASL. *Ca.*
251 Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ has been described for the first time in 2013 by Bing
252 *et al.* (20) in *B. tabaci* samples from China belonging to the China1 biotype. Since then, it has
253 also been found in China2, Asia (I and II), Indian Ocean and SSA (SSA6) genetic groups, but
254 only in countries of the Asian continent: Indian and Pakistan (22, 38, 39). In the present study,
255 *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ was detected in the African continent, with a
256 very high prevalence in the MED and ASL genetic groups from Burkina Faso: 53% in MED-
257 Q3, 77% in MED-Q1, reaching 82% in ASL. On the other hand, it has not been found in SSA2.

258 Since its description, *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ has been relatively under-
259 studied. To our knowledge, only two field surveys have been done so far. They both found the
260 presence of *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ in Asia I and II species but with high
261 differences in prevalence: the infection rate was high in Central India (89%: 51 out of 57
262 individuals checked were infected; 38), but lower in Pakistan (between 5% and 39%; 22). Based
263 on all these results, the presence of *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ should be
264 sought in population studies when *a priori* methods based on specific PCRs are used to describe
265 the bacterial community associated with the *B. tabaci* complex species. This is especially true
266 as we describe here that *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ can also infect the
267 worldly distributed MED-Q1.
268
269 Our phylogenetic analyses on *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ based on three
270 genes, 16S rRNA~~*16SrRNA*~~, *GroEL* and *GltA* revealed that 3% of nucleotide sites differ between
271 the MED strain identified in the present study and the reference YH-ZHJ isolate described in
272 the China *B. tabaci* species (20). It also revealed a substitution in the *GroEL* sequence between
273 the *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ found in MED and in ASL, yet found in
274 sympatry. Therefore, the present data ~~confirm~~ reveal the existence of polymorphism in the *Ca.*
275 Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ genus with at least three strains present in *B. tabaci*.
276 Naturally, in further studies, an extended sampling should be done in more continents,
277 countries, on more species/genetic groups, and more molecular markers should be developed
278 in order to get a more accurate idea of the diversity of *Ca.* Hemipteriphilus
279 asiaticus~~*Hemipteriphilus*~~ strains in this complex species. Anyway, *Ca.* Hemipteriphilus
280 asiaticus ~~*Hemipteriphilus*~~ is not an isolated case: several S-symbionts (*Wolbachia*,
281 *Arsenophonus*, *Cardinium* and *Rickettsia)* are represented by more than one strain in *B. tabaci*
282 (35, 40), with up to 6 phylogenetic groups identified for *Arsenophonus* (41).

To date the influence of *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ on its host is not known. In *B. tabaci*, it has been suggested that some S-symbionts could play a nutritional role, in collaboration with the P-symbiont. Indeed, several data suggest that *Hamiltonella* and *Arsenophonus* could have an impact on the *B. tabaci* metabolism and dietary requirements. These two S-symbionts are almost fixed in some genetic groups (27, 41, present study). For instance, *Hamiltonella* is widespread in MEAM1 and MED-Q1 (review in 21 and 35). Moreover, *Hamiltonella* possesses some genes involved in amino-acid biosynthesis pathways that are lost or non-functional in the P-symbiont (18). In addition, recent experiments demonstrated that this S-symbiont can supply *B. tabaci* with the production of B vitamins (42-43). Even if there is no evidence of fixation in any genetic group in the field, *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ could confer a benefit to its host under some environmental conditions, for example, according to the nutritional quality of the host plants. Indeed, previous research demonstrated that aphid performance is associated with the amino-acid composition of the phloem sap (44-45). It could explain why *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ was not present in the SSA species which, contrary to MED and ASL which have been found in several host plant species, has only been detected on cassava in Burkina Faso (34). Analysis of the genome of *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~*,* with a special focus on genes involved in metabolic pathways, would give further insight into its putative nutritional role.

The presence of another closely relative S-symbiont, *Sitobion miscanthi L Type Symbiont* (SMLS), has also been recently highlighted in the aphid *Sitobion miscanthi* (46). It also belongs to the Rickettsiaceae family and, similarly to *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~, is widely distributed in some populations of its host (see survey in China in 47). It has been

suggested that SMLS could stimulate the proliferation of the P-symbiont *Buchnera* and thus improve the aphids' fitness. Indeed, *Buchnera*'s density is significantly higher in SMLS-infected individuals and laboratory experiments revealed that infected individuals show higher values of some fitness traits (37). However, our results did not reveal any influence of *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus*~~ on the *Ca.* Portiera aleyrodidarum~~*Portiera*~~ density. Clearly, life history traits should be measured on *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~-infected and -free *B. tabaci* individuals. Anyway, the high frequency of these two newly reported S-symbionts in some field populations of these phloemophagous insects could suggest they bring benefit to their hosts. F~~Clearly, f~~urther research on the infection dynamics of these S-symbionts, and their role on their host phenotype and adaptation are needed.

Compared to the previous field survey done in Burkina Faso by Gnankiné *et al.* (27), data on the infection with S-symbionts are highly similar except for the presence of *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~, which was not described at that time. In particular, the infection rate involving at least one S-symbiont were higher than 90% in the two studies. In MED-Q1 genetic group, *Hamiltonella* is the more frequent S-symbiont (96% in the present study, 89% in 27) while *Arsenophonus* is the most common bacteria in MED-Q3 and ASL individuals (previous study/present study, respectively 93%/79% and 40%/50%). Interestingly, *Hamiltonella* and *Arsenophonus* are mutually exclusive which is not the case of *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus*~~ that is often found in co-infection with these S-symbionts: it was involved in almost 80% of co-infection by two S-symbionts.

Conclusion

In summary, these data confirmed the variability of the symbiotic community in the *B. tabaci* complex species~~,~~ ~~showed~~ despite its~~the~~ high temporal stability ~~of the symbiotic community~~ in populations ~~in the *B. tabaci* complex species found in~~from Burkina Faso, ~~–~~and reveal the presence of another player whose role deserves to be studied. The stability and the high indicence of S-symbionts in *B. tabaci* ~~(in the present study, we found that more than 98% of individuals harbour at least one S-symbiont)~~, together with genomic studies, suggest that they can have central roles in shaping the fitness of this pest in different environments. Understanding their possible contribution to successfull invasion, widespread distribution, and more generally, on the population dynamics of this whitefly is critical for the implementation of effective pest management programs.

344 ## Materials and methods

345

346 **Sampling**

347 Sampling was done in nine localities in Burkina Faso, Western Africa (Figure 31), in March
348 and April (dry season) 2015 and 2016 on vegetables, ornamental plants and weeds as described
349 in Romba *et al.* (34). The species and biotypes (hereafter genetic groups) of the individuals
350 (adults) were determined in Romba *et al.* (34) according to the PCR-RFLP method developed
351 in Henri *et al.* (48). Total DNA was extracted from single whiteflies. Three species were found,
352 ASL (Africa Silver-Leafing), SSA (Sub-Saharan Africa) and MED (Meditteerranean). Within
353 the MED species, two genetic groups, MED-Q1 and MED-Q3, were identified.

