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Investigation of *Capitella* spp. symbionts in the context of varying anthropic pressures: First occurrence of a transient advantageous epibiosis with the giant bacteria *Thiomargarita* sp. to survive seasonal increases of sulfides in sediments

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4 **Investigation of *Capitella* spp. symbionts in the context of varying anthropic pressures:**
5 **First occurrence of a transient advantageous epibiosis with the giant bacteria**
6 ***Thiomargarita* sp. to survive seasonal increases of sulfides in sediments**
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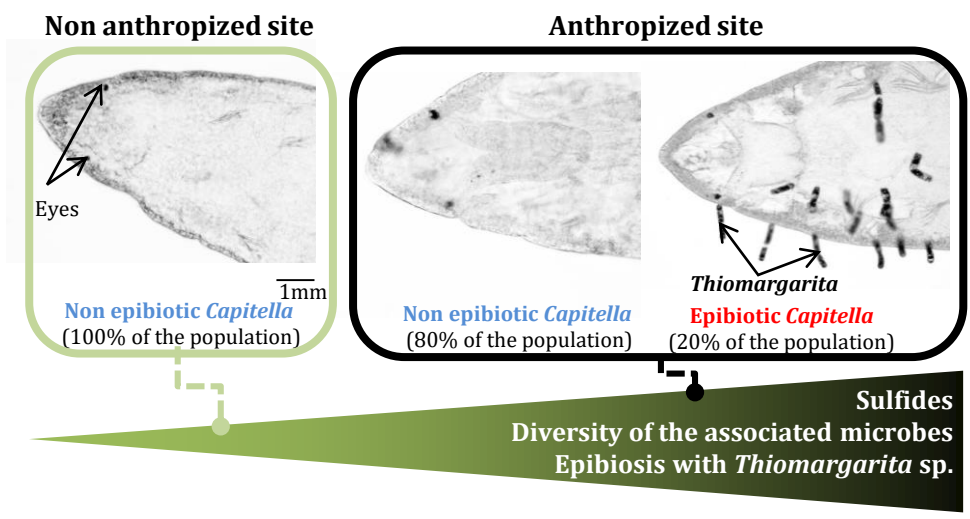
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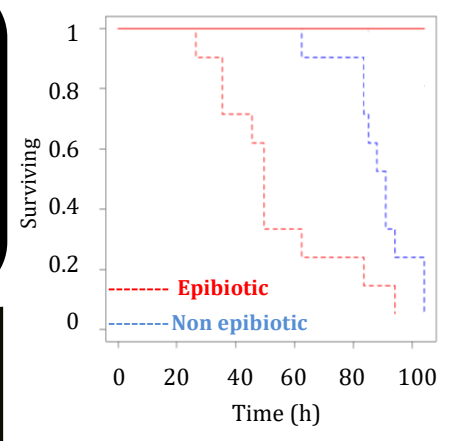
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Worm's survival to sulfides



Highlights

- Local sediment geochemistry and diversity of symbionts associated to a common coastal worm are compared between specimens from an anthropized *versus* a non-anthropized site
- A peculiar development of a transient epibiosis with the giant sulfur oxidizing bacteria *Thiomargarita* spp was observed in *Capitella* spp. exposed to high level of sulfides
- The transient epibiosis allows the worms to survive toxic levels of sulfides during the summer
- This is the first evidence of an adaptive advantage of a facultative ectosymbiosis to face changing habitats

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1 **Abstract**

2 *Capitella* spp. is considered as an important ecological indicator of eutrophication due to
3 its high densities in organic-rich, reduced, and sometimes polluted coastal ecosystems. We
4 investigated whether such ability to cope with adverse ecological contexts might be a
5 response to the microorganisms these worms are associated with. In populations from the
6 French Atlantic coast (Roscoff, Brittany), we observed an epibiotic association covering the
7 tegument of 20-30% specimens from an anthropized site while individuals from a
8 reference, non-anthropized site were devoid of any visible epibionts. Using RNAseq,
9 molecular and microscopic analyses, we described and compared the microbial
10 communities associated with the epibiotic *versus* the non-epibiotic specimens at both
11 locations. Interestingly, data showed that the epibiosis is characterized by sulfur-oxidizing
12 bacteria amongst which the giant bacterium *Thiomargarita* sp., to date only described in
13 deep sea habitats. Survey of *Capitella* combined with the geochemical analysis of their
14 sediment revealed that epibiotic specimens are always found in muds with the highest
15 concentration of sulfides, mostly during the summer. Concomitantly, tolerance tests
16 demonstrated that the acquisition of epibionts increased survival against toxic level of
17 sulfides. Overall, the present data highlight for the first time a peculiar plastic adaptation
18 to seasonal variations of the habitat based on a transient epibiosis allowing a coastal
19 species to survive temporary harsher conditions.

