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Schistosomiasis vector snails and their microbiota display a phylosymbiosis pattern

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Abstract

Planorbidae snails are the intermediate host for the trematode parasite of the *Schistosoma* genus which is responsible for schistosomiasis, a disease that affects both humans and cattle. The microbiota for *Schistosoma* has already been described as having an affect on host/parasite interactions, specifically through immunological interactions. Here we sought to characterize the microbiota composition of seven Planorbidae species and strains. Individual snail microbiota was determined using 16S rDNA amplicon sequencing. The bacterial composition was highly specific to host strain with limited inter-individual variation. In addition, it displayed complete congruence with host phylogeny, revealing a phylosymbiosis pattern. These results were confirmed in common garden, suggesting that the host highly constrains microbial composition. This study presents the first comparison of bacterial communities between several intermediate snail hosts of *Schistosoma* parasites, paving the way for further studies on the understanding of this tripartite interaction.

1 Introduction

A microbiota consists of microbial communities in association with a host. Here, we defined the microbiota as all microorganisms involved in a long lasting interaction with a host, excluding parasites and pathogen microorganisms (Bordenstein & Theis, 2015). The microbiota is involved in numerous functions, including nutrition (McCutcheon *et al.*, 2009), development (Fraune & Bosch, 2010; McFall-Ngai, 2002), reproduction (Perlman *et al.*, 2008; Werren *et al.*, 2008) and immunity (Hahn & Dheilly, 2016; Lee & Mazmanian, 2010). For example, the bacterial microbiota of the mosquito gut is involved in the immune response of its host against DENV pathogen virus, through direct inhibition of the virus towards bacterial metabolites as well as through indirect effects by stimulating its basal immunity (Saraiva *et al.*, 2016). This illustrates the importance of considering microbiota in host-pathogen interactions.

1 Numerous studies have already explored the factors shaping microbiota composition in several
2 models and highlighted the role of neutral processes (Burns *et al.*, 2015), environment (Roder *et al.*,
3 2015), host genetic background (Brucker & Bordenstein, 2011) or host physiology/immunity (Chu &
4 Mazmanian, 2013; Hahn & Dheilly, 2016). Results from these studies demonstrate the effect of the
5 host immune system in microbiota homeostasis. In *Hydra*, the nature and combination of
6 antimicrobial peptides belonging to the arminin family are involved in the species-specificity of host
7 microbial communities that follow host phylogeny (Franzenburg *et al.*, 2013).

8 Here, we characterized the microbiota of several genera of Planorbidae, a family of freshwater snails.
9 These snails are the intermediate hosts for the parasite *Schistosoma spp.*, a genus of trematode
10 parasites which develop asexually in the snails before infecting vertebrates where sexual
11 reproduction takes place. Human *Schistosoma* species, mainly *S. mansoni*, *S. haematobium* and *S.*
12 *japonicum*, infect about 250 million people (Hotez *et al.*, 2014) annually, and each year, more than
13 200 000 people die as a result of the infection worldwide (WHO, 2019). While *Biomphalaria*
14 *glabrata* and *Biomphalaria pfeifferi* snails can be infected with *Schistosoma mansoni* (responsible for
15 human intestinal schistosomiasis), *Planorbarius metidjensis* is responsible for the transmission of *S.*
16 *bovis*, and *Bulinus truncatus* snails are natural hosts for *S. bovis* as well as *S. haematobium* (agent of
17 the human urinary schistosomiasis). Interestingly, it has been shown that within the *B. glabrata*
18 species, some strains can be completely refractory to infection depending on the parasite strain, a
19 phenomenon called compatibility polymorphism (Galinier *et al.*, 2017; Theron *et al.*, 2014). These
20 snail-parasite interactions, resulting from coevolution dynamics, reflect differences in host immune
21 capacities or differences in immunobiological interactions between different host-parasite
22 combinations.