354

355 **Characterization of the bacterial community**

356 Seventy-two field-collected whiteflies from Burkina Faso were used to characterize the
357 bacterial community through qPCRs using primers specific to each symbiont. They were
358 chosen in order that all genetic groups, all localities and all host plants were represented (Table
359 1). We also included 18 adults coming from laboratory lines belonging to MED-Q1 and MED-
360 Q2 genetic groups reared for years in the « Laboratoire de Biométrie et Biologie Evolutive ».
361 The universal bacterial primer set 341419F-*TCG-TCG-GCA-GCG-TCA-GAT-GTG-TAT-AAG-*
362 *AGA-CAG*-CCT-ACG-GGN-GGC-WGC-AG and 805R-*GTC-TCG-TGG-GCT-CGG-AGA-*
363 *TGT-GTA-TAA-GAG-ACA-G*GA-CTA-CHV-GGG-TAT-CTA-ATC-C was used to amplify
364 486 464 bp of the V3-V4 hypervariable regions of the 16Sr rRDNA gene (49). The primers
365 were synthesized with overhang adapters (in italic) for index attachment and Illumina
366 sequencing adapters. For each sample, which consisted in one individual, triplicates were
367 performed, consisting of three PCR reactions using the KAPA ReadyMix (KAPA) containing
368 using 200nM of each primer, 12.5µL of KAPA Hifi HotStart Ready Mix and 2.5 µL4µL of

Mis en forme : Police :Non Italique

Mis en forme : Police :Non Italique

Mis en forme : Non Surlignage

Mis en forme : Non Surlignage

369 DNA template, in a final volume of 25 µL. ~~and 25 cycles of PCR.~~ The conditions of reactions

370 were ~~-~~ 95°C for 3min followed by 25 cycles of 95°C for 30sec, 55°C for 30sec and 72°C for

371 30sec. Then a final elongation was done at 72°C for 5min. Each amplification product was

372 checked on an agarose gel to verify that there was specific amplification only. For some of

373 them, a bioanalyzer verification was also performed. Then, all the replicates were pooled by

374 sample before purification and to proceed to the further preparation of the library according to

375 the protocol outlined by Illumina (« 16S metagenomic Sequencing Library Preparation »),

376 n°15044223 Rev.B. The pooled library was PE-sequenced using the Illumina MiSeq reagent

377 kit version 3 for 600 cycles (2x300pb) by Biofidal (Vaulx enVelin, France).

378 The sequencing data in FASTQ format were processed and analyzed with the QIIME2 software

379 suite version 2021.11~~18.8~~ (50). The raw Illumina reads were imported into QIIME2,

380 demultiplexed, and then denoised, trimmed and filtered with DADA2 pipeline to remove noisy

381 and chimeric sequences, to construct denoised paired-end sequences and to dereplicate them

382 (51). This produced a table containing representative sequences also called amplicon sequence

383 variant or ASV. The taxonomy assignment ~~of ASVs~~ was then performed by using feature-

384 classifier classify-sklearn. ~~against the database Greengenes 13_8~~. For that, reads from the Silva

385 138 SSURef NR99 reference database were extracted to match on the primer set 341F/805R.

386 The Naive Bayes classifier has been trained before the classification ~~(99% OTUs from~~

387 ~~515F/806R region of sequences)~~ (52-53). A taxa barplot has been done on all the data with

388 "qiime taxa bar plot" (except chloroplast sequences that have been removed; Dryad,

389 https://doi.org/10.5061/dryad.547d7wm91) and a heatmap has been produced only on field

390 samples for major taxa with "qiime feature table heatmap" (Figure 1).

391

392 **Primer design**

Mis en forme : Motif : Transparente

Mis en forme : Non Surlignage

Mis en forme : Non Surlignage

Mis en forme : Anglais (États-Unis)

Mis en forme : Non Surlignage

Mis en forme : Non Surlignage

Mis en forme : Non Surlignage

393 We designed new primers for *Ca.* Hemipteriphilus asiaticus*Hemipteriphilus* using sequences

394 available in Genbank (20) with the Primer3 software (https://bioinfo.ut.ee/primer3-0.4.0/). We

395 aligned sequences of *Ca.* Hemipteriphilus asiaticus *Hemipteriphilus* with sequences of

396 *Rickettsia* from *B. tabaci* in order to design primers specific to *Ca.* Hemipteriphilus

397 asiaticus*Hemipteriphilus* that do not amplify sequences from *Rickettsia*. These primers were

398 tested on individuals harbouring *Rickettsia* but not *HemipteriphilusHemipteriphilus*

399 *asiaticusCa. Hemipteriphilus asiaticus* and we didn't detect any amplification. Multiple

400 sequence alignments were done using the MUSCLE algorithm (54) implemented in CLC DNA

401 Workbench 8.0 (CLC Bio). Three sets of primers targeting three different genes of *Ca.*

402 Hemipteriphilus asiaticus*Hemipteriphilus*, ADNr16S16S rRNA, the citrate synthase *GltA* and

403 the chaperonin *GroEL* were designed (Table 2).

404

405 **Phylogenetic analysis of *HemipteriphilusCa.* Hemipteriphilus asiaticus *asiaticus* strains**

406 PCRs targeting *GltA*, *GroEL* and 16S rRNA*ADNr16S* genes of *Ca.* Hemipteriphilus asiaticus

407 *Hemipteriphilus* were carried out on 20 positive samples (Table 1) with sets of primers designed

408 specifically for this study (Table 2). DNA was amplified in a final volume of 25µL containing

409 200µM dNTPs, 200nM of each primer, 0.5IU DreamTaq DNA polymerase (ThermoFisher) and

410 2µL of DNA. All PCR amplifications were performed under the following conditions: initial

411 denaturation at 95°C for 2min followed by 35 cycles at 94°C for 30sec, 56°C for 30sec, 72°C

412 for 1min and a final extension at 72°C for 10min. PCR products were sequenced using the

413 Sanger method by the platform Biofidal (Vaulx en Velin, France).

414 The nucleotide polymorphism was analyzed by aligning the sequences obtained using the

415 MUSCLE algorithm (54) implemented in CLC DNA Workbench 8.0 (CLC Bio) and inspecting

416 them by eye. Moreover, we studied the phylogenetic relationships of the *Ca.* Hemipteriphilus

417 asiaticus *Hemipteriphilus asiaticus* strains found in our samples with the reference strain

Code de champ modifié

Mis en forme : Police :Non Italique

Mis en forme : Police :Non Italique

418 described in *B. tabaci* (isolate YH-ZHJ; 20) as well as other symbionts belonging to the
419 Rickettsiales family, *Rickettsia*, *Orientia tsutsumagushi* and *Sitobion miscanthi L Type*
420 *Symbiont* (SMLS). Phylogenetic trees were constructed with CLC DNA Workbench 8.0 (CLC
421 Bio) using maximum-likelihood method for each sequence separately and for the concatenated
422 data set (substitution model: GTR + G + T, choosed by using the "Model Testing" tool of CLC
423 DNA Workbench 8.0). The robustness of nodes was assessed with 100 bootstrap replicates. We
424 also constructed a phylogenetic tree with Bayesian inference using the program MrBayes
425 (version 3.2.6) and a GTR+G model (55). For these concatenated gene dataset, 20000
426 generations were run and the first 25% of these were discarded as burn-in.
427