20 **INTRODUCTION**

21 The past decades have seen an increasing number of studies with the aim of characterizing
22 the biology of bacterial symbionts in a wide variety of invertebrates and plants, as well as
23 their role on community structure and ecosystem functioning (Brooks et al., 2017; Carrier
24 and Reitzel, 2017; Ferrari and Vavre, 2011; Gilbert et al., 2015; Moran and Wernegreen,
25 2000). It is now widely admitted that symbiotic associations can be responsible for some

26 of the most noticeable changes in phenotypes, as they constitute a low-cost source of
27 evolutionary innovation for their host (Margulis, 1991). The very short generation time of
28 associated microorganisms could allow a faster acclimatization of the host to changing
29 environments than the fixation of favorable alleles in the host genome, and therefore
30 accelerate the acquisition of new phenotypes more adapted to novel ecological conditions.
31 For instance, it is now well established that diagnostic traits of numerous symbiotic species
32 are in fact a response to the microorganisms they are associated with (McFall-Ngai, 2008;
33 McFall-Ngai et al., 2013). Symbioses have been shown to affect adaptive traits, from trophic
34 niche (Kohl et al., 2014) to temperature dependence (Morsy et al., 2010), salinity tolerance
35 (Nougué et al., 2015), resistance to oxidative stress (Richier et al., 2005), or resistance
36 against pathogens (Kaltenpoth and Engl, 2014; Tasiemski et al., 2015) that may have an
37 early effect during organism development (Gasnier-Fauchet et al., 1986; Gilbert et al.,
38 2015). Consequently, understanding the adaptation of marine species to changing
39 environments requires the further exploration of how the environment impacts the host-
40 symbiont associations and their evolution for either endo- or ecto-symbioses (epibiosis).
41 Until now, the symbiotic microflora of marine animals was often considered as a random
42 consortium (McFall-Ngai, 2008). However, multiple lines of evidence show that this
43 microflora corresponds in fact to a highly specialized microbial community forming a
44 specific and stable symbiosis with its host, with dedicated roles. The discovery of the
45 association of chemoautotrophic bacteria with the deep-sea hydrothermal vent tube worm,
46 *Riftia pachyptila* revolutionized our view about the morphological and physiological impact
47 of bacteria on the host (Bright and Lallier, 2010; Cavanaugh et al., 1981; Felbeck, 1981).
48 Chemoautotrophic bacteria use sulfur compounds, particularly hydrogen sulfide, a
49 chemical highly toxic to most known organisms, to produce organic material through the
50 process of chemosynthesis. Interestingly, *R. pachyptila* develops from a non-symbiotic

51 trochophore larva, which enters juvenile development, becoming sessile, and subsequently
52 acquiring symbiotic bacteria through skin infection. After chemoautotrophic bacteria are
53 established in the midgut of the juveniles, it undergoes substantial remodelling and
54 enlargement to become the trophosome, while the remainder of the digestive tract fully
55 disappears in adults (Stewart and Cavanaugh, 2006). Lacking a mouth and a gut and being
56 unable to obtain organic compounds by diffusion, adults gain the latter *via* sulfur oxidation-
57 CO₂ fixation driven by the endosymbionts confined into peculiar cells (namely
58 bacteriocytes) of the trophosome. The tubeworm depends completely on the
59 chemoautotrophic bacteria for the byproducts of their carbon fixation cycles needed for its
60 growth. Reciprocally, endosymbionts rely on *R. pachyptila* for the assimilation of nutrients
61 needed for the array of metabolic reactions they employ (Bright and Lallier, 2010).
62 Soon after this first description of chemoautotrophic symbiosis, additional thiotrophic
63 symbioses were described at oxic-anoxic interfaces of more accessible coastal shallow-
64 waters also recognized as chemosynthetic based ecosystems (Dubilier et al., 2008; Petersen
65 et al., 2011; Stewart et al., 2005). Gutless oligochaetes' (annelids) and stilbonematids'
66 (nematodes) symbioses are among them and constitute a remarkably well-described and
67 interesting mode of nutrition (Bulgheresi, 2016; Dubilier et al., 2006; Polz et al., 1992).
68 More recently, the nematode *Metoncholaimus albidus*, reported in the Roscoff Harbor
69 (Brittany, France), has also been shown to be associated with distinct microbial
70 communities known to be involved in sulfur metabolism (Bellec et al., 2019).
71 Marine worms belonging to the genus *Capitella* represent the most common component
72 species of benthic communities in organically enriched ecosystems throughout the world
73 (Kitamori, 1975; Pearson and Rosenberg, 1978; Reish, 1979). This so-called sediment
74 "black zone" - previously considered to be azoic - is characterized by strongly reducing,
75 micro- to anaerobic conditions with high concentrations of reduced sulfur species like

76 dissolved sulfides and polysulfides, thiols... and sulfide precipitates such as MeS (where Me
77 can be Fe, Pb, Zn, Cd...), Fe₃S₄ and FeS₂ (Wood, 1992). Differential tolerance to sulfide has
78 been observed between sibling species of *Capitella*, leading to the hypothesis that these
79 ecophysiological differences were genetically fixed and that sulfidic environments could
80 have been the driving force of such species diversification (Gamenick et al., 1998). The
81 tolerance to sulfides in *Capitella* sp1 from North America (subsequently identified as
82 *Capitella teleta* (Blake et al., 2009)) was evaluated through experimental exposure of the
83 annelids to H₂S under laboratory conditions. Sulfide concentrations up to 2mM were
84 considered as a cue for *Capitella* sp1 larval settlement (Cuomo, 1985) whereas those
85 exceeding 10mM were detrimental to their survival (Dubilier et al 1988). The presence of
86 sulfides up to 7mM was also shown to favor the burrowing activity of adults thus
87 stimulating the respiratory activities of the bacteria associated with the mucus-lined
88 burrow of the worm in soft agar microcosm (Wada et al., 2006). This was coupled with an
89 enhanced growth and survival rates of the adults observed in sediments supplied with
90 sulfides for 6 weeks (Tsutsumi et al., 2001). Consequently, *Capitella* species does not seem
91 to favor organically enriched sediment with sulfides but rather prefers the environments
92 that sulfides provide. As mentioned before, hydrogen sulfide can be exploited for the
93 chemosynthesis of organic matter by chemoautotrophic bacteria. *Capitella* species are not
94 gutless worms and an examination of *Capitella* sp. I for the presence of enzymes commonly
95 associated with chemoautotrophic bacteria ~40 years ago has led to the conclusion by the
96 authors that adults were not associated with chemoautotrophic symbionts (Cavanaugh,
97 1983; Cuomo, 1985).