23 The snail immune response in this interaction is complex with a specificity according to the parasite
24 strain (Portela *et al.*, 2013). Indeed, snails immune effectors and receptors seem to be specific to the
25 parasite and the type (cellular or humoral) and efficiency of immune response is linked to the
26 infection type (primo-infection or challenge (homologous or heterologous) (Pinaud *et al.*, 2016). A
27 shift in microbiota composition following an infection was observed after an immune challenge,
28 where humoral immunity took place (Portet *et al.*, 2018). This highlights the importance of further
29 in-depth studies of the relationship between the host's immune and vectorial capacities and its
30 microbiota composition. To do this, it is essential to first characterize the factors that shape microbial
31 communities and their host specificity.

32 To identify the effect of host identity in bacterial microbiota composition, we used 16S rDNA
33 amplicon sequencing to analyse the bacterial communities at the individual level (10 to 15 samples
34 per condition/strain) for six snail strains: four different strains of *Biomphalaria glabrata*, one strain
35 of *Biomphalaria pfeifferi*, one strain of *Planorbarius metidjensis*, and one strain of *Bulinus truncatus*.
36 Our results provided the first characterization of microbiota for several strains of molluscs, the
37 intermediate hosts of the *Schistosoma sp.* parasite.

38

39 **2 Material & Methods**

40 **2.1 Rearing conditions**

41 2.1.1 Individual tank experiment

42 To determine the bacterial microbiota composition and specificity, we used four strains of
 43 *Biomphalaria glabrata*, one from Guadeloupe (B. gla GH2), two from Brazil (B. gla BAR2 and B.
 44 gla BRE2) and one experimentally selected for reduced compatibility to different *S. mansoni* parasite
 45 strains (B. gla BS902) (Theron et al., 2014). In addition, *Biomphalaria pfeifferi* (Oman) as well as
 46 another Planorbinae genus, *Planorbarius metidjensis* (Spain), and a Planorbidae non-Planorbinae
 47 species, *Bulinus truncatus* (Spain) were used (Table 1).

48 All strains were reared in the same conditions: 20 individuals of each strain were maintained in
 49 separate tanks (3L) and fasted one week before sampling to avoid changes in microbiota composition
 50 associated with diet. Snail shell size (diameter 7-8 mm), which is directly correlated to age, was
 51 similar for each experimental group.

52 2.1.2 Common garden experiment

53 Thirty molluscs of each strain were maintained for two months within the same 8L tank, where
 54 perforated baskets separating the strains were used in order to avoid mixing and potential
 55 antagonistic interactions, but which favoured the potential exchange of microbiota as they were
 56 reared in the same tank. Molluscs were fed with lettuce every 2 days (and fasted one week before
 57 sampling) and 50% of the water was renewed weekly.

58 2.2 Sampling

59 The mollusc shells were cleaned with cotton buds soaked in bleach (to avoid transfer of
 60 contaminants), and molluscs were then removed from the shell by dissection and flash-frozen
 61 individually in liquid nitrogen before being kept at -80°C until DNA extraction. DNA extraction and
 62 sequencing

63 DNA was extracted with the Nucleospin® tissue extraction kit from Macherey-Nagel and quantified
 64 with Qubit 2.0 Fluorometer following the procedure described in the Qubit™ dsDNA HS Assay Kit,
 65 to check its purity and yield.

66 For samples with highest DNA yield and quality (11 to 15 depending on snail strains for individual
 67 tank experiment, and 10 per strain for the common garden experiment, Table S1), 16S rRNA gene
 68 (V3-V4 regions) (Klindworth et al., 2013) libraries were generated using PCR primers 341F (5'-
 69 CCTACGGGNGGCWGCAG-3') and 805R (3'-GACTACHVGGGTATCTAATCC-5') following
 70 the standard Illumina two-step procedure. Libraries were paired-end sequenced with 250 bp read
 71 length on three different flow cells using the MiSeq system (Illumina) at the Génome Québec
 72 Innovation Centre, McGill University Montréal, Canada. A blank sample was sequenced in each of
 73 the three runs but as very few sequences were obtained, this dataset was not further analyzed.