428 **Prevalence of bacterial endosymbionts**

429 Three hundred and thirty four individuals were screened for the presence of the P-symbiont Ca.
430 Portiera aleyrodidarum ~~*Portiera aleyrodidarum*~~ and six secondary symbionts, *Hamiltonella*
431 *defensa*, *Arsenophonus* sp., *Cardinium hertigii*, *Rickettsia* sp., *Wolbachia pipientis* and Ca.
432 Hemipteriphilus asiaticus ~~*Hemipteriphilus asiaticus*~~ (hereafter *Portiera, Hamiltonella,*
433 *Arsenophonus*, *Cardinium*, *Rickettsia*, *Wolbachia* and *Ca.* Hemipteriphilus
434 asiaticus~~*Hemipteriphilus*~~) using specific PCR primers (Table 3). We did not check for the
435 presence of *Fritschea bemisiae* in these individuals because this bacterium was not detected in
436 the 16S rRNA ~~metagenomic~~ metabarcoding analysis. We also amplified one host gene (actin)
437 using the following primers: wf-B actin-For:5'-TCT-TCC-AGC-CAT-CCT-TCT-TG-3' and
438 wf-B actin-Rev: 5-CGG-TGA-TTT-CCT-TCT-GCA-TT-3' to ensure the quality of DNA
439 extractions. DNA was amplified in a final volume of 10µL containing 5µL of Sso Advanced
440 SYBR Green Supermix (Bio-Rad), 2µL of water, 0.5µL of each primer (final concentration of
441 500nM) and 2µL of DNA samples. The reaction conditions for amplification were 95°C for
442 30sec followed by 40 cycles of 95°C for 10sec, 55°C to 63°C (according to the primers' set)

Mis en forme : Police :(Par défaut) Times New Roman, 12 pt, Anglais (États-Unis)
Mis en forme : Anglais (États-Unis)
Mis en forme : Police :(Par défaut) Times New Roman, 12 pt, Anglais (États-Unis)
Mis en forme : Anglais (États-Unis)
Mis en forme : Police :(Par défaut) Times New Roman, 12 pt, Anglais (États-Unis)
Mis en forme : Anglais (États-Unis)
Mis en forme : Police :(Par défaut) Times New Roman, 12 pt, Anglais (États-Unis)
Mis en forme : Police :Non Italique
Mis en forme : Police :Non Italique
Mis en forme : Police :Non Italique

443 for 30sec (see Table 3 ; 63°C for Actine) and 72°C for 30sec. The specificity of the amplified
444 products was controlled by checking melting curves (65°C to 95°C). To assess the efficiency
445 of the reaction, standard curves were plotted using dilutions of previously amplified and
446 purified PCR products. Amplification and detection of DNA were done on the real-time CFX96
447 instrument (Bio-Rad).
448
449 **Statistical analysis**
450 Data analysis were performed using the R statistical software version 3.2.2 -(http:// www.R-
451 project.org).
452 The infection status of *B. tabaci* individuals was graphically represented using the Mondrian
453 shiny application (Siberchicot, Charif, Terraz & Vavre: https://cran.r-
454 project.org/web/packages/Mondrian/).
455
456 **Data availability**
457 Nucleotide sequences obtained in this study are accessible in GenBank database under
458 accession numbers MW353022 and MW353023 for *GroEL* (MED and ASL species
459 respectively), MW343733 for 16S rRNA and MW353021 for *GltA*.
460 All datasets generated and analyzed on the bacterial community characterization are available
461 in Dryad at: https://doi.org/10.5061/dryad.547d7wm91.
462

Code de champ modifié

Mis en forme : Police :Non Italique

Mis en forme : Anglais (États-Unis)

463 **Acknowledgments**
464 This study was funded by Campus France, the Agence Nationale de la Recherche (HMicMac,
465 ANR16-CE02-00014) and the Scientific Breakthrough Project Micro-be-have (Microbial
466 impact on insect behavior) of Université de Lyon, within the programme 'Investissements
467 d'Avenir' (ANR-11-IDEX-0007; ANR-16-IDEX-0005). The authors thank Agnès Nguyen

468 from the Biofidal company for her precious advice and collaboration on the ~~metagenomic~~

469 metabarcoding part.

470

471 Conflict of interest disclosure

472 The authors of this article declare that they have no financial conflict of interest with the content

473 of this article. Fabrice Vavre and Laurence Mouton are ones of the PCI Zool recommenders.

474

475

Mis en forme : Justifié

Mis en forme : Police :12 pt, Non Gras

476 # References

477

478 1. Douglas AE. 2009. 'The microbial dimension in insect nutritional ecology'. Funct Ecol 23 : 38–47.

479

480 2. Moran NA, McCutcheon JP, Nakabachi A. 2008. Genomics and evolution of heritable bacterial
481 symbionts. Annu Rev Genet 42: 165-90.

482

483 3. Buchner P. 1965. 'Endosymbioses of animals with plant microorganisms' *New York : Interspecience*
484 *Publisher*.

485

486 4. Baumann, P. 2005. 'Biology of Endosymbionts of Plant Sap-Sucking Insects' Ann Rev Microbiol
487 155–89.

488

489 5. McCutcheon JP, Moran NA. 2011. Extreme genome reduction in symbiotic bacteria. Nat
490 Rev Microbiol. 10: 13-26.

491

492 6. Moran NA, Bennett GM. 2014. The tiniest tiny genomes. Annu Rev Microbiol 68: 195–215.

493

494 7. Gil R, Silva FJ, Pereto J, Moya A. 2004. Determination of the core of a minimal bacterial gene set.
495 Microbiol Mol Biol R 68:518-537.

496

497 8. Latorre A, Manzano-Marín A. 2017. Dissecting genome reduction and trait loss in insect
498 endosymbionts. Ann NY Acad Sci 1389: 52-75.

499

500 9. Manzano-Marín A, Simon JC, Latorre A. 2016. Reinventing the wheel and making it round again:
501 evolutionary convergence in *Buchnera-Serratia* symbiotic consortia between the distantly related
502 Lachninae Aphids *Tuberolachnus salignus* and *Cinara cedri*. Genome Biol Evol 8:1440-1458.

Mis en forme : Espace Après : 8 pt, Interligne : Multiple 1,08 li

503

504 10. Ferrari J, Vavre F. 2011. Bacterial symbionts in insects or the story of communities affecting
505 communities. Philos Trans R Soc Lond B Biol Sci 366 : 1389-1400.

506

507 11. Meseguer AS, Manzano-Marín A, Coeur d'Acier A, Clamens AL, Godefroid M, Jousselin E. 2017.
508 *Buchnera* has changed flatmate but the repeated replacement of co-obligate symbionts is not associated
509 with the ecological expansions of their aphid hosts. Mol Ecol 26: 2363-2378.