98 The main purpose of this paper was to explore both the microhabitat and microbial
99 diversities associated with the complex of *Capitella* species recently identified as "*Capitella*
100 spp. from the English Channel" (Boidin-Wichlacz et al., Under review) to first report

101 whether such host-symbiont interactions (notably with chemoautotrophic bacteria) exist
102 and to evaluate secondarily whether changing environmental conditions, and especially
103 sulfides can affect these associations and the worm tolerance to this chemical.
104 For this end, *Capitella* specimens collected from two sites with differing levels of anthropic
105 influence and sulfides were compared. Biogeochemical characteristics of the sites were
106 documented, and microbial communities associated with specimens of *Capitella* sp. were
107 assessed using a RNASeq-based approach. Finally, the cost *versus* benefit of the transient
108 association with sulfur-oxidizing ectosymbionts was studied in animals exposed to lethal
109 doses of sulfides.

110

111 **1. MATERIALS AND METHODS**

112 **1.1. Specimen collection**

113 Sediment and *Capitella* specimens were collected together at two different sites: the
114 Roscoff Harbor and Le Laber near Roscoff (Brittany, France). For the « Tolerance tests to
115 experimental exposure to sulfides” worms were only sampled at the Roscoff Harbor.

116 A map with the GPS coordinates is presented in Fig. 1. The sampling dates and locations for
117 each experiment as well as the number of collected worms are detailed in the
118 supplementary data (Table S1). *Capitella* spp. were collected at low tide. At both locations,
119 *Capitella* individuals were abundant, representing the most dominant species in the Roscoff
120 Harbor, and with abundance similar to that of oligochaetes in the nearby site Le Laber. The
121 sediment was sieved on a 500 µm mesh in the field and animals were brought back to the
122 laboratory for sorting under a dissecting microscope.

123 **1.2. Sediments**

124 The methods used to determine the “Total metal concentrations”, the “Carbon and nitrogen
125 contents”, the “AVS, CRS and HCl-extractable metals” and the “Granulometry” of the
126 sediments are provided as supplementary data.

127
128 **Sampling and pretreatments** – Sediments of the two study sites were characterized in
129 terms of trace metals concentrations (total metals and metals extracted with 1M HCl),
130 reduced sulfur species content (AVS: Acid Volatile Sulfides and CRS: Chromium Reducible
131 Sulfur), dissolved sulfides and additional environmental parameters. Sediments were
132 collected using a 5 cm long (for the top 0–5-cm surface layer of sediment) or along cores of
133 35 cm long (for sediment profiles) using Perspex tubes (internal diameter: 7.5 cm). Cores
134 sampled with the Perspex tubes were put into a glove box, previously flushed with nitrogen,
135 and sliced every 1 cm at both sites. Each sediment sample was then stored under nitrogen
136 untreated in a plastic bag at -18°C prior to perform AVS, CRS and metal analyses. A slice of
137 each core was also dried to measure granulometry and total carbon and nitrogen contents.
138 Additional sediment cores were sampled for exposure to DGT (Diffusive Gradients in Thin
139 films) - AgI passive samplers used for dissolved sulfide determination.

140 **Enrichment factor and toxicity index calculation** – The enrichment factor (EF)
141 normalized towards aluminum (Al) has been used to compare the level of metal pollution
142 between our sediment samples. This factor is defined as follows:

$$143 \quad EF = \frac{\frac{[Me]_{sample}}{[Al]_{sample}}}{\frac{[Me]_{reference}}{[Al]_{reference}}}$$

144 Where $[Me]_{sample}$ and $[Me]_{reference}$ are the concentrations of metal (Me: Cd, Co, Cu, Ni, Pb or
145 Zn) in our samples and in the reference material, respectively (Audry et al., 2004; Davide
146 et al., 2003). To avoid using average world values for the reference material that do not

147 reflect the local geology of the area studied, reference geochemical background values from
148 pristine loess deep horizons in the North of France (Boulogne, Gravelines and Authie) has
149 been considered (Sterckeman et al., 2006).

150 The toxicity index (TI) was calculated as the ratio SEM/AVS to predict metal sediment
151 toxicity towards benthic invertebrate species (Ankley et al., 1993). Its relevance has been
152 demonstrated *via* toxicity tests on several benthic organisms (notably the polychaetes
153 *Capitella capitata* and *Neanthes arenaceodentata*), *in natura* or through experimental
154 exposure to contaminants (Lee et al., 2000). For each sample, the TI has been calculated,
155 according to the following relation: $TI = \log ([SEM]/[AVS])$ (Ankley et al., 1993). Previous
156 studies have shown that sediments with $TI > 0$ are toxic for animals whereas sediments
157 with $TI \leq 0$ are not (Hansen et al., 2005)., AVS and SEM data of the 5 first cm of the sediment
158 were used to calculate the TI values for both study sites over a period of time from 28 of
159 July to 8 of December 2015.