74 2.3 Analysis of 16S sequences

75 The FROGS (Find Rapidly OTU with Galaxy Solution) pipeline implemented on a galaxy instance
 76 (<http://sigenae-workbench.toulouse.inra.fr/galaxy/>) was used for data processing (Escudié et al.,
 77 2017). In brief, paired reads were merged using FLASH (Magoč & Salzberg, 2011). After denoising
 78 and primers/adapters removal with CUTADAPT (Martin, 2011), de novo clustering was performed
 79 using SWARM with a local clustering threshold (Mahé et al., 2014), with aggregation distance $d = 3$
 80 after denoising. Chimeras were removed using VSEARCH (Rognes et al., 2016). We filtered the

81 dataset for singletons and performed affiliation using Blast+ against the Silva database (release 128,
82 September 2016) for 16S rRNA gene amplicons. Finally, OTU tables were produced in a standard
83 BIOM format for subsequent analyses.

84 We then used the packages phyloseq 1.24.2 (Mcmurdie & Holmes, 2013) and vegan 2.5-4 (Oksanen
85 et al., 2018) with RStudio (R Core Team (2017)). Sample B. gla BRE_JC_7 had too low coverage
86 (155 reads) and was thus discarded from subsequent analyses. Non-bacterial sequences as well as
87 singletons remaining after all the secondary filtering steps were discarded from the dataset. We
88 rarefied the data according to the sample with fewer sequence numbers (18299 reads for the
89 Individual Experiment and 15969 reads for the Common Garden) in order to normalize for
90 sequencing coverage. We characterized the beta-diversity dissimilarities using Principal Coordinates
91 Analyses (PCoA) and Hierarchical Clustering on Bray-Curtis distance matrix (ranging from 0 for
92 identical communities to 1 for completely different communities).

93 2.4 Core microbiota

94 To determine the core microbiota, which is the most stable part of the microbiota, we identified the
95 families and genera that were either present in 100% of individuals or absent from a maximum of one
96 individual for each strain, and represented at least 0.5% of sequences for each strain.

97 2.5 Snail Phylogeny

98 Phylogenetic analysis was performed using 28S rRNA gene sequences from the NCBI database (*B.*
99 *glabrata*: AF435694.1, *B. pfeifferi*: MG461588.1, *P. metidjensis*: AF435671.1 and *B. truncatus*:
100 AF435659.1). The 28S rRNA gene sequence of a Physidae species, *Physa sp.* (Egypt) (sister family
101 of the Planorbidae) was used as an outgroup (AF435654.1). The sequences were aligned using
102 MUSCLE (v3.8.31) and the tree was reconstructed using the maximum likelihood method
103 implemented in the PhyML program (v3.1/3.0 aLRT) with 500 bootstraps on
104 <http://www.phylogeny.fr> (Dereeper et al., 2008; Dereeper et al., 2010).

105 2.6 Statistical analyses

106 We analysed the variance due to host effect on dissimilarity matrices using Permutational
107 Multivariate Analysis of Variance (PERMANOVA). PERMANOVA were done with 999
108 permutations. For all analyses, the threshold significance level was set at 0.05.

109 We used an indicator value index and 999 permutations (multipatt, {indicspecies}) (Caceres and
110 Legendre 2009) to identify OTUs associated with the different host species.
111 P-values were corrected for multiple comparisons using Benjamini and Hochberg's method
112 (Benjamini and Hochberg 1995) (p.adjust, {stats}).

113 3 Results

114 3.1 Bacterial composition

115 At the phylum level, the composition of bacteria was similar in all *Biomphalaria* samples (Figure 1),
116 with Proteobacteria being the predominant phylum for the different strains, in which the
117 Flavobacteriaceae, Rhodobacteraceae, Comamonadaceae and Xanthomonadaceae families were the
118 most represented. This is consistent with the results found by Portet et al. (2018). *P. metidjensis*
119 composition also displayed a high proportion of Proteobacteria, in particular Alphaproteobacteria,

120 represented by Rhodobacteraceae (Table S1). In the case of *B. truncatus*, more pronounced
 121 differences were visible at the phylum level, where Proteobacteria and Tenericutes were dominant
 122 (Figure 1), with the latter represented mainly by Mycoplasmataceae, and more specifically by the
 123 genus *Mycoplasma* (Table S1).

