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## **Evidences that host genetic background more than the environment shapes the microbiota of the snail *Bulinus truncatus* , an intermediate host of *Schistosoma* species**

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### ► To cite this version:

Mathilde J Jaquet, Philippe Douchet, Eve Toulza, Thierry Lefevre, Bruno Senghor, et al.. Evidences that host genetic background more than the environment shapes the microbiota of the snail *Bulinus truncatus* , an intermediate host of *Schistosoma* species. 2025. hal-04879778

**HAL Id: hal-04879778**

**<https://hal.science/hal-04879778v1>**

Preprint submitted on 10 Jan 2025

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1 Evidences that host genetic background more than the environment shapes the  
2 microbiota of the snail *Bulinus truncatus*, an intermediate host of *Schistosoma*  
3 species.

4  
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13  
14 **Key-words:** *Bulinus truncatus*, Microbiota, Spatial structure, Population genetics, Trematodes,  
15 Multiple regressions on distance matrices

16 **Abstract:**

17 Microbiota are increasingly recognized as key players in regulating host biological functions, influencing  
18 both the ecology and evolution of organisms. However, the factors shaping microbiota diversity and  
19 structure in natural environments remain underexplored, especially the relative importance of host  
20 genetics versus environmental factors. In this study, we address this gap using the freshwater snail  
21 *Bulinus truncatus*, an intermediate host for some human and animal *Schistosoma* parasites, as a model  
22 species. We developed 31 new microsatellite markers to assess the population structure of *B. truncatus*  
23 across 9 sites in Senegal. We then applied metabarcoding to characterize the diversity and structure of  
24 individual snail bacterial microbiota and environmental communities associated with each sampling  
25 site using environmental DNA. We also used molecular diagnostics to determine trematode infection  
26 status of *B. truncatus* individuals. By integrating these data through multiple regression on distance  
27 matrices (MRM) analyses, we quantified the influence of *B. truncatus* population genetics, spatial  
28 distribution, environmental bacterial communities, and infection status on the snail's microbiota  
29 structure. Our results show that the genetic structure of *B. truncatus* populations, and to a lower extent  
30 geographic distribution, are the main factors explaining the snail's microbiota compositions. Neither  
31 the environmental bacterial communities nor trematode infection status significantly contributed to  
32 microbiota structure. A portion of the variance in microbiota composition remains unexplained,  
33 suggesting that additional ecological or intrinsic factors might be involved. These findings provide new  
34 insights into the drivers of microbiota structure in natural populations and highlight the complexity of  
35 host-microbiota-environment interactions.

36 **Data availability statement**

37 All sequence data generated have been submitted to the Sequence Read Archive of NCBI.

38 **Funding statement**

39 This work was funded by the MICROVECT Project (défi clé RIVOC Occitanie Region, University of  
40 Montpellier); the European and Developing Countries Clinical Trials Partnership (EDCTP2) program. and  
41 by the French Agency for Food, Environmental and Occupational Health & Safety. This study is set

42 within the framework of the “Laboratoire d’Excellence (LabEx)” TULIP (ANR-10-LABX-41) and LabEx  
43 CeMEB (ANR-10-LABX-04-01).). The funders had no role in study design, data collection and analysis,  
44 decision to publish, or preparation of the manuscript.

45 **Conflict of interest disclosure**

46 The authors have no conflict of interest to disclose.

47 **Ethics approval statement**

48 The project has received approval from the National Ethical Committee (CNERS) of Senegal (agreement  
49 number: 00061/MSAS/CNERS/SP).

## 50 1. Introduction

51 The 'microbiota', consisting in all microorganisms associated with a host, is now broadly  
52 acknowledged as a crucial factor in various aspects of organismal biology, encompassing  
53 developmental, physiological, reproductive, behavioral and immune phenotypes (Liberti and Engel  
54 2020; Daisley *et al.* 2020; Yuan *et al.* 2021; Davidson *et al.* 2024). The microbiota therefore partly  
55 influences the ecology and the evolution of their hosts and that of biotic interactions between host  
56 species (Sharon *et al.* 2010; Henry *et al.* 2021; Lange *et al.* 2023). Despite recent efforts to understand  
57 the factors shaping host microbiota, identifying and understanding the ecological and evolutionary  
58 determinants of microbiota composition and structure in natural host populations remains a major  
59 scientific challenge.

60 The composition and structure of natural hosts' microbiota is particularly complex and depend  
61 on multiple environmental and host genetic factors (Benson *et al.* 2010). On one hand, the microbiota  
62 is partly composed of some either obligatory or facultative symbionts that are transmitted, generally  
63 via maternal inheritance, from one host generation to another (Funkhouser and Bordenstein 2013).  
64 Their evolutionary history hence follows that of their hosts with which they have co-evolved at both  
65 macroevolutionary and microevolutionary timescales leading to phylosymbiosis (Kohl 2020; Dapa *et al.*  
66 2023). These microorganisms constitute the 'core microbiota' which consists of species consistently  
67 associated with the host regardless of environmental conditions (Risely 2020). Accordingly, the  
68 composition and structure of hosts' microbiota is expected to be partly shaped by a combination of  
69 stochastic (e.g. dispersal, drift) and deterministic (e.g. selection) processes associated with hosts  
70 ecology and evolution (Benson *et al.* 2010; Furman *et al.* 2020; Hayashi *et al.* 2024).

71 On the other hand, most microorganisms that constitute hosts' microbiota are facultative and  
72 are acquired from the environment throughout the host's lifelong development. This is well illustrated  
73 by some species of Lepidopteran that lack a resident microbiota and generally acquire bacterial  
74 communities from their host plants or from the environmental microbial communities (Montagna *et al.*  
75 2016; Phalnikar *et al.* 2018; Minard *et al.* 2019; Liu *et al.* 2020). Moreover, several environmental  
76 biotic factors including the communities of free-living microorganisms present in the environment  
77 (Díaz-Sánchez *et al.* 2018), and abiotic factors (e.g. temperature, pH) can influence the composition of  
78 the hosts' microbiota which can in turn modify hosts biology (Bernardo-Cravo *et al.* 2020). In other  
79 word, the ecology of hosts (e.g. behavior, diet) also influences their microbiota (Archie and Tung 2015;  
80 Kennedy *et al.* 2020). Thus, it is expected that organisms from a common genetic pool and established  
81 in different environments harbor different communities of microorganisms (Berg *et al.* 2016).  
82 Accordingly, part of hosts' microbiota is also expected to be partly shaped by ecological stochastic and

83 deterministic (e.g. niche-based) processes (Hayashi *et al.* 2024). This more flexible microbiota  
84 compartment which includes taxa that are stable or predictable over time within individuals or across  
85 populations and is called the ‘temporal/dynamic core microbiota’ (Risely 2020). This more flexible  
86 microbiota compartment which includes taxa that are stable or predictable over time within individuals  
87 or across populations and is called the ‘temporal/dynamic core microbiota’ (Risely 2020). Our  
88 comprehension of factors shaping hosts’ microbiota hence requires accounting for the ecological  
89 context in which hosts are established and the genetic background of host populations.

90 Surprisingly however, few studies have yet specifically investigated the relative contribution of  
91 organisms’ genetic diversity and that of their environment in shaping natural hosts’ microbiota and  
92 lead to distinct conclusions. For instance, Suzuki and collaborators found that the structure of gut  
93 microbiota of wild house mouse (*Mus musculus domesticus*) populations is well predicted by genetic  
94 distances between host populations computed from an extensive exome genomic dataset and not by  
95 different environmental conditions, including temperature and diet (Suzuki *et al.* 2019). Conversely,  
96 the structure of the gut microbiota associated with California voles (*Microtus californicus*) populations  
97 across a contact zone between two recently diverged lineages of this species was best explained by the  
98 spatial distribution of hosts and not by lineage divergence, hence suggesting a strong influence of the  
99 environment on the structure of voles’ gut microbiota (Lin *et al.* 2020). In the same vein, Rothschild *et*  
100 *al.* (2018) found that the genetic background among 1046 healthy human individuals have minor role  
101 in determining individuals’ gut microbiota while environmental factors such as housing, diet and  
102 anthropometric measurements can explain some of the inter-individual variability in microbiota  
103 (Rothschild *et al.* 2018). Finally, in the *Nematostella vectensis* sea anemone model, Fraune *et al.* (2016)  
104 have shown that both the environment and genetics play a role in shaping the microbiota, and that  
105 these elements are complexly interconnected (Fraune *et al.* 2016). In the light of these studies, it  
106 appears that the importance of the genetic and environmental determinants of microbiota are  
107 potentially species and/or context dependent.