160 ***Dissolved Sulfides*** – Dissolved sulfides were measured using DGT-AgI probes (Gao et al.,
161 2009). Briefly, dissolved sulfides were measured from a coloration which turns from white
162 to black when forming Ag_2S with sulfides after diffusing from pore-water through an
163 acetate cellulose filter (0.45 μm pore size) into a polyacrylamide gel containing the AgI
164 precipitate. After a known exposure time of the filter in pore-water samples, the precipitate
165 is scanned using a commercial flatbed scanner and color intensity is then digitized and
166 calibrated to calculate the concentrations initially present (Lourino-Cabana et al., 2014;
167 Teasdale et al., 1999). Calibration of the DGT-AgI probes in standard sulfide solutions were
168 performed using the same conditions.

169 **1.3. Microbial communities associated with the worms**

170 ***worms sampling*** –For the RNAseq, animals collected in 2013, were checked for
171 filamentous epibionts under the microscope and separated into three groups: 1/ non

172 epibiotic animals from the Le Laber 2/ non epibiotic animals from the Roscoff Harbor and
173 3/ epibiotic animals from the Roscoff Harbor (Fig 2B). For each group, 30 individuals were
174 placed in RNA-later. At the time of sampling for transcriptome sequencing, *Capitella*
175 covered by epibionts were only found at the Roscoff Harbor site; no epibiotic individuals
176 were found in Le Laber. For the morphological analyses, five specimens of each group were
177 fixed in glutaraldehyde 2.5% for electron microscopy and five were fixed in
178 paraformaldehyde 4%, for fluorescence *in situ* hybridization in 2013 and in 2014.

179 ***Seasonal survey of associations with Thiomargarita on Capitella spp*** – From March to
180 December 2015, samples were collected at two-week intervals from both Le Laber and the
181 Roscoff Harbor sites (19 sampling events per site). Each individual worm was then
182 preserved in 85% ethanol. Fifty-two individuals were used for the genetic analysis (see
183 below) and the remaining worms were later observed individually under a dissection
184 microscope to check for presence of epibiotic microorganisms and measure the width of
185 the body at the fifth setiger (Pardo et al., 2010). In total, 5900 worms were sampled (with
186 150-160 worms collected at each sampling event at each site). To obtain a better estimation
187 of the association prevalence among the worms, the association (presence/absence) of
188 large epibiotic microorganisms was modeled as a Bernoulli random variable through a
189 generalized linear model (GLM) with binomial error and logit link between the explanatory
190 variables and their effect on the association probability. We built 166 different GLM based
191 on the “complete model”, which incorporated the effects of site (Le Laber vs. Roscoff
192 Harbor), worm size and Julian date (number of days since last change of year). The other
193 165 models were obtained as the sub-models nested within the complete one (*i.e.* models
194 lacking one or more explanatory variables or interactions thereof). The goodness-of-fit of
195 each model and its corrected Akaike Information Criterion (AICc) were computed and
196 models were ranked from best to worst following increasing values of AICc. To obtain a

197 more robust estimation of model predictions, model averaging procedures were used
198 based on the Akaike weight of each model (Burnham et al., 2011). For all these statistical
199 analyses, R (v 3.2.3) was used with package ‘fields’ to make the heatmaps and package
200 ‘MuMIn’ for automated model goodness-of-fit comparisons and model averaging.

201 **1.4. Morphological observations of associated microorganisms**

202 ***Optical microscopy*** - For each sample of Le Laber and the Roscoff Harbor, worms with and
203 without large epibionts were examined alive or fixed (paraformaldehyde 4%) using an
204 optical microscope (Zeiss Axio Imager M2) and a stereomicroscope (Zeiss Stemi 305).

205 ***Electron microscopy of the epibiotic microflora*** - Specimens of the three groups (epibiotic
206 from the Roscoff Harbor and non-epibiotic from the Roscoff Harbor or from Le Laber) fixed
207 in 2.5% glutaraldehyde were dehydrated in a series of ethanol solutions of progressively
208 increasing concentrations (75–100%), critical-point-dried with a Balzers SCD 30
209 (temperature 37°C and pressure 70 kg cm⁻²), mounted on stubs, covered with a layer of 10–
210 20 nm of gold, and observed under the SEM using a JEOL JSM-840A Scanning Electron
211 Microscope at 20 kV accelerating voltage.

212 ***Fluorescence in situ hybridization (FISH) of epibiotic microflora*** - FISH experiments
213 were performed using generalist probes targeting Eubacteria (EUB338),
214 Gammaproteobacteria (GAM42), and the probe NON338 (antisense of EUB338) as a
215 negative control {Amann, 1990 #159}. All hybridizations were conducted using 30%
216 formamide at 46°C for 3 hours, followed by a 15 minutes rinse in appropriate buffer using
217 the protocol described in (Duperron, 2017). FISH hybridizations were performed on whole
218 specimens of *Capitella* fixed in paraformaldehyde 4% to visualize epibionts, as well as on
219 8µm-thick cross sections of specimens that were previously embedded in Steedman Wax
220 as described in (Duperron et al., 2008), using DAPI as a background stain. Hybridized
221 samples were visualized under a BX61 epifluorescence microscope (Olympus, Japan).