124 The core microbiota was determined as all bacterial families that were either present in 100% of
 125 individuals or absent from a maximum of one individual. The core microbiota was composed of 44
 126 families, for all strains included (Table S2). The core microbiota composition varied between strains;
 127 whereas seven bacterial families were common to all strains, where *Cloacibacterium* (a
 128 Flavobacteriaceae genus) were found as part of the core microbiota in all *Biomphalaria* strains and
 129 species, except for *B. glabrata* BS90, which was absent in the core microbiota of *P. metidjensis* and
 130 *B. truncatus*.

131 3.2 Beta-diversity, ordination and clustering

132 An ordination using PCoA was performed on Bray-Curtis (BC) distance matrix to visualize the
 133 similarities between individuals according to their bacterial composition (Figure 2). The first two
 134 axes explained 30% of the variability observed. Individuals tended to group according to host
 135 species. *Biomphalaria* strains were grouped at the exception of the *B. glabrata* BS902 strain.
 136 Individuals of the two other species, *Planorbarius metidjensis* and *Bulinus truncatus* were separated
 137 from *Biomphalaria* individuals.

138 The hierarchical clustering analysis based on BC distance on the core microbiota confirmed a
 139 grouping between individuals of the same strain or species. Moreover, the dendrogram of bacterial
 140 communities reflected host phylogeny (Figure. 3). *B. truncatus* and *P. metidjensis* were separated
 141 from *Biomphalaria* species, and *B. pfeifferi* was separated from *B. glabrata* strains. The microbiota
 142 specificity according to host genetic background was confirmed by Manova on Bray-Curtis
 143 dissimilarity matrix on core microbiota ($p < 0.001$).

144 The core microbiota beta-diversity was analysed using the same approach with a PCoA ordination
 145 based on the Bray-Curtis dissimilarity index (Figure. 3). Individuals belonging to the same strain tend
 146 to cluster together and *Biomphalaria* strains were grouped, except for, again, the BS902 strain. In
 147 addition, individuals from the two other species, *Planorbarius metidjensis* and *Bulinus truncatus*
 148 were separated from *Biomphalaria* individuals. This analysis of the core microbiota composition
 149 confirmed the pattern obtained for the whole microbiota with specific core microbiota associations
 150 for individuals belonging to the same phylogenetic group (strain, species or genus), suggesting a
 151 phyllosymbiosis pattern, driven by host species among snail intermediate hosts of schistosomiasis.

152 We used indicator value index and permutation tests to identify OTUs significantly associated with
 153 each host species. In average, each species had 37 specific OTUs belonging to 88 genera (Table S3).
 154 Although the 88 genera were mostly (77%) specific of each host species, this analysis highlighted
 155 that specific OTUs belonging to 5 genera (*Pirellula*, *Planctomyces*, *Candidates Odysella*,
 156 *Mesorhizobium*, and *Pseudomonas*) were found in more than 50% of host species. Strikingly, specific
 157 OTUs from *Mesorhizobium* and *Pseudomonas* showed identical distribution within host species
 158 (presence in BRE, BS90, Pfe, and Plan), suggesting that these bacteria might cooperate within host
 159 microbiome.

160 3.3 Common Garden

161 Environmental conditions and/or host genetics can both affect microbiota composition. To
162 investigate the main key drivers for core microbiota composition, we performed an additional
163 experiment with all strains raised together in the same water for two months. However, we couldn't
164 include *B. pfeifferi* in this analysis because most individuals did not survive until the end of the
165 experiment as they escaped their basket and were predated.

166 The PCoA ordination revealed a similar microbiota specificity by strain to those observed in the first
167 experiment with a grouping by strain then species (Figure S1), as confirmed by Manova analysis on
168 host effect on Bray-Curtis dissimilarities between host strains ($p < 0.001$).

169 4 Discussion

170 To understand the host effect in shaping microbiota in Planorbidae schistosomiasis vector snails, we
171 characterized the individual bacterial communities associated with several strains of *Biomphalaria*
172 *glabrata*, *B. pfeifferi*, *P. metidjensis* and *B. truncatus* snails. Working on individuals reared in lab
173 conditions favoured the control of most of the parameters that can influence microbiota composition.