510

511 12. Gil R, Latorre A. 2019. Unity makes strength: a review on mutualistic symbiosis in representative
512 insect clades. Life 9: 21.

513

514 13. Thao MLL, Baumann P. 2004. Evolutionary relationships of primary prokaryotic endosymbionts of
515 whiteflies and their hosts. App Env Microbiol 70: 3401–3406.

516

517 14. Santos-Garcia D, Farnier PA, Beitia F, Zchori-Fein E, Vavre F, Mouton L, Moya A, Latorre A, Silva
518 FJ. 2012. Complete genome sequence of "*Candidatus Portiera aleyrodidarum*" BT-QVLC, an obligate
519 symbiont that supplies amino acids and carotenoids to *Bemisia tabaci*. J Bacteriol 194:6654-6655.

520

521 15. Sloan DB, Moran NA. 2012. Genome reduction and co-evolution between the primary and
522 secondary bacterial symbionts of psyllids. Mol Biol Evol 29: 3781–3792.

523

524 16. Sloan DB, Moran NA. 2013. The evolution of genomic instability in the obligate endosymbionts of
525 whiteflies. Genome Biol Evol 5 : 783-793.

526

527 17. Jiang ZF, Xia F, Johnson KW, Brown CD, Bartom E, Tuteja JH, Stevens R, Grossman RL, Brumin
528 M, White KP, Ghanim M. 2013. Comparison of the genome sequences of "*Candidatus* Portiera
529 aleyrodidarum" primary endosymbionts of the whitefly *Bemisia tabaci* B and Q Biotypes. Appl Env
530 Microbiol 79: 1757-1759.
531
532 18. Rao Q, Rollat-Farnier PA, Zhu DT, Santos-Garcia D, Silva FJ, Moya A, Latorre A, Klein C, Vavre
533 F, Sagot MF, Liu SS, Wang XW, Mouton L. 2015. Genome reduction and metabolic complementation
534 of the dual endosymbionts in the whitefly *Bemisia tabaci*. BMC Genomics 16: 226.
535
536 19. Bing XL, Ruan YM, Rao Q, Wang XW, Liu SS. 2012. Diversity of secondary endosymbionts among
537 different putative species of the whitefly *Bemisia tabaci*. Insect Sci 20 : 194-206.
538
539 20. Bing XL, Yang J, Zchori-Fein E, Wang XW, Liu SS. 2013. Characterization of a newly discovered
540 symbiont of the whitefly *Bemisia tabaci* (Hemiptera : Aleyrodidae). Appl Env Microbiol 79 : 569-575.
541
542 21. Zchori-Fein E, Lahav T, Freilich S. 2014. Variations in the identity and complexity of
543 endosymbiont combinations in whitefly hosts. Front Microbiol 5: 310.
544
545 22. Wang HL, Lei T, Xia WQ, Cameron SL, Liu YQ, Zhang Z, Gowda MMN, Navas-Castillo J,
546 Omongo CA, Delatte H, Lee KY, Patel MV, Krause-Sakate R, NG J, Wu SL, Fiallo-Olivé E, Liu SS,
547 Colvin J, Wang XW. 2019. Insight into the microbial world of *Bemisia tabaci* cryptic species complex
548 and its relationships with its host. Sci Rep 9 : 6568.
549
550 23. Gottlieb Y, Ghanim M, Gueguen G, Kontsedalov S, Vavre F, Fleury F, Zchori-Fein E. 2008.
551 Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. FASEB J. 22 :
552 2591-2599.

24. Lv N, Peng J, Chen XY, Guo CF, Sang W, Wang XM, Ahmed MZ, Xu YY, Qiu BL. 2020. Antagonistic interaction between male-killing and cytoplasmic incompatibility induced by *Cardinium* and *Wolbachia* in the whitefly, *Bemisia tabaci*. Insect Sci 28 : 330-346.

25. Brumin M, Kontsedalov S, Ghanim M. 2011. *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. Insect Sci 18 : 57-66.

26. Yang K, Yuan MY, Liu Y, Guo CL, Liu TX, Zhang YJ, Chu D. 2021. First evidence for thermal tolerance benefits of the bacterial symbiont *Cardinium* in an invasive whitefly, *Bemisia tabaci*.Pest Manag Sci 77: 5021-5031 ~~In press~~.

27. Gnankiné O, Mouton L, Henri H, Terraz G, Houndeté T, Martin T, Vavre F, Fleury F. 2013. Distribution of *Bemisia tabaci* biotypes (Homoptera: Aleyrodidae) and their associated symbiotic bacteria on host plants in western Africa. Ins Cons Div 6: 411-421.

28. Gnankiné O, Traore D, Sanon A, Traore NS, Ouédraogo AP. 2007. Traitements insecticides et dynamique des populations de *Bemisia tabaci* en culture cotonnière au Burkina Faso. Cahiers Agriculture 16: 101–109.

29. Dinsdale A, Cook L, Riginos C, Buckley YM, De Barro P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhynca: Aleyrodoidae: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genomic boundaries. Ann Entomol Soc Am 103: 196-208.

30. De Barro PJ, Liu SS, Boykin LM, Dinsdale AB. 2011. *Bemisia tabaci*: a statement of species status. Ann Rev Entomol 56: 1-19.

31. Firdaus S, Vosman B, Hidayati N, Supena EDJ, Visser RGF, van Heusden AW. 2013. The *Bemisia tabaci* species complex : additions from different parts of the world. Insect Sci 20: 723-733.

Mis en forme : Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

32~~9~~. Roopa HK, Asokan R, Rebijith KB, Hande RH, Mahmood R, Kumar NK. 2015. Prevalence of a new genetic group, MEAM-K, of the whitefly *Bemisia tabaci* (Hemiptera : Aleyrodidae) in Karnataka, India, as evident from mtCOI sequences. Florida Entomol 98 : 1062-1071.

33. Hu J, Zhang X, Jiang Z, Zhang F, Liu Y, Liu Z, Zhang Z. 2018. New putative cryptic species detection and genetic network analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China based on mitochondrial COI sequences. Mitochondrial DNA Part A. 29: 474-484.

34. Romba R, Gnankine O, Drabo SF, Tiendrebeogo F, Henri H, Mouton L, Vavre F. 2018. Abundance of *Bemisia tabaci* Gennadius (Hemiptera : Aleyrodidae) and its parasitoids on vegetables and cassava plants in Burkina Faso (West Africa). Ecol Evol 8 : 6091-6103.

35. Kanakala S, Ghanim M. 2019. Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. PLoS One 14: e0213946.

36. Petti CA. 2007. Detection and identification of microorganisms by gene amplification and sequencing. Clin inf dis 44 : 1108-14.

37. Li T, Wu XJ, Jiang YL, Zhang L, Duan Y, Miao J, Gong ZJ, Wu YQ. 2016. The genetic diversity of SMLS (*Sitobion miscanthi* L type symbiont) and its effect on the fitness, mitochondrial DNA diversity and *Buchnera aphidicola* dynamic of wheat aphid, *Sitobion miscanthi* (Hemiptera : Aphididae). Mol Ecol 25 : 3142-3151.