222 **1.5. Assessing microorganism's biodiversity associated with *Capitella* by**
223 **RNAseq sequencing**

224 ***RNA extraction and sequencing*** – To assess microorganisms co-occurring with *Capitella*,
225 RNAs from the three groups (see worm sampling) were extracted and sequenced to obtain
226 transcriptomes representative of eukaryotes and prokaryotes associated with the worms.
227 The total RNAs of each group were extracted with the TRI-Reagent solution (Sigma),
228 following the manufacturer's protocol. The RNAs were re-suspended in DEPC-treated
229 water and the quality and quantity were evaluated on a Nanodrop. An Illumina library was
230 prepared for each of the three groups. Each library was sequenced on one lane of HiSeq
231 2000 (100 million clusters, 2x100 bases paired-end). RNAseq sequencing was performed
232 at Genoscreen (Lille, France).

233 ***Assembly and determination of the abundance of assembled contigs*** –The analyses were
234 all carried out in the Galaxy environment and the computing power was provided by the
235 ABiMS platform (Station Biologique de Roscoff, France). The 100-bp paired-ends reads for
236 each group were first filtered for quality with Prinseq-lite, and the pairs of sequences of
237 sufficient quality were established (GetPairs) (Schmieder and Edwards, 2011). The
238 ribosomal sequences were separated from the remaining sequences based on similarity
239 with a rRNA database (riboPicker) (Schmieder et al., 2011). These reads targeted rRNA of
240 both the hosts and the associated microfauna (typically about 25 million paired reads per
241 library) were then assembled with Trinity after normalization to reduce the size of the
242 dataset. This was performed on the three libraries and the resulting contigs were
243 concatenated. Redundancy was removed with CAP3 (Huang and Madan, 1999). The final
244 assembly of rRNA sequences was then used as a reference for quantification of the contigs
245 for each habitat-driven library of worms with RSEM (Li and Dewey, 2011). The results were
246 normalized for the size of the contigs, and the sequencing effort, and are expressed in

247 Fragment Per Kilobase of transcript per Million reads of sequencing (FPKM). The closest
248 sequences in GenBank were identified by Blastn and the identifier recovered for all contigs
249 (Altschul et al., 1997).

250 **1.6. Molecular identification of the large epibionts using 16S rRNA**

251 Clone libraries of the 16SrRNA-encoding gene were built from 4 specimens, 2 displaying
252 and 2 devoid of large epibionts using standard bacterial 16SrRNA primers 8F and 1492R
253 as described in (Duperron et al., 2005). Among the distinct bacterial sequences identified,
254 one found only in specimens displaying epibionts was used to design specific primers
255 targeting these epibionts (Forward 5'- GCTGGTCTGAGAGGACGAAC-3'; Reverse 3'-
256 TTCATGGAGTCGAGTTGCAG-5) with the Primer3 Input software
257 (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3www.cgi>).

258 Large epibionts were also isolated from debris pellets after centrifuging each worm of the
259 2015 collection (at 4000 rpm for 5 min) in an ethanol solution as they immediately detach
260 from *Capitella* in presence of ethanol. Microbial DNA was extracted using the NucleoSpin
261 Tissue kit for bacteria (Macherey-Nagel) according to the manufacturer's instructions, and
262 amplified with a GoTaq® G2 DNA Polymerase (Promega) using *Thiomargarita*-specific
263 primers. Reaction mixture for PCR amplification contained 10 µM of each primer, 10 µM of
264 each (dNTP), 1X Go Taq® Flexi buffer (Promega), and 5U of GoTaq G2 Flexi DNA
265 polymerase (Promega). The final volume was adjusted to 25 µl with water. DNA
266 amplification was performed under the following conditions: (1) An initial denaturation
267 step at 95°C for 3 min without enzyme, followed by (2) a series of 39 cycles of denaturation
268 at 95°C for 45 s, of annealing at 55°C for 45 s, and elongation at 72°C for 1 min with the
269 enzyme, and (3) a final elongation step at 72°C for 7 min. PCR products were purified with
270 the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel) and were then sequenced
271 according to the Sanger method on a 310 ABI prism (Applied Biosystems).

272 Sequence alignments and phylogenetic tree: Sequences were aligned using ClustalX (Larkin
273 et al., 2007). A 16S rRNA dataset was built by collecting sequences available from
274 *Thiomargarita* and related groups. Phylogenetic relationships were estimated based on
275 maximum likelihood using a General Time Reversible (GTR) model and a 5-category
276 discrete Gamma distribution of rates with invariants. Positions with gaps and missing data
277 were not used, resulting in a 1107-bp dataset. Phylogenetic reconstructions were
278 generated using the software MEGA 7 (Kumar et al., 2016).