108 Here, we took advantage of high-throughput sequencing technologies to identify and assess  
109 the relative contribution of the ecological and host genetic factors structuring the microbiota  
110 associated with natural populations of the freshwater snail *Bulinus truncatus* as a model. *Bulinus*  
111 *truncatus* constitutes the main intermediate host for several trematode species including *Schistosoma*  
112 *haematobium*, the parasite responsible for urogenital bilharziasis in human populations in Africa  
113 (Toledo and Fried 2014). Ecological studies aimed at characterizing the communities of microorganisms  
114 associated with natural snail populations have recently flourished, especially in freshwater snails that  
115 are associated with disease transmission (Li *et al.* 2023). Based on cultivation-independent molecular  
116 methods, Van horn *et al.* (2012) have shown highly diverse intestinal microbial communities among

117 three freshwater planorbid snails collected from the field and two of which serve as intermediate  
118 hosts of several parasites including digenetic trematodes of the genus *Schistosoma* the etiologic agent  
119 of Schistosomiasis (Van Horn *et al.* 2012). Similarly, Huot *et al.* (2020) demonstrated that seven species  
120 and strains of Planorbidae exhibited highly specific core bacterial composition that are closely related  
121 to the host strain. This bacterial composition shows high congruence with the host phylogeny, hence  
122 revealing a phyllosymbiotic pattern (Huot *et al.* 2020). More recently, (McCann *et al.* 2024), showed  
123 that the microbiota of *Galba truncatula*, the main intermediate host for the zoonotic trematode  
124 *Fasciola hepatica*, vary between natural populations established at two geographically and ecologically  
125 distinct sites, hence highlighting the existence of natural diversity in the composition of bacterial  
126 communities associated with this snail species. However, whether such microbiota diversity in natural  
127 populations is driven by snails' genetic background or by environmental factors remains to be fully  
128 elucidated. If the freshwater snails' microbiota is important in the circulation of associated pathogens,  
129 as has been suggested, it is essential to study the natural diversity, the structure and the factors  
130 structuring these communities in the field.

131 Here, we characterized the diversity and structure of the whole-body *B. truncatus* microbiota  
132 over 9 freshwater aquatic sites in Northern Senegal. The presence of trematodes developing within  
133 each snail was molecularly diagnosed and the genetic background of each snail host was characterized  
134 based on 31 newly developed microsatellite markers using a genotyping-by-sequencing approach.  
135 Aquatic bacterial communities associated with each of the 9 studied freshwater sites were also  
136 characterized and used as a proxy of the ecological context in which *B. truncatus* populations are  
137 established. We used these complementary datasets to identify the main factors structuring the  
138 microbiota associated with *B. truncatus* in natural populations.

139

## 140 **2. Materials and Methods**

### 141 **2.1. Field collection of samples**

142 Field sampling was conducted in February 2022 during the dry season in the Senegal River  
143 basin region (**Fig. 1**). We focused on nine natural sites of *S. haematobium* transmission, where  
144 urogenital schistosomiasis' prevalence among school-aged children was previously reported (Senghor  
145 *et al.* 2022). These sites differ in terms of ecological contexts and in terms of *B. truncatus* abundance  
146 (Douchet *et al.* 2024). Six transmission sites are located near the villages of Ndiawara, Ouali Diala,  
147 Dioundou, Fonde Ass and Khodit, all of which being located along river "le Doue", a tributary of the  
148 Senegal River in the middle valley. One transmission site, near the village of Guia, is also located in the  
149 middle valley and consists of an irrigation canal that drains water from the river "le Doue". Two



150 transmission sites, near the villages of Mbane and Saneinte are located along the east shore of Guiers  
151 lake. One transmission site near the village of Lampsar is located in the lower valley and consists of an  
152 inlet of the Senegal River delta (**suppl. Table 1**).



153

154 **Figure 1:** Locations of the studied aquatic transmission sites (black dots) nearby the closest established  
155 villages, on a satellite map of northern Senegal.

156

### 157 2.1.1 Field collection of environmental samples

158 At each site, we first filtered water along the water column from the surface to the water-  
159 sediment interface. We used filtration capsules of 0.45  $\mu\text{M}$  mesh size (Waterra USA Inc.) connected to  
160 an electric water pump as previously described in Douchet and collaborators (Douchet *et al.* 2022).  
161 Filtration was performed until the filtration capsule was clogged and the final filtration volume was  
162 retrieved. Once the filtration completed, the filtration capsule was depleted from its water content,  
163 filled with 50 mL of Longmire solution, vigorously shaken, and preserved at ambient temperature until  
164 used for environmental DNA (eDNA) extractions. At each site but Khodit, a technical field negative  
165 control was obtained by filtering 1.5L of commercial spring water, following the same protocol for  
166 preservation.

### 167 2.1.2 Field collection and preservation of snails' samples

168 Following eDNA sampling, we manually collected all encountered aquatic snails by scooping  
169 the aquatic vegetation using a colander for about 30 minutes to 1 hour, along a 10 to 30-meters long  
170 transect along the shore of the targeted waterbody (either a river or a lake). After taxonomic  
171 identification (following the keys of (Mandahl-Barth 1962)), up to 17 non-emitting *B. truncatus* snail  
172 were extracted from their shell, after a quick heating step at 70°C for 1 minute, with decontaminated  
173 forceps and individualized into a sterile 1.5ml tube containing 70% Ethanol. Forceps were

174 decontaminated between each sample using a DNA AWAY solution. The extraction of snail bodies from  
175 their shells was achieved to avoid DNA contamination with microorganisms, including bacteria,  
176 established on snail shells. Overall, 124 (mean = 14; min = 5; max = 17; **suppl. Table 1**) adult *B. truncatus*  
177 snails that displayed a shell size of approximately 6 mm long, were prepared as above.

178

## 179 **2.2. DNA Extraction from eDNA samples and *B. truncatus* snails**

180 All pre-PCR steps including DNA extraction and the preparation of PCR mixes were conducted  
181 under a sterile hood decontaminated before and after each use with 10% bleach, 70% ethanol, a DNA  
182 AWAY solution followed by a UV light exposure for 20 minutes.

183 Total eDNA from water filtrations were extracted following (Douchet et al. 2022). Briefly, the  
184 Longmire buffer contained within each filtration capsule was equally split into three 50 mL tubes. For  
185 the field negative controls (i.e. Spring water filtrates), each capsule content was recovered in one single  
186 50 ml tube. The tubes were centrifuged for 20 min at 16,000 x g and the supernatant was discarded.  
187 We then collected up to 1g of sediment from the pellet from each tube or 500 µl of Longmire remaining  
188 at the base of the tube when not enough material was observed. Thus, total eDNA from each capsule  
189 were extracted in triplicate. No pellets were observed for filtration negative controls (consisting in  
190 spring water) after centrifugation. For these samples, 500 µL of Longmire were retained and  
191 resuspended after removing the supernatant and were processed as the other samples. This step led  
192 to the processing of 35 samples (i.e. 3 extraction replicates for each of the nine eDNA samples and one  
193 field negative control per site except for Khodit). The total eDNA of these 35 samples was extracted  
194 using the DNeasy PowerSoil Pro kit (Qiagen) according to manufacturer's protocol performing the  
195 physical lysis with a MagNA Lyser at a speed of 7000 x g for 30 s.

196

197 Total genomic DNA from each of the 124 snails removed from their shell was extracted using  
198 DNeasy 96 Blood and Tissue Kit (Qiagen), according to the manufacturer's protocol. Two negative  
199 control extractions consisting of empty 1.5 ml tubes same as those used for snail preservation were  
200 added in the process.

201

## 202 **2.3. Infection status and genotyping of *B. truncatus* snails**

### 203 **2.3.1. Infection status of *B. truncatus* snails**

204 The presence of developing trematodes within each of the 124 snails was diagnosed using the  
205 Trem\_16S\_F1 (GACGGAAAGACCCCRAGA) and Trem-16S\_R2 (CRCCGGTYTTAACTCARYTCAT) 16S  
206 trematode metabarcode (Douchet *et al.* 2022). DNA extracts were diluted to 1/100<sup>th</sup> to minimize PCR  
207 inhibitors and hence limit PCR false negatives. PCR reactions were prepared and run under standard  
208 conditions, with detailed methodologies provided in **Appendix 1**. Positive samples were reamplified



209 and sent to the Bio-Environment platform (University of Perpignan Via Domitia, France) to identify the  
210 trematodes potentially developing within snail hosts and accounting for possible coinfections. Libraries  
211 were performed using Illumina Nextera index kit and Q5 high fidelity DNA polymerase (New England  
212 Biolabs). Indexed PCR products were then normalized with SequelPrep plates (ThermoFisher) and  
213 paired-end sequenced on a MiSeq instrument using v2 chemistry (2 x 250 bp).