174 In the present study, the whole microbiota was characterized using 16S amplicon sequencing. We
175 identified 31207 OTUs among the six different snail strains. Most of OTUs were not assigned to the
176 species level and 63% were assigned to the genus level. This corresponds to the limitation of the 16S
177 V3V4 marker resolution. In addition, the blast+-based pipeline we use for taxonomic affiliation
178 avoids false affiliation when a sequence matches with several sequences in the database. If several
179 blast results have identical scores for a given OTU, and these taxonomies differ across hits, the OTU
180 is set to "Multi-affiliation" (Escudié et al. 2017).

181 A few studies have characterized the cultivable flora of *B. glabrata* and have identified
182 Aeromonadaceae, Enterobacteriaceae, Moraxellaceae and Pseudomonadaceae as being the most
183 prevalent bacterial families in this species (Ducklow *et al.*, 1979; Ducklow *et al.*, 1981; Silva *et al.*,
184 2013). The dominant families described in the previous studies were also represented in our dataset.
185 However, the relative composition of microbiota at the phylum level revealed that Proteobacteria
186 were dominant for most of the different *Biomphalaria* strains, represented by three main families:
187 Rhodobacteraceae (Alphaproteobacteria), Comamonadaceae (Betaproteobacteria) and
188 Xanthomonadaceae (Gammaproteobacteria). Not all bacterial families can easily be cultivated; the
189 MiSeq technology allows identifying the whole bacterial diversity. Our results are consistent with
190 those found for *B. glabrata* BRE bacterial microbiota in Portet *et al.* (2018), in which these three
191 families were the most abundant of the core microbiota. The microbiota of a Guadeloupian strain of
192 *B. glabrata* was also described using a similar approach (Allan *et al.*, 2018) and similarly, the
193 dominant phyla were Proteobacteria and Bacteroidetes.

194 Proteobacteria have been described as key factor in marine bivalve digestion, like the great scallop
195 *Pecten maximus*, as they are involved in the degradation of major alimentary components contained
196 in their diet (Lasa *et al.*, 2016). This phylum is also dominant in other molluscs, as is the case for
197 oysters *Crassostrea corteziensis*, *C. gigas* and *C. sikamea* (Trabal *et al.*, 2012). As this is the first
198 study to characterize the bacterial microbiota of *B. glabrata* BS902, *P. metidjensis*, *B. truncatus*, it is
199 not possible to compare with previous results and to draw any definitive conclusions.

200 Interestingly, the bacterial families that comprise the core microbiota for each mollusc strain were
201 also among the most abundant taxa in the whole microbiota, which is consistent with previous studies
202 on this model (Portet *et al.*, 2018). In corals, for example, the OTUs belonging to core microbiota are
203 among the rare taxa and are difficult to detect within the whole microbiota (Ainsworth *et al.*, 2015).

204 Due to the high inter-individual variation, in some studies, the core microbiota in the coral model was
205 defined by OTUs present in a limited proportion of individuals, 30% in Ainsworth *et al.* (2015) and
206 50% in Brener-Raffalli *et al.* (2018). In our study, the most impressive case of high abundance in
207 core taxa concerns *B. truncatus*, with the Mycoplasmataceae family, and more precisely the Genus
208 *Mycoplasma*, that represents more than 47% of the whole microbiota. The Genus *Mycoplasma* was
209 originally described as an obligate vertebrate parasite and the causative agent of human genital and
210 respiratory diseases with a high tissue specificity (Razin *et al.*, 1998). This genus has been described
211 in other models including algae and several invertebrates such as oysters (Clerissi *et al.*, 2018; King
212 *et al.*, 2012), abalone (Huang *et al.*, 2010) and Sacoglossans (Davis *et al.*, 2013). It has also been
213 described as being one of the most abundant microorganisms in the deep-sea bone-eating snail,
214 *Rubyspira osteovora* (Aronson *et al.*, 2016). Its role in these organisms remains unclear but some
215 authors hypothesized that they may help with digestion (Aronson *et al.*, 2016; Duperron *et al.*, 2012;
216 Fraune & Zimmer, 2008), notably because of its presence in the digestive tract. In the present study,
217 bacteria belonging to the genus *Cloacibacterium* have been found in all *Biomphalaria* strains and
218 species, and *C. haliotis* has been described in another mollusc, the sea snail *Haliotis discus* (Hyun *et*
219 *al.*, 2014).