279 **1.7. Genotyping of epibiotic and non-epibiotic *Capitella* individuals**

280 **DNA extraction and barcoding** – After the epibiont recovery, fifty-two *Capitella* collected
281 during our 2015 temporal survey (see before) in both Le Laber and the Roscoff Harbor
282 were used entirely for DNA extraction using a NucleoSpin Tissue XS (Macherey-Nagel)
283 according to manufacturer's protocol. A 569 bp fragment of the cytochrome oxidase
284 subunit 1 (*Cox-1*) mitochondrial gene was then amplified using *Capitella*-specific primers
285 CO1F and CO1R: Forward 5'- GTACAGAACTTGCGCGTTCCT-3' and Reverse 5'-
286 CCACCACCAGTAGGATCAAA -3'. Amplifications were carried out with a GoTaq® G2 DNA
287 Polymerase (Promega). Reaction mixture for PCR amplification contained 10 µM of each
288 primer, 10 µM of each desoxynucleotide triphosphate (dNTP), 1X Go Taq® Flexi buffer
289 (Promega), and 5U of GoTaq G2 Flexi DNA polymerase (Promega). The final volume was
290 adjusted to 25 µl with sterile water. DNA amplification was performed on a Thermocycler
291 (Eppendorf) with the following conditions: (1) an initial denaturation step at 95°C for 15
292 min without enzyme, followed by (2) a series of 39 cycles of denaturation at 95°C for 30 s,
293 of annealing at 56°C for 30 s, and elongation at 72°C for 1 min with the enzyme, and (3) a
294 final elongation step at 72°C for 5 min. The PCR products were then visualized onto a 1.5%
295 agarose gel with ethidium bromide following electrophoresis at 100 volts for half an hour.
296 PCR products were then purified with nucleofast 96 PCR cleanup kit and then Sanger-

297 sequenced on an ABI 3100 using BigDye (PerkinElmer) terminator chemistry following the
298 manufacturer's protocol. (Applied Biosystems, Foster City, CA).

299 **Sequence analysis** – Chromatograms were checked manually using SeqScape V2.5. The
300 sequence data were aligned manually with BioEdit v.7.2.5. Maximum likelihood tree
301 reconstructions were performed on our subset of barcoded specimens and additional
302 referenced sequences from Genbank using the software Mega7 following the HKY model of
303 substitutions with the pairwise deletion option (Kumar et al., 2016) to check whether
304 *Capitella* spp. populations found at Le Laber and at the Roscoff Harbor represent cryptic
305 species.

306 **1.8. Tolerance tests to experimental exposure to sulfides**

307 Animals collected in July 2020 at the Roscoff Harbor were checked for filamentous
308 epibionts under the microscope and then split into two groups: non-epibiotic *Capitella* (3
309 batches of 10 individuals each) and epibiotic *Capitella* (3 batches of 10 individuals each).
310 Each batch was placed in a petri dish (35mm) containing 2 mL of artificial seawater (Instant
311 Ocean). The 3 “non epibiotic” batches and the 3 “epibiotic” batches were separately
312 exposed to increasing concentrations of sulfides (batch 0 mM, 1 mM and 3 mM of Na₂S 9H₂O
313 (SIGMA) in artificial seawater (Instant Ocean® Sea Salt) for 4 days in a moisture chamber
314 in the dark at 16°C. Mortality was assessed every 3 hours, dead animals were counted and
315 immediately removed. The sulfide concentration was also measured and adjusted when
316 required at the same intervals by using the N, N-dimethyl-p-phenylenediamine
317 colorimetric method (Walkley and Black, 2003).

318 Survivorship data were analyzed through Cox proportional hazard models (Andersen and
319 Gill, 1982), using the ‘coxph’ function within the ‘survival’ package in R programming
320 language (Jackson, 2016). All survival data were analyzed together (same mortality
321 baseline) for the sake of effect comparability. Mortality was assumed to depend on the

322 phenotype of the worms (epibiotic and non-epibiotic) and the concentration of sulfides (0,
323 2 and 3mM). We used robust variance estimation (Horvitz-Thompson estimate) assuming
324 correlation among individuals from the same batch (same experiment x same phenotype x
325 same treatment).

326

327 **2. RESULTS**

328 **3.1. Geochemical characterization of sediments in both sites**

329 **General parameters** - Sediment granulometry was very similar for both sites (Fig. S1): silts
330 (2-63 μm) are the most abundant fraction (40-50%), and their proportions increased
331 toward the sediment-water interface. In the fine fraction, smaller than 63 μm , the amount
332 of Ca, Fe and Al were higher at the Roscoff Harbor than at Le Laber, suggesting that sandy
333 particles, less reactive than clays, carbonates and iron oxides, were more frequent in
334 sediments of Le Laber (Table 1). In the Roscoff Harbor, the layer with the highest
335 proportion of silts extends to a depth of about 3 cm when compared with the site Le Laber
336 (less than 2 cm depth). In this top layer, organic and inorganic carbon contents were greater
337 at the Roscoff Harbor (Table S2). Total nitrogen contents however, are very similar. At
338 sediment depth greater than 3.5 cm, no significant difference between the two sites was
339 noticeable.

340 **Reduced Sulfur Species** - At the time when the worms were collected for NGS sequencing
341 (October 2013), the two locations greatly differed by the amount of sulfide in the upper
342 layer of the sediment (Fig. 2A). At the water-sediment interface, the concentrations of solid
343 reduced sulfur species increased in sediments of Roscoff Harbor but not at Le Laber. At one
344 cm depth, concentrations of reduced sulfur species were 5-6 times higher at the Roscoff
345 Harbor than at Le Laber site. Below the depth of 3 cm, concentrations of AVS (the less stable
346 fraction of solid reduced sulfur to oxidation) and CRS (the less reactive fraction of solid

347 reduced sulfur) ranged from 141 to 978 mgS kg⁻¹ and from 447 and 712 mgS kg⁻¹ for the
348 Roscoff Harbor and Le Laber sites, respectively.