214

### 215 **2.3.2. Microsatellite development**

216 The *B. truncatus* genome (GenBank accession GCA\_021962125.1, (Young *et al.* 2022)) was used  
217 for microsatellite discovery, leading to the selection of 96 primer pairs optimized for multiplex PCR  
218 (details in **Appendix 2**).

219

### 220 **2.3.3. Sequence-based microsatellite genotyping**

221 Multiplex PCR amplification and sequencing libraries were constructed, with detailed methods  
222 provided in **Appendix 3**.

223

### 224 **2.3.4. Genetic analyses**

225 Descriptive statistics of genetic diversity including the mean number of alleles, the mean allelic  
226 richness (computed based on the minimal number of genotypes obtained over sampling sites,  $N = 5$ ),  
227 the mean expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity, and the mean  $F_{is}$  were computed at each  
228 sampling sites using the SPAGeDi software (v. 1.5d; (Hardy and Vekemans 2002)) accounting for the  
229 allotetraploidy nature of *B. truncatus* (Njiokou *et al.* 1993).

230 We next computed pairwise Nei's genetic standard distance  $D_s$  (Nei 1978) and  $F_{st}$  values  
231 between each sampling sites using the SPAGeDi software (v. 1.5d; (Hardy and Vekemans 2002)). From  
232 the obtained  $D_s$  values, we ran a Mantel test to test for possible isolation by distance pattern among  
233 sampling sites using matrices of pairwise  $D_s$  values and pairwise linear geographical distances obtained  
234 between each sampling site. The statistical significance of the Mantel coefficient  $r$  was tested based  
235 on 10000 permutations as implemented in the '*adeget*' R package (Jombart 2008). A similar Mantel  
236 test analysis was run using pairwise ( $F_{st} / (1 - F_{st})$ ) values obtained between sampling sites.

237

### 238 **2.4. Sequencing of bacterial communities and metabarcoding analyses**

239 A total of 198 bacterial 16S metabarcoding libraries were prepared following the Illumina two-step  
240 PCR protocol; including the DNA extracts of the 124 snails, 70 eDNA replicates consisting in 54 eDNA  
241 samples (each extraction triplicate was amplified in duplicate) and 16 technical field negative controls  
242 (each control was amplified in duplicate), and 4 negative PCR controls (millip water) (**suppl. Table 3**).  
243 We targeted the variable V3-V4 loops region of the 16S sDNA gene using the 341F (5'-

244 CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') primers (Klindworth *et al.*  
245 2013) combined with universal Illumina adapters.

246 The first PCRs were performed using the Q5® High-Fidelity 2X Master Mix (New England Biolabs),  
247 in a 25µL final volume containing 2 µL of template DNA and using a PCR program consisting in an initial  
248 denaturation step of 30s at 98°C followed by 32 cycles containing a denaturation step of 6 sec at 98°C,  
249 an annealing step of 30 sec at 55°C, and an elongation step of 8 sec at 72°C and ending with a final  
250 elongation step of 60 sec at 65°C. We used 5 µL of the PCR products to check the PCR products quality  
251 and integrity through electrophoresis on agarose gels. The 20µL left were sent to the Bio-Environment  
252 platform (University of Perpignan Via Domitia, France). Libraries were performed using Illumina  
253 Nextera index kit and Q5 high fidelity DNA polymerase (New England Biolabs). Indexed PCR products  
254 were then normalized with SequalPrep plates (ThermoFisher) and paired-end sequenced on a MiSeq  
255 instrument using v2 chemistry (2 x 250 bp).

256 Raw data were processed using R (version 2023.03.0+386) using a pipeline based on the '*dada2*'  
257 package (v. 1.28.0) (Callahan *et al.* 2016) on the IFB (French Institute of Bioinformatics) cloud. Briefly,  
258 this pipeline consists in removing primers from the obtained sequenced reads (max.mismatch = 1),  
259 trimming and quality filtering reads (minLen = 150, maxN = 0, maxEE = c(3, 3), truncQ = 2), denoising,  
260 dereplicating, merging paired-end reads (maxMismatch = 0), building the Amplicon Sequence Variant  
261 (ASV) table and removing potential chimeric sequences produced during the process (method =  
262 'consensus'). The taxonomic assignment (multithread = TRUE, minBoot = 60) of the resulting filtered  
263 and cleaned ASVs was performed using the Ribosomal Database Project (RDP) classifier (Wang *et al.*  
264 2007) and based on the Silva\_train\_set and silva\_species\_assignment 138.1 datasets (Quast *et al.*  
265 2012). Eukaryotic, mitochondrial, and chloroplast sequences were removed, as well as ASVs not  
266 affiliated to 'Bacteria' at the Kingdom taxonomic levels. The obtained ASV sequence table was finally  
267 merged with the taxonomy database and the sample metadata matrix for subsequent analyses using  
268 the '*phyloseq*' R package (v. 1.22.3) (McMurdie and Holmes 2013).

269 The final ASV dataset was next cleaned from possible contamination during field collection and  
270 biomolecular processing (DNA extraction, PCR). To this aim, ASVs obtained from negative PCR controls  
271 were removed from all samples through the 'prune\_taxa' function of the '*phyloseq*' package. Then, the  
272 'merge\_phyloseq' function of the '*phyloseq*' package was used to generate the whole object containing  
273 all the samples. Moreover, ASV present at low abundance (< 0.005%; (Bokulich *et al.* 2013)) were  
274 filtered from the whole dataset to account for possible sequencing errors. Briefly, the whole phyloseq  
275 object was transformed in relative abundance with the 'transform\_sample\_count' function of the  
276 '*phyloseq*' R package. Then, the low abundant taxa were removed through the 'prune\_taxa' function  
277 of the '*phyloseq*' R package.

278 To assess whether sequencing depth was satisfactory across samples, rarefaction curves were  
279 generated using ‘rarecurve’ function from the ‘*vegan*’ R package (v. 2.6.4) (Oksanen *et al.* 2022). The  
280 abundance of each ASV was normalized based on the lowest sample in terms of ASV counts (i.e. 7000,  
281 see **suppl. Table 3**) by random sub-sampling using the ‘rarefy\_even\_depth’ function of the ‘*phyloseq*’  
282 package (rngseed = 1000) (McMurdie and Holmes 2013). To quantify read counts per sample during  
283 preprocessing steps, refer to **suppl. Table 3**.

284

## 285 **2.5. Statistical analyses**

### 286 **2.5.1. Diversity in environmental and snail-associated bacterial communities and characterization of** 287 ***B. truncatus* core microbiota**

288 The diversity of bacterial communities associated with environmental matrices and *B.*  
289 *truncatus* snails was assessed at the sampling site level based on the absolute number of ASVs, the  
290 Shannon and Simpson’s diversity indices as implemented in the ‘*microbiome*’ R package (v. 1.22.0)  
291 (Lahti and Shetty 2012). Kruskal-Wallis nonparametric tests were computed to compare each of these  
292 alpha-diversity indices between sample type (i.e. snails *versus* eDNA samples) and between sites using  
293 the ‘*stats*’ R package (v. 4.3.0) (R Core Team 2023). Those tests were followed by pairwise multiple  
294 comparisons using the ‘*FSA::dunnTest*’ of the ‘*FSA*’ R package (v. 0.9.5) with the Benjamini-  
295 Hochberg method for adjusting p-values (Dunn 1964).

296 To characterize the core microbiota of *B. truncatus in natura*, which consists in the most stable  
297 part of the bacterial communities shared among all individuals (Risely 2020), we followed established  
298 guidelines (Neu *et al.* 2021). The core microbiota was assessed at the family level using the  
299 ‘*core\_members*’ function of the ‘*microbiome*’ R package (Lahti and Shetty 2012) and setting the  
300 detection parameter to 0 and the prevalence parameter to 90%.

301

### 302 **2.5.2. Identification of factors structuring environmental and snail bacterial communities**

303 To investigate for possible structuration of bacterial communities in environmental samples  
304 and *B. truncatus* snails we conducted a Principal Coordinate Analysis (PCoA) using the ‘*pco*’ function  
305 of the ‘*ecodist*’ package (v. 2.1.3) (Goslee and Urban 2007). Pairwise Jaccard and Bray-Curtis indices  
306 were conducted using the ‘*distance*’ function of the ‘*phyloseq*’ R package. Based on these beta diversity  
307 indices, we ran permutational multivariate analyses of variance (PERMANOVA) to test for: 1-  
308 Differences between aquatic bacterial communities (i.e. eDNA samples) and snails associated  
309 communities, accounting for the sampling site as an additional explanatory variable and for a possible  
310 interaction between sampling site and the nature of the sample (environment *versus* snail). 2- An  
311 effect of trematode infection status (all trematode species combined) on the difference in *B. truncatus*

312 microbiota. Here we accounted for the sampling site as an additional explanatory variable and an  
313 interaction term between the sampling site and the infection status. PERMANOVA were conducted  
314 using the 'adonis2' function implemented in the 'vegan' R package (Oksanen *et al.* 2022), and setting  
315 the number of permutations to 10 000.