38. Ansari PG, Singh RK, Kaushik S, Krishna A, Wada T, Noda H. 2017. Detection of symbionts and virus in the whitefly *Bemisia tabaci* (Hemiptera : Aleyrodidae), vector of the Mungbean yellow mosaic India virus in Central India. Appl Entomol Zool 52 : 567-579.

609 39. Paredes-Montero JR, Zia-Ur-Rehman M, Hameed U, Haider MS, Herrmann HW, Brown JK. 2020.
610 Genetic variability, community structure, and horizontal transfer of endosymbionts among three Asia
611 II-*Bemisia tabaci* mitotypes in Pakistan. Ecol Evol 10 : 2928-2943.
612
613 40. Gueguen G, Vavre F, Gnankine O, Peterschmitt M, Charif D, Chiel E, Gottlieb Y, Ghanim M,
614 Zchori-Fein E, Fleury F. 2010. Endosymbiont metacommunities, mtDNA diversity and the evolution of
615 the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. Mol Ecol 19 : 4365-4376.
616
617 41. Mouton L, Thierry M, Henri H, Baudin R, Gnankiné O, Reynaud B, Zchori-Fein E, Becker N, Fleury
618 F, Delatte H. 2012. MLST diversity and recombination evidence in *Arsenophonus* symbionts of the
619 *Bemisia tabaci* species complex. BMC Microbiol 12(Suppl. 1): S10.
620
621 42. Ren FR, Sun X, Wang TY, Yao YL, Huang YZ, Zhang X, Luan JB. 2020. Biotin provisioning by
622 horizontally transferred genes from bacteria confers animal fitness benefits. ISME J. 14: 2542-2553.
623
624 43. Wang YB, Ren FR, Yao YL, Sun X, Walling LL, Li NN, Bai B, Bao XY, Xu XR, Luan JB. 2020.
625 Intracellular symbionts drive sex ratio in the whitefly by facilitating fertilization and provisioning of B
626 vitamins. ISME 14 : 2923–2935.
627
628 44. Sandström J, Pettersson J. 1994. Amino acid composition of phloem sap and the relation to
629 intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. J Insect Phys 40: 947–955.
630
631 45. Stout MJ, Thaler JS, Thomma BPHJ. 2006. Plant-mediated interactions between pathogenic
632 microorganisms and herbivorous arthropods. Ann Rev Entomol 51 : 663-689.
633
634 46. Li T, Xiao JH, Xu ZH, Murphy RW, Huang DW. 2011. A possibly new *Rickettsia*-like genus
635 symbiont is found in Chinese wheat pest aphid, *Sitobion miscanthi* (Hemiptera : Aphididae). J Inv Pathol
636 106 : 418-421.

47. Li T, Xiao JH, WU YQ, Huang DW. 2014. Diversity of bacterial symbionts in populations of *Sitobion miscanthi* (Hemiptera : Aphididae) in China. Env Entomol 43 : 605-611.

48. Henri H, Terraz G, Gnankiné O, Fleury F, Mouton L. 2013. Molecular characterization of genetic diversity within the Africa/Middle East/Asia Minor and Sub-Saharan Africa groups of the *Bemisia tabaci* species complex. Int J Pest Management 59: 329-338.

49. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41: e1.

50. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857.

51. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Meth 13:581–583.

52. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JG. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6 : 90.

53. Robeson MS II, O'Rourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, Bokulich NA. RESCRIPt: Reproducible sequence taxonomy reference database management for the masses. bioRxiv 2020.10.05.326504; doi: https://doi.org/10.1101/2020.10.05.326504

Mis en forme : Police :11 pt

664

665 54. Edgar RC. 2004. MUSCLE : multiple sequence alignment with high accuracy and high throughput.

666 Nucl Acids Res 32 : 1792-1797.

667

668 55. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard

669 MA, Huelsenbeck JP. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model

670 selection across a large model space. Syst. Biol. 61:539-542.

671

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

Mis en forme : Anglais (États-Unis)

Mis en forme : Police :(Par défaut) Times New Roman, Anglais (États-Unis)

672

Tables and figures

Mis en forme : Police :(Par défaut) Times New Roman, 14 pt

673

674

675

676

Table 1: Samples used for the phylogenetic analyses of *Ca.* Hemipteriphilus asiaticus~~*Hemipteriphilus asiaticus*~~

677

Locality	Host plant	Sample name	Biotype
Tanghin/Ouagadougou	Eggplant	TangO22	MED-Q1
	Lantana camara	TangOE56	MED-Q3
	Potato	TangO14	ASL
	Tomato	TangOE35	ASL
Tiebele/Pô	Chilli pepper	TiebPE11	MED-Q1
	Potato	TiebP13	ASL
Boulmiougou/Ouagadougou	Bell pepper	BoulOE33	MED-Q1
	Cucumber	BoulOE44	MED-Q3
	Cucumber	BoulOE46	MED-Q3
	Zucchini	BoulOE51	MED-Q3
	Tomato	BoulOE15	ASL
Boulbi/Komsilga	Eggplant	BoulkE11	MED-Q1
	Eggplant	BoulKE33	MED-Q3
	Zucchini	BoulkE39	MED-Q3
Koubri/Kombissiri	Bell pepper	KoubKE11	MED-Q1
	Zucchini	KoubK45	MED-Q3
Werra/Koudougou	Eggplant	WerrkE24	MED-Q1
	Tomato	WerrE11	ASL
Loumbila/Oubritenga	Chilli pepper	LoumOE14	MED-Q1
Bonyolo/Réo	Eggplant	BonyRE13	MED-Q1

678

679 **Table 2:** PCR primers and conditions used for phylogenetic analysis of *Ca.* Hemipteriphilus
680 asiaticus~~*Hemipteriphilus asiaticus*~~
681

Targeted gene	Primers	Primer sequences	Annealing temperature/ Product size
GltA	**HA_GltA_62F** **HA_GltA_259R**	5'- AGCAGCAGGTATTGCCTCAT -3' 5'- TGCCCTGGGATCATAATTCTT -3'	56°C / 198bp
16S rRNA~~***AD Nr16S***~~	**HA_16S_36F** **HA_16S_555R**	5'- ATTAGTGGCAAACGGGTGAG -3' 5'- CTCTAGCCTAGCAGTTTTAG -3'	56°C / 519bp
GroEL	**HA_GroEL_318F** **HA_GroEL_1008R**	5'- GCCAATGGCGATAGTGAGAT -3' 5'- GCACTGCTACACCAGTTTGC -3'	56°C / 691bp

682
683 All these primers have been designed in this study.
684

Mis en forme : Police :Non Italique

Table 3: PCR primers and conditions used for symbionts' screening (qPCR)