349 A survey of dissolved sulfide concentrations performed two years later (from July to
350 December 2015) monitored with DGT-AgI probes showed that these species were more
351 abundant in a deeper part of the cores (*i.e.* below 4-5 cm depth). Interestingly, sulfide
352 concentrations were on average higher at the Roscoff Harbor (from 8.2 to 11.60 mg L⁻¹)
353 than at Le Laber (from 0.58 to 5.52 mg L⁻¹) (Fig. S2), in a way similar to the AVS and CRS
354 concentrations. More precisely, in the first 3 cm, where the worms live, the inter-site
355 differences were even more marked, with levels ranging between 1.08 and 5.75 mg L⁻¹ for
356 the Roscoff Harbor as opposed to 0 and 0.27 mg L⁻¹ for Le Laber (Fig. S2 and Table 2).

357 **Trace metals** - Total metal concentrations (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) in the
358 sediments clearly indicate that the Roscoff Harbor was enriched in metals when compared
359 to Le Laber site (Table 1 and S2), especially in Cu for which the ratio reaches 6.1 (Roscoff
360 Harbor/Le Laber). Following the normalization of values, it is worth noting that all the EF
361 values in the sediments of Le Laber site were lower than or equal to 1, excepted for Pb (EF
362 = 1.4). These findings clearly suggest that sediments from Le Laber can be used as an
363 unmodified environment regarding metal concentrations. Conversely, the EF values for Cd,
364 Cu, Zn and Pb were much higher (3.5, 2.8, 2 and 1.6) in the Roscoff Harbor, suggesting a low
365 to moderate anthropic contamination of sediments.

366 The toxicity index (TI) was calculated for each sample (averaged over the first 5 cm of
367 sediment) from July to December 2015 (Fig. S3). During this period, all TI values were
368 below zero, indicating that no significant toxicity has been encountered in sediments (*i.e.*
369 most of trace metals are efficiently trapped by sulfides forming AVS). The bioavailability of
370 metals should therefore be extremely limited as sulfides are in excess. The TI values are

371 however consistently greater in the Roscoff Harbor throughout the sampling period
372 because of higher Zn concentrations.

373 **3.2. Micro-organisms associated with *Capitella* differ between localities and** 374 **associated habitats**

375 **Morphological observations** – In October 2013 (RNAseq sampling date), around 20% of
376 worms exhibited an epibiosis with long white hair-like projection at the Roscoff Harbor
377 (Figs. 2B, 3B, D). This association was also observed in some *Capitella* worms from Le Laber
378 during the 2015 temporal survey of the two localities, notably during the summer period.

379 Electron microscopy and FISH hybridizations using the probe EUB338 evidenced dense
380 assemblages of filamentous structures (Figs 3D, 4A), with small bacteria attached to larger
381 and more visible ones strongly anchored in the tegument (Figs. 3B, 4B, C) of the epibiotic
382 *Capitella* worms only (Fig. 4, Figs. 3F, G). The larger epibiotic microorganisms were easily
383 observable under transmission or light microscopy (Fig. 3A-D) displaying a size reaching
384 50 microns from basal to apical ends with refringent cytoplasmic inclusions resembling
385 sulfur granules typical of sulfur-oxidizing bacteria, the lack of a nucleus based on DAPI
386 staining (Fig. 4) and the presence of a large vacuole in the center of the cells (Figs 4D, E).
387 Large *Capitella* epibionts also displayed a larger basal bacterium with an elongated rod
388 shape, atop of which a second, spherical-to-elongate bacterium is budding.

389 A few worms were also parasitized by nematodes (*Trophomera sp.*) living in the coelomic
390 cavity of the worm (Figs.S4D, E), by vorticellid ciliates attached to the tegument (Figs. S4A,
391 B, C) or by gut gregarines (*Ancora saggitata*) (Figs. S4F, G).

392 **Abundance of symbionts lineages based on RNAseq data** – The most abundant
393 assembled sequences regroup three different *Capitella* rRNAs as expected (Boidin-
394 Wichlacz et al., Under review). These sequences were not considered in the following
395 analyses. The other recovered contigs corresponded to organisms associated to *Capitella*,

396 which could be either epibionts (tegument), part of the gut contents, or parasites. In the
397 following analyses, we only considered contigs with abundances greater than 100 FPKM in
398 at least one of the libraries. Some of these may correspond to different fragments of the
399 same organism (*e.g.* fragment of 28S, another fragment of 28S, fragment of 18S, etc.).
400 The sequence assembly followed by quantification allowed us to identify contigs
401 corresponding to associated organisms that are found in all three groups (1/ non epibiotic
402 *Capitella* from Le Laber 2/ non epibiotic *Capitella* from the Roscoff Harbor and 3/ epibiotic
403 *Capitella* from the Roscoff Harbor) but in variable abundances (Table 3), contigs that are
404 more common at Le Laber (Table 4), and contigs that are more abundant in the group
405 corresponding to animals with epibiotic microorganisms (Table 5). *Capitella* from the three
406 compared groups are host to a variety of eukaryotes at intermediate occurrence (Tables 3-
407 5).