316 To assess the relative effect of the environmental bacterial communities and the genetic  
317 background of snails on whole-body snail microbiota, we tested for correlation between four pairwise  
318 matrices including, the geographical distance matrix between each sampling site, the Jaccard indices  
319 computed between environmental bacterial communities at each sampling site, the Jaccard indices  
320 computed between bacterial communities associated with *B. truncatus* individuals and the Nei's  
321 genetic distance between snail hosts. To ensure comparable dimensions for the four matrices, 14  
322 samples from the original matrices were discarded using the 'subset\_samples' function from the  
323 'phyloseq' R package. This step led to two matrices of 109\*109 dimensions. Based on those matrices,  
324 we pseudoreplicated the geographic distance and the Jaccard indices computed between  
325 environmental bacterial communities at each sampling site matrices to transform them in the same  
326 dimensions.

327 Based on these four matrices, we conducted a multiple regression on distance matrices (MRM)  
328 fixing the Jaccard distance matrix obtained from the bacterial communities between *B. truncatus*  
329 individuals as the dependent variable and using three matrices as explanatory variables: the Jaccard  
330 distance between bacterial communities at each site (pseudoreplicated at the individual level), the  
331 Nei's genetic distance computed between individuals and the euclidean geographic distance between  
332 sites (also pseudoreplicated at the individual level). This MRM analysis was ran using the 'MRM'  
333 function of the 'ecodist' R package and the significance of this multiple regression test was assessed  
334 based on 9999 permutations.

335 Results were visually assessed using a custom function to plot matrices against one another,  
336 with Mantel test statistics computed using the 'mantel' function of the 'vegan' R package.

337

### 338 **3. Results**

#### 339 **3.1. Abundance of *B. truncatus*, trematode prevalence among sites**

340 Overall, from the 124 *B. truncatus* extracted from their shell, the total trematode infection  
341 prevalence was 15.3% ranging from 0% (Dioundou, Mbane, Saneinte) to 29.4% (Fonde Ass) (**suppl.**  
342 **Table 1**). Nine species of trematodes were identified. Aside from *Schistosoma haematobium* and  
343 *Schistosoma bovis*, we also found *Haematolochus* sp., two species of Paramphistomoidea,  
344 *Orientocreadium batrachoides*, *Petasiger* sp. and two species of Diplostomoidea.

345

#### 346 **3.2. Genetic diversity and structure of *B. truncatus* among sampling sites**

347 Based on the 31 newly developed microsatellite markers, we found little genetic diversity with  
348 little variation in genetic diversity among sites. Allelic richness (Ar) ranged from 2.13 (Dioundou) to  
349 2.75 (Mbane) and observed heterozygosity (Ho) ranged from 0.38 (Ndiawara and Saneinte) to 0.41  
350 (Guia and Mbane) (**Table 1**). Inbreeding coefficients ( $F_{is}$ ) were different from zero for all but the two  
351 sites Mbane and Saneinte located on the Guiers lake. Pairwise  $D_s$  genetic distances computed between  
352 sites ranged from 0.0019 between Ndiawara and Dioundou both sites being located along the Doué  
353 river approximately 4.8 km one from each other; and 0.4354 between Ouali Diala (Doué River) and  
354 Lampsar distant by approximately 160.3 km. The observed genetic differentiation between sites  
355 followed an isolation by distance pattern (Mantel  $r = 0.76$  ;  $P_{val} = 0.0009$ ) (**suppl. Fig. 1**).

356  
357 **Table 1.** Genetic diversity statistics for *B. truncatus* at each sampling site based on the 31 microsatellite  
358 markers developed in this study. Na: Number of alleles; Ar: Allelic richness; He: gene diversity (Nei  
359 1978); Ho: Observed heterozygosity; Fis: Inbreeding coefficient; Pval of Fis after 10000 randomization  
360 of gene copies among individuals.

Site	Sample size	Na	Ar	He	Ho	Fis	Pval (Fis<>0)
Ndiawara	7	2.52	2.42	0.405	0.382	0.063	0.028
Ouali Diala	11	2.42	2.27	0.375	0.389	-0.042	0.054
Guia	16	2.58	2.21	0.384	0.406	-0.06	0.001
Dioundou	5	2.13	2.13	0.369	0.396	-0.087	0.011
Fonde Ass	15	3.06	2.53	0.417	0.39	0.069	0.001
Khodit	15	2.97	2.63	0.453	0.396	0.133	0
Mbane	12	3.23	2.75	0.401	0.41	-0.023	0.239
Saneinte	16	3.26	2.68	0.383	0.382	0.004	0.806
Lampsar	13	2.9	2.57	0.421	0.392	0.072	0
Overall sites	110	5.52	3.18	0.499	0.394	0.211	0

361  
362 **3.3. Diversity in environmental and snail-associated bacterial communities and**  
363 **characterization of *B. truncatus* snails' core microbiota**

364 In terms of alpha diversity, the bacterial communities from the environment displayed equal  
365 alpha diversity among sites except for Lampsar that displayed lower alpha diversity whatever the index  
366 used (i.e. Species Richness, Shannon, Simpson) (**suppl. Fig. 2**). Moreover, *B. truncatus* microbiota  
367 displayed lower diversity than aquatic bacterial communities, except for the Lampsar site, when  
368 considering the ASV richness (Kruskall-Wallis chi squared = 95.394;  $P_{val} < 0.0001$ ), the Shannon  
369 diversity index (Kruskall-Wallis chi squared = 92.187;  $P_{val} < 0.0001$ ) and the Simpson index (Kruskall-  
370 Wallis chi squared = 89.132 ;  $P_{val} < 0.0001$ ) (**suppl. Fig. 2 and suppl. Table 4**). We found significant  
371 differences in the mean alpha diversity indices in the microbiota of *B. truncatus* from the different  
372 sample sites (all  $p < 0.05$ , **suppl. Table 5 and suppl. Fig. 3A**). Pairwise post-hoc tests revealed that only  
373 the alpha diversity of the sampling site Mbane was lower than that of Saneinte ( $Z = 6.78$ ,  $P_{val} < 0.01$ ),