Symbiont	Targeted gene	Primers	Primer sequences	Annealing temperature/ Product size	Reference
Ca. Portiera aleyrodidarum~~***Portiera***~~	**16S rRNA**~~***AD Nr16S***~~	**Port73-F** **Port266-R**	5' – GTGGGGAATAACGTACGG – 3' 5' – CTCAGTCCCAGTGTGGCTG – 3'	60°C / 193bp	Caspi-Fluger *et al.*, 2011
Rickettsia	***GltA***	**GltA 375F_Rick** **GltA 574R_Rick**	5'- TGGTATTGCATCGCTTTGGG-3' 5'- TTTCTTTAAGCACTGCAGCACG-3'	60°C / 199bp	Caspi-Fluger *et al.*, 2011
Hamiltonella	***dnaK***	**dnaK-F** **dnaK-R**	5'- GGTTCAGAAAAAAGTGGCAG -3' 5'- CGAGCGAAAGAGGAGTGAC -3'	60°C / 155bp	Moran *et al.*, 2005
Cardinium	**16S rRNA**~~***AD Nr16S***~~	**CFB-F** **CFB-R**	5'-GCGGTGTAAAATGAGCGTG-3' 5'-ACCTMTTCTTAACTCAAGCCT-3'	59°C / 395bp	Weeks *et al.,* 2003
Wolbachia	***FtsZ***	**F2** **R2**	5'- TTGCAGAGCTTGGACTTGAA -3' 5'-CATATCTCCGCCACCAGTAA-3'	55°C / 400bp	Vavre *et al*, 1999
Arsenophonus	**23S rRNA**~~***AD Nr23S***~~	**ArsF3** **ArsR3**	5'- GTCGTGAGGAARGTGTTARGGTT -3' 5'- CCTYTATCTCTAAAGGMTTCGCTGGATG -3'	63°C / 765bp	Duron *et al.*, 2008
Ca. Hemipteriphilus asiaticus~~***Hemipteriphilus asiaticus***~~	***GltA***	**HA_GltA_62F** **HA_GltA_259R**	5'- AGCAGCAGGTATTGCCTCAT -3' 5'- TGCCCTGGGATCATAATTCTT -3'	56°C / 198bp	This study

Mis en forme : Police :10 pt

Mis en forme : Police :Non Italique

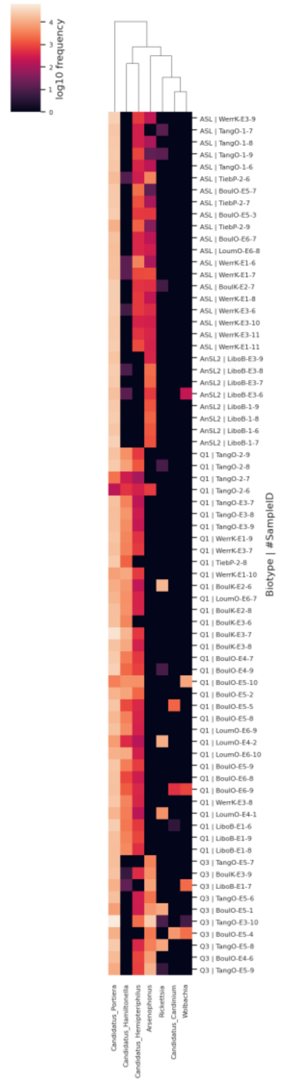
Mis en forme : Police :10 pt

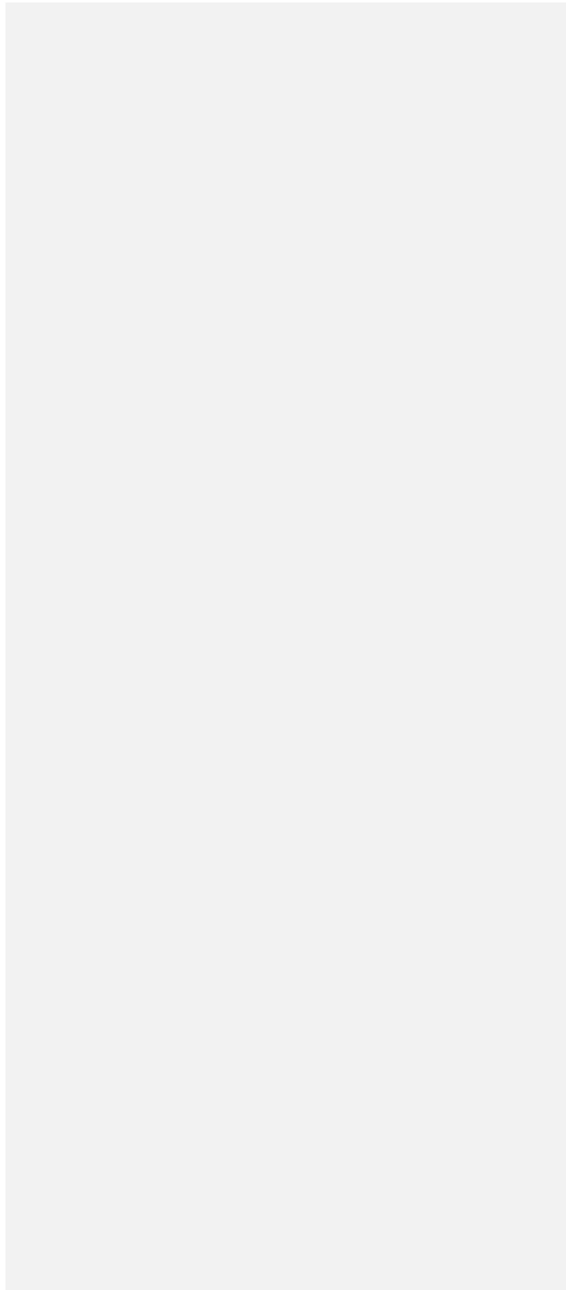
Mis en forme : Police :10 pt

691 **Figure 1:** Heat-map showing the major taxa identified in the metabarcoding analysis

692 Heatmap showing differences in bacterial communities based on taxonomic classifications of DNA 16S amplicons generated in QIIME 2 using the
693 SSU SILVA 138 taxonomy. The heatmap was generated from the log-transformed relative abundance values of the 7 major taxa at the genus level
694 (level-6). The relative abundance of each genus is indicated by a gradient of color from darkest (low abundance) to lightest color (high abundance).