220 The results of dissimilarity between strains revealed that the bacterial microbiota of *B. truncatus*
221 individuals was distinct from other species with most of the Bray-Curtis distance values ranging
222 between 0.8 and 0.99. The phylogenetic distance of this species from the others could explain this
223 difference. Indeed, this is the only species, in this study, belonging to Bulinae sub-family, whereas all
224 the others are classified in Planorbinae sub-family. Moreover, the bacterial microbiota of this species
225 seems to be very specific, as suggested by the high abundance of *Mycoplasma*. Interestingly,
226 individuals of the strain Bg BS90 also displayed strong dissimilarities with the other species and even
227 the other strains of *B. glabrata*, with most of the dissimilarity values also ranging from 0.8 and 0.99.

228 As the different strains were maintained in separate tanks, we performed a common garden
229 experiment to circumvent potential biases due to mollusc maintenance and tested whether the same
230 microbial environment would lead to a homogeneous distribution of the bacterial communities
231 between snail strains. This result confirmed a specificity of the microbiota by strain/species,
232 suggesting that the importance of the host effect in microbiota composition is higher than the effect
233 of rearing conditions. The microbiota can nevertheless vary during the host lifespan, with an initial
234 recruitment of bacterial communities occurring during early development. It would be interesting to
235 test the possibility of microbiota transfers from the environment in different developmental stages
236 when the definitive flora is not yet fully established. A recent study showed a loss of microbial
237 communities from one generation to the next in laboratory reared mosquitoes (Akorli *et al.*, 2019),
238 which presents another avenue for our model to be further investigated.

239 In both individual and common garden experiments, almost every individual of each strain grouped
240 together in the dendrogram, supporting the specificity according to the host. Additionally, the
241 topology of microbiota dissimilarities was congruent with the mollusc phylogeny, despite a limited
242 number of strains but that covers species, genera and sub-families of Planorbidae. This revealing
243 suggests a pattern of phyllosymbiosis ~~driven by~~ at the host species level among snail intermediate
244 hosts of schistosomiasis. This has already been described in other models, for both vertebrates and
245 invertebrates. In vertebrates, for example, a loose phyllosymbiosis pattern was identified between 44
246 species of coral reef fishes and their skin microbiota (Chiarello *et al.*, 2018), possibly related to a
247 plasticity in the immune system. Host immune genes and other factors like nutrient production by the
248 host and vertical transmission have also been hypothesized to explain phyllosymbiosis between

249 several populations of American pika, *Ochotona princeps* (Kohl *et al.*, 2017). For invertebrates, this
 250 pattern was shown in three *Nasonia* species, in a controlled environment, with such a
 251 codiversification and coevolution that there is a lethality of hybrids from a breed between two
 252 *Nasonia* species (Brucker & Bordenstein, 2013). This codiversification as a mechanism leading to
 253 phylosymbiosis has also been hypothesized in a study comparing microbiota composition of 15
 254 *Cephalotes* species (Sanders *et al.*, 2014), whereas it would not be the main driver of this
 255 phenomenon in corals, in which phylosymbiosis would be led by other mechanisms like
 256 biogeography or host traits (Pollock *et al.*, 2018). Similar findings of phylosymbiosis driven by the
 257 host have been identified between two different species of *Hydra* (Fraune & Bosch, 2007) and many
 258 studies have shown that the host genetic background shape the microbiota in numerous models
 259 (Chaston *et al.*, 2016; Coon *et al.*, 2016; Paniagua Voirol *et al.*, 2018; Parker *et al.*, 2017; Sánchez-
 260 Cañizares *et al.*, 2017). In our model, this correlation between host and microbiota indicates that host
 261 phylogeny highly constrains the microbiota composition and structuration (Brooks *et al.*, 2016;
 262 Chiarello *et al.*, 2018). However, this pattern may not be ubiquitous and a few studies on *Drosophila*
 263 (Chandler *et al.*, 2011), mosquitos (Osei-Poku *et al.*, 2012) or flea beetles (Kelley & Dobler, 2011)
 264 identified no correlation between host phylogeny and microbiota composition. Nevertheless, we
 265 could not assess the phylosymbiosis pattern at a lower phylogenetic level (i.e. the strain) as we
 266 cannot determine the genetic distance between the different *Biomphalaria glabrata* strains because of
 267 inbreeding in the laboratory and high differentiation between strains.