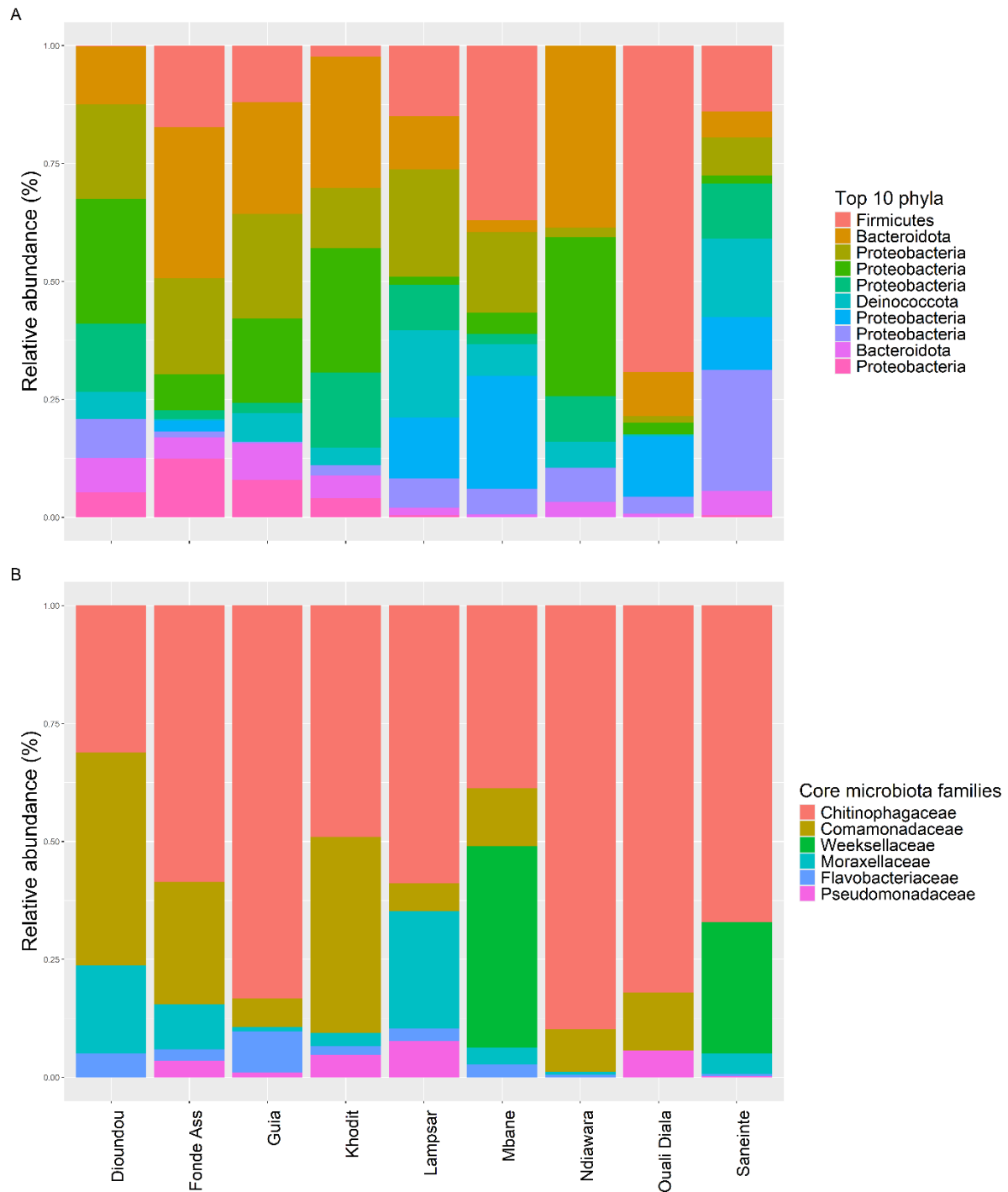
374 Guia ( $Z = 3.16$ ,  $P_{\text{val}} = 0.014$ ) and Lampsar ( $Z = 8.17$ ,  $P_{\text{val}} < 0.01$ ). Moreover, we found no significant  
375 differences in alpha diversity between infected and uninfected snails (all  $p > 0.05$ , **suppl. Table 6 and**  
376 **suppl. Fig. 3B**).

377

378 Proteobacteria were predominant in the microbiota of *B. truncatus* across all sampling sites,  
379 followed by Bacteroidota and Firmicutes (**Fig. 2A**). Among the phyla identified in the environmental  
380 samples, only two were common to both the snail microbiota and the environmental samples:  
381 Cyanobacteria and Planctomycetota (**suppl. Fig 4**). Cyanobacteria was the second most abundant  
382 phylum in the environmental samples, while Planctomycetota ranked seventh among the 28 phyla  
383 found. Interestingly, Planctomycetota were more abundant in the snail samples than in the  
384 environmental samples, whereas the opposite was true for Cyanobacteria.

385 The core microbiota of *B. truncatus* was characterized at the family level by considering  
386 bacterial families present in at least 90% of the overall snails. The core microbiota consisted of 6  
387 families out of the 169 families identified in the snails. These families, which included  
388 Chitinophagaceae (69.3% of core microbiota reads), Weeksellaceae (12.9%), Comamonadaceae  
389 (8.9%), Moraxellaceae (3.4%), Flavobacteriaceae (2.9%), and Pseudomonadaceae (2.6%), were  
390 common to at least 90% of individuals across all sampling sites, although their relative abundances  
391 varied (**Fig. 2B**).





392

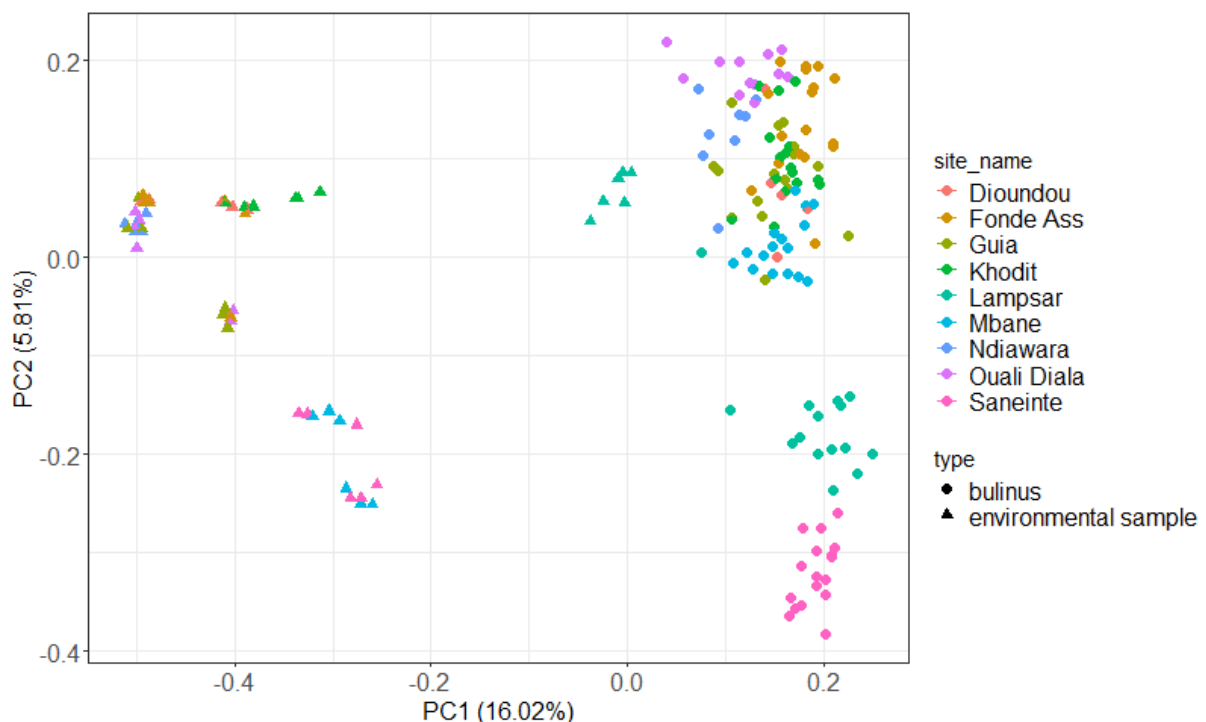
393 **Figure 2:** (A) Relative abundance of the ten most abundant phyla in the microbiota of *B. truncatus* from  
394 the nine different sites. (B) Bacterial composition of the core microbiota of *B. truncatus* snails at the  
395 family level across the 9 studied sites.

396

### 397 3.4. Identification of factors structuring environmental and snail bacterial communities

#### 398 3.4.1. The microbiota of *B. truncatus* snails differ from environmental bacterial communities

399 Based on the microbial communities characterized from the 54 environmental samples and the  
400 124 *B. truncatus* individuals, we found that the total microbiota associated with whole-body *B.*  
401 *truncatus* differs from bacterial communities present in the environment. The first two axes of the  
402 Principal Coordinates Analysis (PCoA) performed on the Jaccard distance matrix obtained among all  
403 samples (i.e. *B. truncatus* snails and environmental samples) and all sampling sites explained 21.83%  
404 of the total variance (**Fig. 3**). *Bulinus truncatus* and environmental samples cluster into two distinct  
405 groups along these two axes. Such difference in the composition in bacterial communities between the  
406 environmental samples and *B. truncatus* is confirmed by the PERMANOVA analysis which indicates  
407 significant effect of the type of sample (i.e. *B. truncatus* snail and environmental sample) (F-value =  
408 39.44; Pval < 0.0001) and of the sampling site (F-value = 6.42 ; Pval < 0.0001). Similar results were  
409 obtained from the PERMANOVA analysis based on the Bray-Curtis distances (nature of the sample: F-  
410 value = 76.60 ; Pval < 0.0001; site: F-value = 10.66 ; Pval < 0.0001; **suppl. Fig. 5**).