695


log10 frequency
0
1
2
3
4
ASL | WerrK-E3-9
ASL | TangO-1-7
ASL | TangO-1-8
ASL | TangO-1-9
ASL | TangO-1-6
ASL | TiebP-2-6
ASL | BoulO-E5-7
ASL | TiebP-2-7
ASL | BoulO-E5-3
ASL | TiebP-2-9
ASL | BoulO-E6-7
ASL | LoumO-E6-8
ASL | WerrK-E1-6
ASL | WerrK-E1-7
ASL | BoulK-E2-7
ASL | WerrK-E1-8
ASL | WerrK-E3-6
ASL | WerrK-E3-10
ASL | WerrK-E3-11
ASL | WerrK-E1-11
AnSL2 | LiboB-E3-9
AnSL2 | LiboB-E3-8
AnSL2 | LiboB-E3-7
AnSL2 | LiboB-E3-6
AnSL2 | LiboB-1-9
AnSL2 | LiboB-1-8
AnSL2 | LiboB-1-6
AnSL2 | LiboB-1-7
Q1 | TangO-2-9
Q1 | TangO-2-8
Q1 | TangO-2-7
Q1 | TangO-2-6
Q1 | TangO-E3-7
Q1 | TangO-E3-8
Q1 | TangO-E3-9
Q1 | WerrK-E1-9
Q1 | WerrK-E3-7
Q1 | TiebP-2-8
Q1 | WerrK-E1-10
Q1 | BoulK-E2-6
Q1 | LoumO-E6-7
Q1 | BoulK-E2-8
Q1 | BoulK-E3-6
Q1 | BoulK-E3-7
Q1 | BoulK-E3-8
Q1 | BoulO-E4-7
Q1 | BoulO-E4-9
Q1 | BoulO-E5-10
Q1 | BoulO-E5-2
Q1 | BoulO-E5-5
Q1 | BoulO-E5-8
Q1 | LoumO-E6-9
Q1 | LoumO-E4-2
Q1 | LoumO-E6-10
Q1 | BoulO-E5-9
Q1 | BoulO-E6-8
Q1 | BoulO-E6-9
Q1 | WerrK-E3-8
Q1 | LoumO-E4-1
Q1 | LiboB-E1-6
Q1 | LiboB-E1-9
Q1 | LiboB-E1-8
Q3 | TangO-E5-7
Q3 | BoulK-E3-9
Q3 | LiboB-E1-7
Q3 | TangO-E5-6
Q3 | BoulO-E5-1
Q3 | TangO-E3-10
Q3 | BoulO-E5-4
Q3 | TangO-E5-8
Q3 | BoulO-E4-6
Q3 | TangO-E5-9
Biotype | #SampleID
Candidatus_Portiera
Candidatus_Hamiltonella
Candidatus_Hemipteriphilus
Arsenophonus
Rickettsia
Candidatus_Cardinium
Wolbachia



697 **Figure ~~1~~2**

698 Phylogeny constructed using Maximum Likelihood analysis (model GTR + G + T) based on the trimmed and concatenated sequences of three

699 genes, 16S rRNA~~*16SrDNA*~~ (483bp), *GroEL* (269bp) and *GltA* (190bp) (942bp in total). Boostrap values are shown at the nodes (100 replicates).

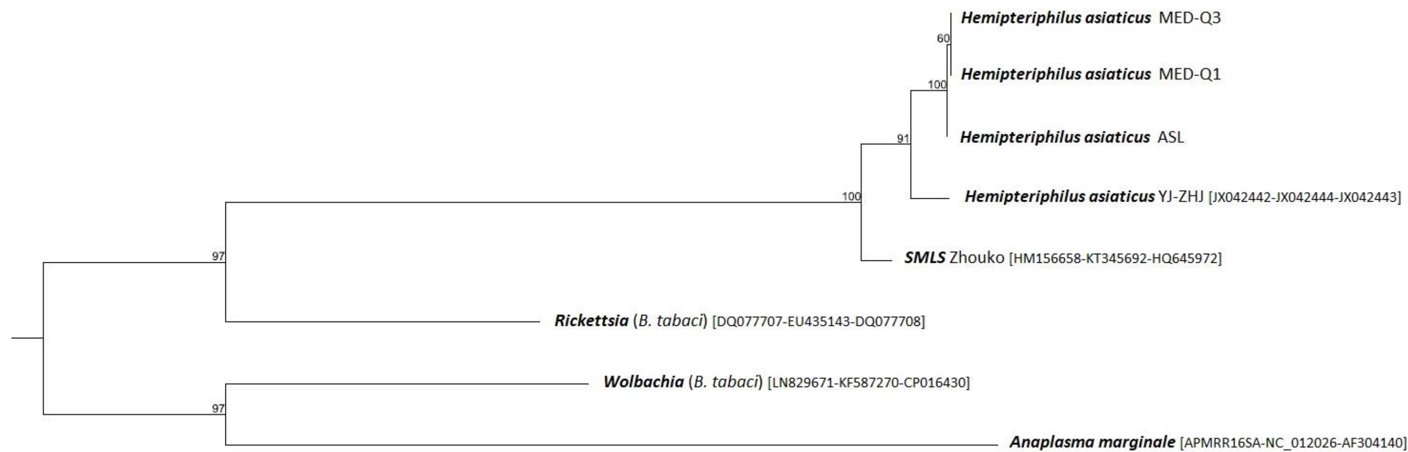
700 Sequences obtained from *Hemipteripilus asiaticus* found in the present study in *B. tabaci* from Burkina Faso (indicated by the biotype of their host,

701 MED-Q1, MED-Q3, ASL) were compared to the YH-ZHJ strain found in China1 biotype individuals of *B. tabaci* from China and to other closely

702 related symbionts belonging to the Rickettsiales family,~~:~~ *Rickettsia* and~~,~~ *Sitobion miscanthi L Type Symbiont* (SMLS)~~ and *Orientia tsutsumagushi*~~.

703 The genbank accession numbers of the sequences obtained from other studies are indicated in the brackets.

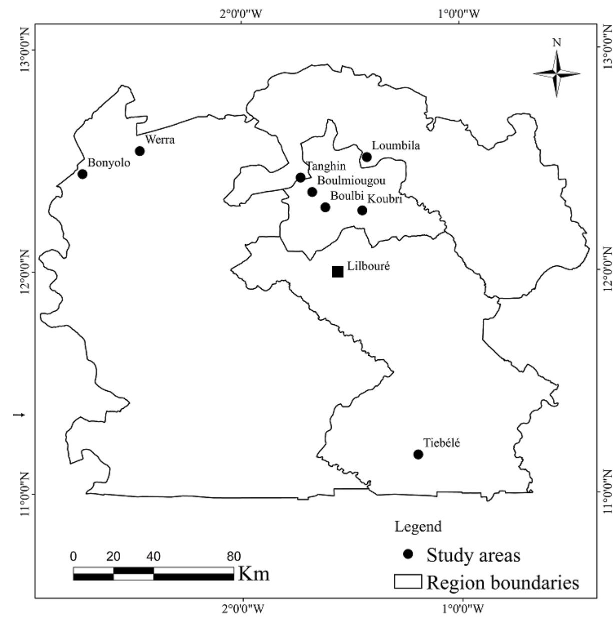
Mis en forme : Police :12 pt, Non Gras



704

705 **Figure ~~2~~3:** Sampling localities in Burkina Faso

706



707

708 Both prevalence of symbionts and phylogenetic analysis of *Ca.* Hemipteriphilus asiaticus ~~*Hemipteriphilus asiaticus*~~ were done on individuals from

709 the nine localities indicated except Lilbouré (indicated by a square) for which no sample was used for the phylogenies.