268 In our case, the phylosymbiosis pattern could not be considered as a hallmark of coevolution because
 269 we focused on the whole bacterial community of a host, with very complex interactions, and not on a
 270 specific symbiont. Here, we defined coevolution according to O'Brien and collaborators (2019), as a
 271 "reciprocal evolution of [...] a broad range of interactions such as predator-prey, host-symbiont and
 272 host-parasite interactions, or interactions among the members of a community of organisms such as
 273 a host and its associated microbiome". O'Brien *et al.* noticed that hosts and their symbiont
 274 phylogenies are often mirrored, which can be interpreted as a parallel divergence called a
 275 codivergence. This codivergence has often led to obligatory symbiosis, as is the case between pea
 276 aphids and bacteria from the genus *Buchnera* (Baumann *et al.*, 2006) and is notably found in
 277 mutualistic symbiosis (O'Brien *et al.*, 2019). In this case, the protagonists have a very close
 278 interaction, with participation in each other's physiological mechanisms.

279 ~~We considered the phylosymbiosis pattern to~~ The host-microbiota specificity illustrates the high
 280 interaction between the snails and their ~~microbiota~~ bacterial communities, suggesting a impact of the
 281 latter on its host fitness toward several functions like nutrition, development, reproduction and
 282 immunity. Given that, in this model, Planorbidae snails are intermediate hosts of *Schistosoma*
 283 parasites, it would be interesting to study the tripartite interaction between the trematodes, the
 284 molluscs and their microbiota.

285 Indeed, previous studies highlighted a variation in the compatibility phenotype between different
 286 combinations of *B. glabrata* strains and *S. mansoni* parasites (Galinier *et al.*, 2017; Theron *et al.*,
 287 2014). Moreover, *P. metidjensis* and *B. truncatus* are not compatible with the same *Schistosoma*
 288 species. This compatibility polymorphism can be seen as a hallmark of differences in immune
 289 capacities. As the phylosymbiosis pattern suggests a strong link between host and microbiota, the
 290 hypothesis of a relationship between the snails' immune capacities and the composition of their
 291 microbiota can be made.

292 The protective role of whole microbiota (or gut microbiota), has indeed been shown in numerous
 293 models like the mosquitoes against dengue virus (Ramirez *et al.*, 2012) or the honey bees with the

294 augmentation in antimicrobial peptide production (Li *et al.*, 2017). Another example is the
295 microbiota of *Dysdercus fasciatus* that acts as a physical barrier to prevent the entry or attachment of
296 a parasite (Onchuru *et al.*, 2018). Chiu and collaborators (2017) also reviewed several examples of
297 microbiota actions against pathogens, such as slowing or preventing the entry, installation,
298 development and expansion of pathogens. In some models, the microbiota has a direct effect against
299 their hosts' pathogens, producing effectors like ROS (Cirimotich *et al.*, 2011), or an indirect effect,
300 promoting some immune pathway. Concerning the interaction between Planorbidae and *Schistosoma*,
301 the immune mechanisms have been well studied, however, there are very few informations
302 concerning the tripartite interactions. Some immune genes located in a Guadeloupe resistance to
303 parasite complex (GRC) region have been shown previously to contribute in shaping microbiota
304 (Allan *et al.*, 2018) highlighting a link between microbiota composition and host immunity.

305 The present study highlighted a ~~phylosymbiosis pattern~~strong host-microbiota specificity, which
306 confirms the link between host genetics, immune capacity and microbiota composition. However,
307 more information are needed to understand if there is a direct or indirect impact of microbiota on the
308 host-parasite interaction.