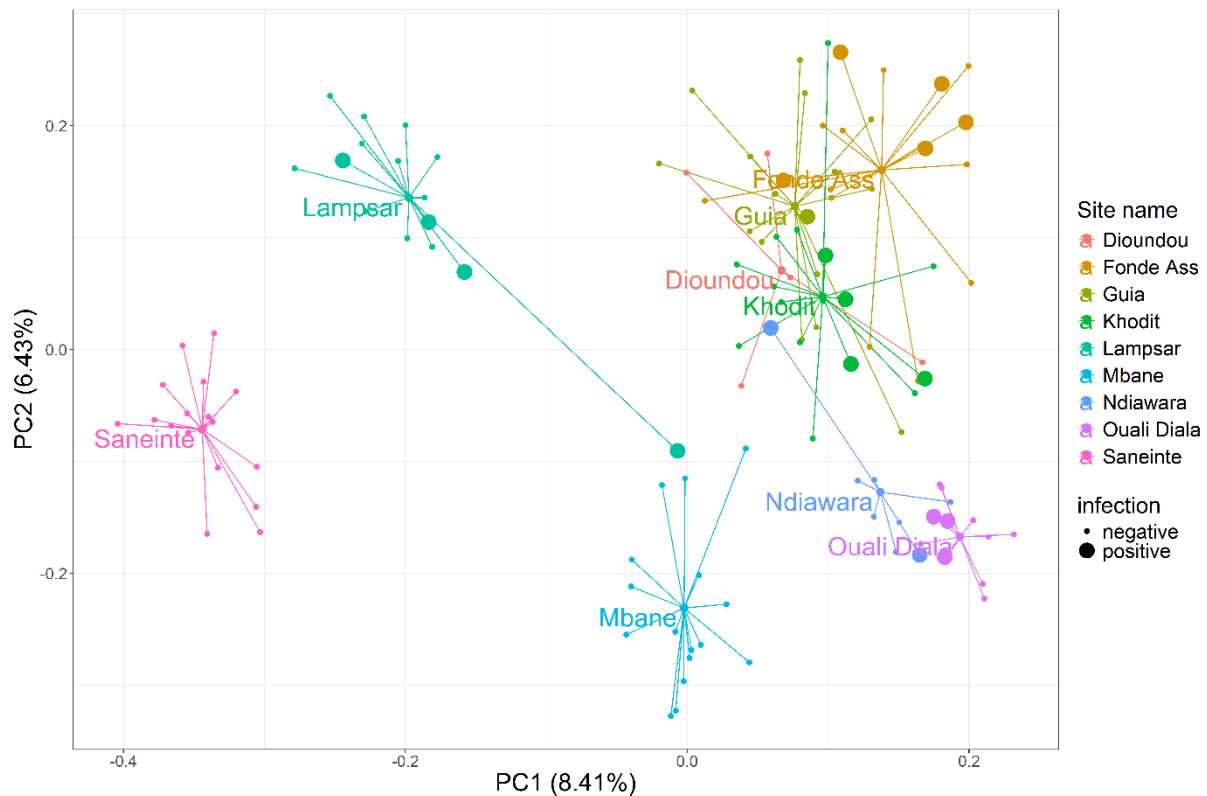


411  
412 **Figure 3** Principal coordinate analysis (PCoA) plot showing environmental samples (triangles) and *B.*  
413 *truncatus* snail associated (dots) bacterial communities distributed along the two first PCoA axes  
414 computed based on pairwise Jaccard distances obtained between each pair of samples.  
415

#### 416 **3.4.2. The microbiota of *B. truncatus* snails is geographically structured, partly driven by 417 their genetic background and the geographic distance and to a lesser extent by the environment.**

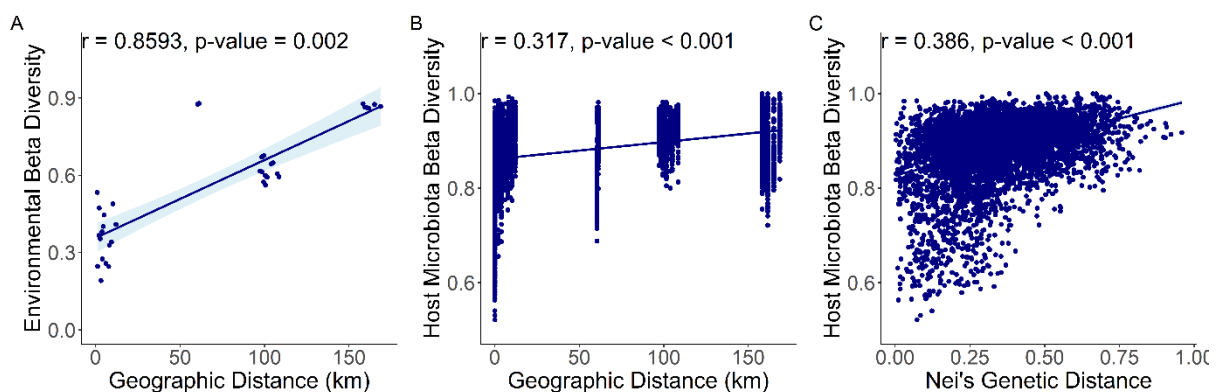
418 The first two axes of the Principal Coordinates Analysis (PCoA) performed based on the Jaccard  
419 distance matrix obtained from *B. truncatus* microbiota explained 28.36% of the total variance (**Fig. 4**).  
420 Our PERMANOVA analysis supports an effect of the sampling site with the structure of *B. truncatus*  
421 microbiota (F-value = 7.84 Pval < 0.0001) (**Fig. 4**). The same results were observed using pairwise Bray-

422 Curtis distances (F-value = 12.20; Pval < 0.0001, **suppl. Fig. 6**). Conversely, we found no effect of the  
423 infection status of *B. truncatus* on the beta diversity of snails' microbiota, neither based on the Jaccard  
424 distances (**Fig. 4**) nor based on the Bray-Curtis distances (all  $p > 0.05$ , **suppl. Table 7** and **suppl. Fig. 6**)  
425 even when accounting for the effect of the sampling site.



426

427 **Figure 4:** Principal coordinate analysis (PCoA) plot showing the bacterial communities of *B. truncatus*  
428 according to their original sampling sites (see color legend), and to their infection status by trematodes  
429 based on a molecular diagnostic (dot size) distributed along the two first PCoA axes based on pairwise  
430 Jaccard similarity indices computed between each sample. Each colored small dot represents one snail  
431 host from a given sampling site. Large dots are snail hosts positive to trematode molecular diagnostic.  
432 Medium dots are the centroids of the bacterial communities of snails originating from each sampling  
433 site.  
434



435

436 **Figure 5:** Distance-decay scatterplots showing bacterial communities distance (determined by the  
437 pairwise Jaccard similarity index) as a function of geographic distance across sampling sites for

438 environmental samples (A), and *B. truncatus* snails (B). Scatterplot of *B. truncatus* snails microbiota  
439 dissimilarity (determined by the Jaccard index) as a function of Nei's genetic distance between *B.*  
440 *truncatus* individuals computed based on 31 microsatellite loci (C). Blue lines of best fit are included  
441 to highlight trends.

442

443 Based on the multiple regression on distance matrices (MRM) and on our series of correlation  
444 tests (Fig. 5), the variables that explain the mean Jaccard indices calculated between *B. truncatus*  
445 microbiota at the individual level are, in order of importance, the Nei's genetic distance between  
446 individuals ( $r = 0.24$  ;  $P_{val} < 0.001$ ), the geographical distance between sites ( $r = 0.22$  ;  $P_{val} = 0.001$ )  
447 and the mean Jaccard indices calculated between the aquatic bacterial communities at the site level ( $r$   
448  $= - 0.05$  ;  $P_{val} = 0.39$ ) both pseudopseudo-replicated at the individual level.

449

#### 450 **4. Discussion**

##### 451 **1. Composition of the microbiota in natural populations of *B. truncatus***

##### 452 **1.a. Bacterial communities display lower alpha-diversity in *B. truncatus* than in** 453 **environmental samples**

454 Our results provide new insights on the composition of the microbiota associated with *B.*  
455 *truncatus* natural populations. Overall, *B. truncatus* display microbiota less diversified than bacterial  
456 communities present in the surrounding freshwater environment. This was somewhat expected  
457 because bacterial communities collected from the environment encompass free-living bacteria,  
458 bacteria that are released by all organisms associated with freshwater habitats and bacteria associated  
459 with freshwater microorganisms that could have been trapped during eDNA sampling (e.g. algae,  
460 invertebrates) (Mikhailov *et al.* 2019; Gendron *et al.* 2019; Sadeghi *et al.* 2021). Moreover, host  
461 microbiota are generally considered as a subsample of environmental bacterial communities resulting  
462 from a filter induced by the intrinsic properties of the hosts (Reese and Dunn 2018; Mazel *et al.* 2018;  
463 Suzzi *et al.* 2023). In particular, the host constitutes an ecological niche, which suitability for bacteria  
464 is largely influenced by hosts immune system that acts as a selective pressure, promoting the growth  
465 of some beneficial bacteria while inhibiting potential pathogens and maintaining the microbiota  
466 homeostasis (Belkaid and Harrison 2017). Such lower alpha-diversity in host microbiota compared to  
467 environmental bacterial communities was also described in recent studies conducted on other  
468 freshwater snails including *Ampullaceana balthica* and *Galba truncatula* (Herlemann *et al.* 2024a;  
469 McCann *et al.* 2024).