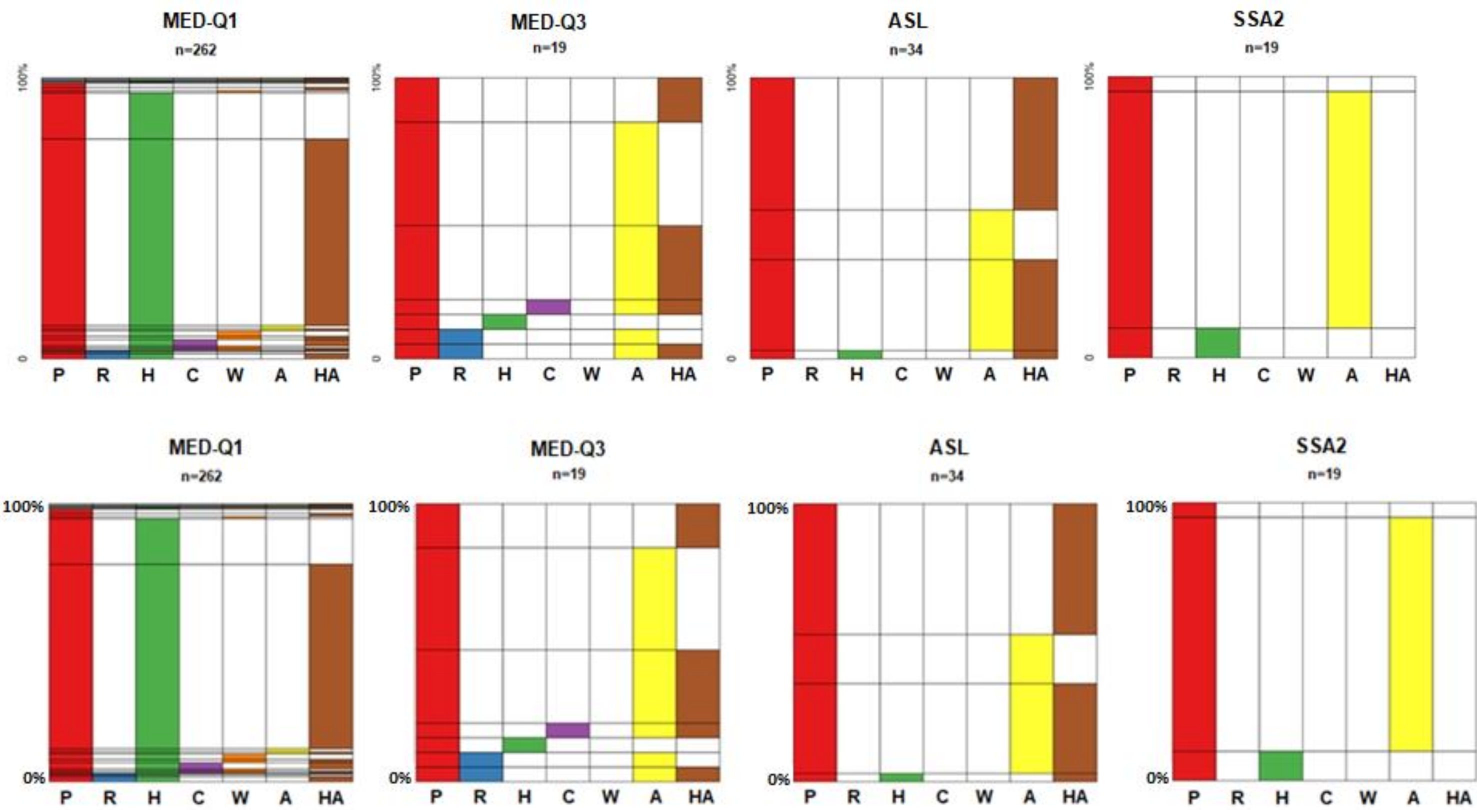
710

711 **Figure 43**

712

713 Infection status of *Bemisia tabaci* individuals collected in Burkina Faso according to their biotype, determined through specific qPCRs. Each graph
714 corresponds to one biotype, with the different bacterial symbionts shown on the x-axis. Each bacterium is represented by a colour (red: *Ca.* Portiera
715 aleyrodidarumPortiera (P), blue: *Rickettsia* (R), green: *Hamiltonella* (H), purple: *Cardinium* (C), orange: *Wolbachia* (W), yellow: *Arsenophonus*
716 (A), brown: *Ca.* Hemipteriphilus asiaticus*Hemipteriphilus* (HA)) and the corresponding coloured bar indicates its prevalence. On the y-axis host
717 individuals are ranked and grouped together according to their infection status: when the graph is read horizontally, the colour combinations
718 represent individuals sharing the same symbiotic community. n indicates the number of individuals checked.

719



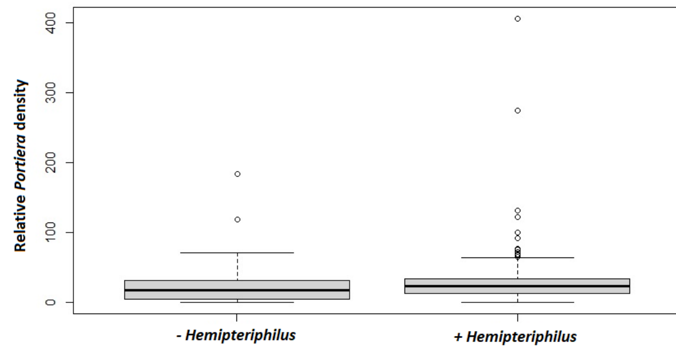
~~**Figure 4:** *Portiera* densities according to the presence of *Hemipteriphilus*~~

725

726 Relative *Portiera* densities (ratio of the number of copies of *Portiera* (ADNr16S) and host (actine) genes) in absence (-) or presence (+) of

727 *Hemipteriphilus* within the same host individuals.

728



729 **Figure 5:** *Ca.* Portiera aleyrodidarum densities according to the presence of *Ca.*

730 Hemipteriphilus asiaticus

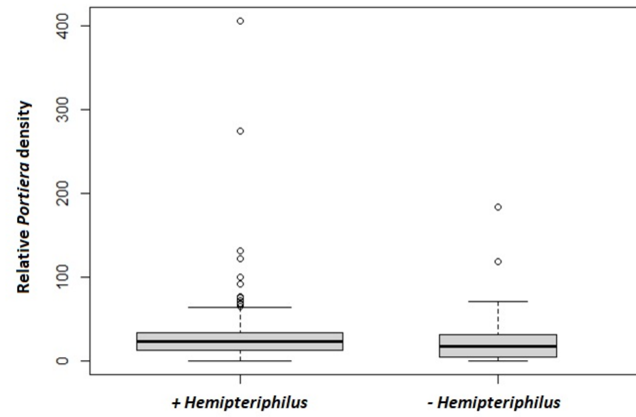
731

732 Relative *Ca.* Portiera aleyrodidarum densities (ratio of the number of copies of *Ca.* Portiera aleyrodidarum (16S rRNA) and host (actine) genes)

733 in absence (- ; n=69) or presence (+ ; n=213) of *Ca.* Hemipteriphilus asiaticus within the same host individuals. The width of the box plots reflect

734 the number of samples for each modality.

735



736
737

Mis en forme : Interligne : 1,5 ligne, Espacement automatique entre les caractères asiatiques et les chiffres