309 The interaction between microorganisms and the host immune system can be complex. The
310 microbiota stability can be affected upon parasite primo-infestation and challenge suggesting a tight
311 control of immune system on bacterial composition (Portet *et al.*, 2018). The next step will be to
312 compare the microbiota dynamics during an infection kinetic with several host/parasite combinations
313 with different immunobiological interactions. Although a shift in microbiota composition during an
314 infection associated with changes in snail immune gene expression was clearly established according
315 to the host/parasite combination (sympatric/allopatric) (Portet *et al.*, 2018), further studies are needed
316 to clarify the link between microbiota and snail host immunity. Phylosymbiosis pattern is a hallmark
317 of tight interactions between host and microbiota, suggesting the role of microbial communities on
318 different host physiological functions, including immunity. This study thus paves the way for future
319 studies to decipher the role of microbiota in host fitness, including the development and transmission
320 of parasites.

321 **Declarations**

322 Conflict of Interest: The authors declare that the research was conducted in the absence of any
323 commercial or financial relationships that could be construed as a potential conflict of interest.

324 Author Contributions: CH, BG, RG, DD and ET were involved in the study concept and design. CH
325 was involved in sampling and data acquisition. CH, CC and ET performed data analysis. CH and ET
326 drafted the manuscript and all authors contributed to critical revisions and approved the final
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- 335 Data Availability Statement: The datasets generated for this study can be found in the Sequence read
336 Archive repository under BioProject PRJNA554540 (sequence data to be released upon publication).

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574

575 **Tables**

576 Table 1: Origin of snail strains used in this study

| Species | Strain | Strain code | Strain origin |
|---------------------------------|--------|--------------|--|
| <i>Biomphalaria glabrata</i> | BAR2 | B. gla BAR2 | Belo Horizonte, Brazil (G. Oliveira, 2013) |
| <i>Biomphalaria glabrata</i> | BRE2 | B. gla BRE2 | Recife, Brazil (A. Théron, 1975) |
| <i>Biomphalaria glabrata</i> | GH2 | B. gla GH2 | Dans Fond, Guadeloupe (2005) |
| <i>Biomphalaria glabrata</i> | BS902 | B. gla BS902 | Salvador, Brazil (1960) |
| <i>Biomphalaria pfeifferi</i> | / | B. pfe | Anakhar, Oman (H. Moné, G. Moné, 2015) |
| <i>Planorbarius metidjensis</i> | / | P. met | Salamanca, Spain (S. Mas-Comà, 2014) |
| <i>Bulinus truncatus</i> | / | B. tru | Almeria, Spain (A. Olega, 2015) |

577

578 **Figure legends**

579 Figure 1: Relative composition for the eight most abundant phyla in the microbiota for each strain of
580 mollusc.

581 Figure 2: Principal Coordinate Analysis (PCoA) on Bray-Curtis dissimilarity matrix for bacterial
582 microbiota composition. Each dot is an individual and each colour, a strain. The labels are displayed
583 at the barycentre.

584 Figure 3: A) Hierarchical clustering based on Bray-Curtis dissimilarity matrix and Ward linkage for
585 OTUs of the core microbiota. Each colour represents a strain. B) Phylogenetic tree of host species
586 based on 28S rRNA gene sequence and using Maximum Likelihood with 500 bootstraps (%) for
587 node support. *Physa sp.* was used as an outgroup. The red numbers are the bootstrap values for the
588 nodes.

589 **Supplementary files**

590 Table S1: OTU table with taxonomic affiliation and read numbers for each sample.

591 Table S2: Core microbiota by strain/species at the family level (representing at least 0.6% of the total
592 sequence read number by strain). The families highlighted in blue are common to all the strains.

593 Table S3: OTUs significantly associated with each single host species using indicpecies.

594 Supplementary Figure S1: Principal Coordinate Analysis (PCoA) of every individual from each
595 strain in the common garden experiment. Each dot is an individual and each colour a strain. The
596 labels are displayed at the barycentre.

597