470 We found only little inter-site differences in *B. truncatus* microbiota alpha diversity among the  
471 nine sampling sites. The microbiota of *B. truncatus* from only one site (i.e. Mbane) harbored lower  
472 alpha-diversity than two other sites (i.e. Saneinte and Lampsar). The low alpha diversity of *B. truncatus*

473 microbiota observed in Mbane comparatively to those of Saneinte is intriguing since these two sites  
474 are geographically distant from only 3.2 km and established along the same shore of the Guiers Lake.  
475 In our study, no obvious technical (e.g. difference in sequencing coverage), or measured biological  
476 factors (e.g. host size, host genetic diversity), can satisfactorily explain this pattern either at an  
477 individual or site scale (data not shown). Ecological differences between these two sites, and in  
478 particular in terms of food resources available for local *B. truncatus* populations, could be one  
479 explanatory factor explaining such difference, although we did not collect this information in the field.  
480 Studies have suggested that, additionally to host physiological traits, dietary diversity (exogenous  
481 microbial diversity) also impact gut microbiota alpha diversity (Reese and Dunn 2018). Fine-scale  
482 geographical studies at the scale of the lake accounting for more detailed ecological factors can be  
483 useful to better understand such result.

#### 484 **1.b. *Bulinus truncatus* microbiota composition in natural populations**

485 We observed that Proteobacteria, Bacteroidota, and Firmicutes were the dominant phyla in  
486 the microbiota of *B. truncatus* across all sampling sites. Among the top 10 bacterial phyla identified in  
487 *B. truncatus*, two were also found in the aquatic bacterial communities: Cyanobacteria and  
488 Planctomycetota. Cyanobacteria was the second most abundant phylum in the water samples, while  
489 it ranked third among the top 10 phyla and seems less abundant in *B. truncatus*. Conversely  
490 Planctomycetota, which ranked seventh out of the 28 total phyla in the water samples, was  
491 significantly enriched in *B. truncatus* snails. This strengthens the idea that the snails may act as a  
492 narrower niche than aquatic environment selecting only some bacteria from the environment (Suzzi  
493 *et al.* 2023; Herlemann *et al.* 2024).

494 At the family level, the 'common' core microbiota of *B. truncatus* included Chitinophagaceae,  
495 Weekselaceae, and Flavobacteriaceae that all belong to the Bacteroidota phylum, as well as  
496 Comamonadaceae, Moraxellaceae, and Pseudomonadaceae belonging to the Proteobacteria phylum.  
497 Interestingly Proteobacteria, Bacteroidota and Firmicutes were previously detected as major bacterial  
498 taxa in a pool of *B. truncatus* initially originating from Spain and maintained in the laboratory for  
499 several generations (Huot *et al.* 2020). In particular, Flavobacteriaceae, Comamonadaceae and  
500 Pseudomonadaceae are families that are shared by this laboratory reared Spanish *B. truncatus* strain  
501 and all natural *B. truncatus* populations from northern Senegal studied here. These bacteria are likely  
502 to display important functions for the development of *B. truncatus*. In this regard, members of the  
503 Comamonadaceae family are known for their denitrifying activities and their action in the sulfur cycle  
504 (Khan *et al.* 2002; Wu *et al.* 2018). Pseudomonadaceae family, apart from containing pathogen bacteria  
505 for humans, can exhibit antimicrobial activity, help in nitrogen fixation and produce antifungal  
506 compounds (Fukuda *et al.* 2021; Saati-Santamaría *et al.* 2021). Pseudomonadaceae are also able to

507 degrade all sort of complex molecular compounds including aromatic nucleus leading to break down  
508 environmental pollutants such as phthalates, hydroxybenzoates, and toluene (Parales and Harwood  
509 1993; Przybylińska and Wyszowski 2016).

510         Conversely, some bacterial families were found only in this study and were absent or present  
511 at low abundance in the previously studied *B. truncatus* laboratory strain. In particular, the  
512 Chitinophagaceae family, as their name implies, can hydrolyze chitin from the environment and are  
513 found primarily in soils and aquatic sediments (Lim *et al.* 2009; Madhaiyan *et al.* 2015). Under natural  
514 conditions, *B. truncatus* feed on recalcitrant food resources including detritus, decaying macrophytes,  
515 diatoms and filamentous algae, and thus potentially require a highly diverse microbial communities  
516 for digestion (Madsen 1992; Van Horn *et al.* 2012). In this regard, a reduction in the complexity of the  
517 food resource available for the laboratory population (i.e. organic washed lettuce) may also explain a  
518 relaxation of selection pressure and a loss of some bacteria taxa involved in snails' digestion.  
519 Alternatively, although nonexclusively, the differences observed in the core microbiota between our  
520 *B. truncatus* field populations from Senegal and that of the Spanish laboratory strain may be a result  
521 of co-evolutionary processes driven in part by the evolutionary history of *B. truncatus* populations; the  
522 population established in Spain having probably diverged from the populations of Senegal for a long  
523 time. This latest hypothesis implies that the genetic structure of *B. truncatus* populations resulting  
524 from their evolutionary history influences the bacterial communities associated with these  
525 populations. This is precisely what our results show at the scale of northern Senegal, as we discuss in  
526 the next section.

527

## 528 **2. Structuring factors of the microbiota of natural *B. truncatus* snails**

529         Our result show that *B. truncatus* microbiota are well structured geographically although they  
530 display a less pronounced 'distance–decay' pattern than that of aquatic bacterial communities.  
531 Communities of free-living bacteria generally display such a pattern, although the robustness of the  
532 relationship may vary depending on the ecological context and the geographical scale (Clark *et al.*  
533 2021). While the factors that lead to this 'distance–decay' pattern are still debated, it is accepted that  
534 it results at least from a limitation in the dispersion of bacterial communities and the heterogeneity of  
535 the environment (Green and Bohannan 2006; Sadeghi *et al.* 2021). We believe that, the strength of  
536 the 'distance–decay' pattern observed among aquatic bacterial communities in our study result from  
537 the fact that ecological heterogeneity and geographical distances between sites are tightly linked, with  
538 closer sites being associated to the same ecological system (e.g. lake de Guiers, River 'Le Doué'). The  
539 less pronounced 'distance–decay' pattern of *B. truncatus* microbiota observed here is in line with the  
540 recent study from Herlemann *et al.* conducted on the freshwater snail *A. balthica* in Northern Europe



541 at a similar geographical scale (Herlemann *et al.* 2024). As the authors suggest in their model, we can  
542 here hypothesize that *B. truncatus* create more uniform environments for their associated bacteria,  
543 compared to the heterogeneity of the aquatic environments where *B. truncatus* and aquatic bacterial  
544 communities are found. Another non-exclusive hypothesis would be that the structure of *B. truncatus*  
545 microbiota is determined by other predominant factors, and particularly some evolutionary factors  
546 specific to *B. truncatus* populations. In this respect, the MRM analysis shows that the genetic distance  
547 between *B. truncatus* hosts explains the beta diversity of their associated microbiota better (albeit  
548 slightly) than the geographical distance. This suggests that the evolutionary history of *B. truncatus*  
549 populations, which is strongly influenced by migration and drift as shown by the strong isolation by  
550 distance (IBD) pattern observed, is a key element in the structuring of *B. truncatus* microbiota. Thus,  
551 while phyllosymbiosis between snail species and their microbiota is already documented at the inter-  
552 specific level (Huot *et al.* 2020; Schols *et al.* 2023), we here argue that phyllosymbiosis could also occur  
553 at the intra-specific level.

554         The strong IBD pattern observed among *B. truncatus* populations was expected and fit well the  
555 results from previous genetic studies conducted on this species in different countries of Africa  
556 including Senegal. Early studies based on allozymes or a limited number of microsatellites have shown  
557 that self-fertilization is frequent in natural populations of *B. truncatus* (Njiokou *et al.* 1993; Jarne *et al.*  
558 1994). On the other hand, populations of *B. truncatus* are often subjected to fluctuating episodes of  
559 extinction and recolonization that follow the alternation of wet and dry seasons that greatly shape the  
560 (sometimes temporary) habitats of this species. Episodes of extinction and recolonization by a small  
561 number of self-fertilizing individuals generally explain the low intra-site genetic diversity and genetic  
562 differentiation between populations, even if they are geographically close (Njiokou *et al.* 1993; Maes  
563 *et al.* 2022). Based on the 31 microsatellite markers newly developed in this study, we found no clear  
564 evidence of self-fertilization and the low values of genetic differentiation observed between  
565 geographically close populations suggest a non-negligible gene flow between these populations that  
566 tends to fade as the geographical distance between populations increases. We explain these results by  
567 the fact that all sites included in this study are permanent habitats with water present all along the  
568 year. This limits (but does not fully exclude) temporary population extinctions and allows populations  
569 to develop demographically, thus reducing the effect of genetic drift and potentially encouraging cross-  
570 fertilization. Moreover, water flow in the river Le Doué certainly promotes gene flow between  
571 geographically close populations and explains the little genetic differentiation observed between these  
572 populations. In this respect, it is interesting to note that the genetic markers used in this study reveal  
573 higher genetic differentiation between the population established in the Guia canal, which is fed and  
574 connected to the Le Doué River, compared to populations established along the Le Doué River at equal

575 or greater geographical distances. Similarly, the sampling sites on the banks of Lake Guiers are only 3.2  
576 km apart and it is likely that the sole effect of wind-generated surface currents facilitates gene flow  
577 between these two populations. Conversely, high values of genetic differentiation were observed  
578 between geographically distant populations and are in line with the genetic patterns recently  
579 documented on *B. truncatus* populations in the same region based on 10750 SNPs (Maes *et al.* 2022).

580

581 Combined together, the genetic structure of *B. truncatus* populations and the geographical  
582 distance between populations do not fully explain the structure of the microbiota of *B. truncatus* and  
583 other factors, probably ecological, which we missed in this study, are certainly at work in shaping the  
584 microbiota of *B. truncatus*. For example, low genetic differentiation and little geographical distance  
585 clearly fail to explain the huge difference observed between the microbiota of *B. truncatus* established  
586 at the two sites along Lake Guiers (Saneinte and Mbane). Among the other potential structuring  
587 ecological factors that we investigated, the structure of aquatic bacterial communities does not predict  
588 the structure of *B. truncatus* microbiota when accounting for the genetic structure of *B. truncatus*  
589 populations and the geographical distances between sites. Thus, environmental bacterial communities  
590 do not directly influence *B. truncatus* microbiota. This was somewhat unexpected for at least two  
591 reasons. First, we might expect that some environmental bacteria are transferred to *B. truncatus*  
592 through several processes including ingestion during snail feeding or colonization of external snail  
593 tissues. Indeed, most freshwater snails are detritivorous or feed upon biofilms hence providing  
594 opportunity for different environmental bacteria to colonize and eventually establish within the  
595 gastrointestinal tract of snails established in different habitats (Madsen 1992; Van Horn *et al.* 2012;  
596 Kivistik *et al.* 2023). The lack of influence from aquatic bacterial communities on *B. truncatus*  
597 microbiota further supports the idea that *B. truncatus* acts as a filter for specific bacteria. This filtering  
598 effect, that might partly depend on the genetic background of *B. truncatus* lineages, limits the  
599 differences between the environmental bacterial communities that occasionally associate with *B.*  
600 *truncatus*. It is worth stressing that we here focused on *B. truncatus* whole microbiota and not  
601 specifically on the gut microbiota. By focusing only on these tissues, it is likely that we would have  
602 found a better congruence between environmental bacterial communities and *B. truncatus* gut  
603 microbiota. In fact, we did find some bacteria that are present both in the environment and in *B.*  
604 *truncatus* such as Chitinophagaceae as previously mentioned.

605 Finally, as part of the biotic environment, the presence of developing trematodes inside the  
606 snails does not seem to influence the snail microbiota although some infected *B. truncatus* displayed  
607 bacterial communities departing from those of non-infected sympatric congeners. In this respect,  
608 Portet *et al.* (2021) previously empirically showed that the experimental infection of laboratory-reared  
609 *Biomphalaria* snails by *Schistosoma mansoni* induced drastic modifications of snail microbiota soon

610 after experimental infection, but that the microbiota gradually returns to its initial state a few days or  
611 weeks after. Consequently, it is likely that most *B. truncatus* diagnosed as infected with trematodes in  
612 our study had been infected for a sufficiently long time, allowing their microbiota to recover.  
613 Conversely, the rare infected *B. truncatus* displaying microbiota distinct from non-infected sympatric  
614 conspecifics could have been infected shortly before sampling. Alternatively, these individuals with  
615 different microbiota may have been exposed to a trematode but were incompatible from an immune  
616 perspective, potentially triggering an immune response that led to a temporary dysbiosis. Lastly,  
617 considering that we analyzed whole snail microbiota, we potentially sequenced the microbiota of the  
618 *B. truncatus* snail and its potential infecting parasites so we could find different microbial signatures  
619 depending on the infecting trematode species as observed by Salloum and collaborators in the mud  
620 snail *Zeacumantus subacarinatus* infected by four different trematode species (Salloum et al. 2023).  
621 However, due to the low observed prevalence of infection in our study, we might have insufficient  
622 results to robustly explore the link between the infection status and the microbiota composition of *B.*  
623 *truncatus* which means that we may have missed such signals.

624

### 625 **3. Conclusions**

626 Our study highlights a well-structured geographical pattern in the microbiota of natural  
627 populations of *B. truncatus* that is best explained by the genetic structure of *B. truncatus* populations.  
628 This suggests an important role of the evolutionary trajectory of *B. truncatus* populations in shaping  
629 their microbiota. This calls for further studies to identify the potentially genomic determinants that  
630 influence the composition of hosts microbiota. Importantly however, some strong differences in the  
631 composition of *B. truncatus* microbiota from genetically and geographically close populations remain  
632 unexplained and suggest that some unexplored ecological factors or hosts' physiological traits (e.g. age  
633 or fitness) could also influence the microbiota of this species. Among these ecological factors we found  
634 that neither the bacterial communities from the surrounding aquatic environment nor the presence of  
635 trematode developing within hosts influence the overall structure of hosts microbiota. The temporal  
636 monitoring of the microbiota associated with hosts populations and that of the environment in which  
637 the hosts are established could provide a valuable approach to identify potential environmental factors  
638 at play in shaping host microbiota. In the case of *B. truncatus*, populations from habitats with high  
639 temporal environmental fluctuations such as temporary ponds should be targeted. More generally,  
640 such temporal monitoring studies in more temperate regions with important seasonal environmental  
641 changes would help better understanding the potential environmental factors influencing the  
642 microbiota of freshwater gastropods. Finally, further research on the interactions between genetic and  
643 environmental variables would be necessary to specifically assess the plasticity of hosts microbiota

644 hence contributing to a better understanding of the relationship between the environment, hosts and  
645 their microbiota at the intra-specific level.

646

#### 647 **4. Acknowledgements**

648 We warmly thank the Bio-Environment platform (UPVD, Région Occitanie, CPER 2007-2013 Technoviv,  
649 CPER 2015-2020 Technoviv2) and Jean-François Allienne, Margot Doberva and Michèle Laudie for  
650 support in library preparation and sequencing. Microsatellites development and genotyping were  
651 performed at the PGTB (doi:10.15454/1.5572396583599417E12) with the help of Zoé Compagnie,  
652 Adline Delcamp and Erwan Guichoux. This study was supported in part by the European and  
653 Developing Countries Clinical Trials Partnership (EDCTP2) program (TMA2018CDF-2370), supported by  
654 the European Union. It was funded by the French Agency for Food, Environmental and Occupational  
655 Health & Safety (PNRES 2019/1/059 Molrisk) and the Occitanie Region (Schistodiag program). This  
656 study was carried out with the support of LabEx CeMEB, an ANR 'Investissements d'avenir' program  
657 (ANR-10-LABX-04-01), and within the framework of the 'Laboratoire d'Excellence (LABEX)' TULIP (ANR-  
658 10LABX-41). It was also supported by the MICROVECT Project (défi clé RIVOC Occitanie Region,  
659 University of Montpellier).

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## 835 **6. Data Accessibility and Benefit-Sharing**

### 836 **6.1. Data Accessibility Statement**

837 All sequence data generated have been submitted to the Sequence Read Archive of NCBI.

### 838 **6.2. Benefit-Sharing Statement**

839 This project obtained from the Nagoya office in Senegal, an exemption from authorization of access  
840 and use of genetic resources (number: 001339 of November 15, 2021, reference: V/L du 28 octobre  
841 2021) by the Competent National Authority (Directorate of National Parks of Senegal).

## 842 **7. Authors Contributions**

843 O.R., P.D., B.G., M.J. were responsible for the research design. O.R., P.D., B.S., J.B. conducted the field  
844 work. M.J. and P.D. conducted the lab work. O.L. and E.C. developed the microsatellite dataset. M.J.,  
845 P.D., O.R., E.T., and T.L. contributed to the data analysis. M.J., O.R. and P.D. wrote the initial draft of  
846 the manuscript and all authors contributed to revisions. O.R. and B.G. supervised the project.

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