408 Among the organisms found in all three groups in variable proportions, there is a total of
409 51 contigs (Table 3). The great majority of these organisms are eukaryotes (86.3%), in
410 particular apicomplexan fish parasites (31.8%) for which *Capitella* could be an
411 intermediary host (*e.g.* *Eimeria leucisci*, *Sphaerospora dicentrarchi*, *Kudoa iwatai*,
412 *Sarcocystis* sp). The contig that ranks second in Table 3 corresponds to the known parasitic
413 gut gregarine *Ancora sagittata* (Apicomplexa, Ancoridae) (Simdyanov et al., 2017)
414 specifically associated to *Capitella* (Fig. 4). Other abundant types of organisms are ciliates
415 (*e.g.* *Vorticella* sp.), nematodes, and annelids that could be part of the gut contents
416 (*Paramphinome jeffreysi*, *Tubificoides brownie*; Fig. 3). Bacteria occupy lower ranks in this
417 list (ranks 28, 31, 34, 35, 44, 49, and 51), which could reflect their lesser abundance and
418 also the fact they are single-celled (as opposed to most eukaryotes mentioned earlier). The
419 15 top-ranking contigs are usually more abundant in the epibiotic animals from the Roscoff
420 Harbor, with the exception of ranks 1 (a polychaete, possibly from the gut contents), 6 and

421 11 (a nematode) that are more abundant in the animals from the control site. The animals
422 from the Roscoff Harbor that do not exhibit an epibiosis tend to have low or very low
423 abundances of these contigs.

424 The animals from Le Laber site exhibited a series of taxa corresponding to the contigs that
425 are found in very low abundances in the Roscoff Harbor (Table 4). Six of these eight contigs
426 correspond to apicomplexan parasites, the two remaining ones correspond to a bivalve
427 (likely found in the gut contents), and *Corynebacterium*.

428 The contigs that are found in much greater abundance in epibiotic animals (Table 5)
429 differed greatly from the organisms identified in Tables 3 and 4. The *Capitella* specimens
430 from the two other groups (non-epibiotic from the Roscoff Harbor and le Laber) have very
431 low abundances of these contigs (FPKM \leq 20). 31 out of the 38 contigs (81.6%) correspond
432 to bacteria, mostly within the Gammaproteobacteria. Eight of these bacterial contigs (21%)
433 correspond to sulfur-oxidizing bacteria (*Thiomargarita*, the most abundant, and
434 *Thioalkalivibrio*). Six of the bacterial contigs (15.8%) correspond to mollicutes, a group
435 usually found in the guts of invertebrates.

436 **3.3. Phylogenetic affiliation of the large bacterial epibiont to the genus** 437 ***Thiomargarita***

438 A near full length 16S rRNA-encoding sequence (6 reads) affiliated with *Thiomargarita* was
439 identified in clone libraries from the two specimens displaying the large epibiont (Table
440 S5). The same sequence was successfully amplified from isolated large epibionts using
441 *Thiomargarita*-specific primers, supporting that this sequence actually corresponds to the
442 large epibiont morphotype.

443 Analysis performed on the near full-length *Thiomargarita* sequences indicate a single 16S
444 rRNA phylotype that shared 98 % of sequence identity and clustered in a 100% bootstrap-
445 supported clade with sequences of *Candidatus* 'Thiomargarita nelsonii' recovered from the

446 Costa Rica margin and the Namibian upwelling zone. This clade is distinct from that
447 containing *Candidatus* 'T. namibiensis' (Salman et al., 2011) (Fig. 5). The sequence was
448 registered in GenBank (accession number MZ053470).

449

450 **3.4. Prevalence of the epibiosis with *Thiomargarita* according to the season, and the** 451 **size and gender of *Capitella*' worms**

452 Observed prevalence of *Thiomargarita* fluctuated between zero and 0.44 among sampling
453 dates (average over the year: 0.10), with 95% of observations between zero and 0.31 and
454 a median prevalence of 0.08. Worm size as estimated from the width at the fifth setiger
455 varied between 0.19 and 1.56 mm (average: 0.54 mm) with a slightly fluctuating average
456 value (between 0.43 and 0.68 mm), without any clear temporal trend. The numbers of
457 males, females and undetermined individuals also do not vary much between sampling
458 dates (Fig. S7). A statistical analysis of time-series was performed using the association
459 occurrences as a quantitative variable and the sampling date, size and gender of the worm
460 as explanatory variables. Overall, the probability of association with *Thiomargarita*
461 increases in summer and increases with the worm's size (Fig. 6). Independently of worm's
462 size, this probability is also higher for males and undetermined individuals than for females
463 (Figs. S5 and S6). As many models have comparable AICc and Akaike values (Table S6),
464 model predictions have been explored using the Akaike-weighted average of all tested
465 models (Figs. 6, S5 and S6). The analysis of evidence ratios (ratio of Akaike weights of
466 models incorporating or not the focal variable) of all explanatory variables (Table S7)
467 indicates that all variables have *likely* effects, except 'site' (*implausible* effect), and
468 'sex:date', 'sex:date²' and 'sex:size:date²' interactions (only *plausible* effects) using the
469 vocabulary of (Massol et al., 2007).

470

471 **3.5. Prevalence of the epibiosis with *Thiomargarita* according to *Capitella***
472 **genotypes**

473 As we know that *Capitella* spp. from the Roscoff Harbor and Laber represent a complex of
474 three cryptic species (Boidin-Wichlacz et al., Under review), series of individuals with and
475 without epibionts from Le Laber and the Roscoff Harbor were barcoded using the
476 mitochondrial *Cox-1* gene to test whether the epibiotic phenotype was species-specific. The
477 obtained phylogenetic tree (Fig. 7) confirmed the co-occurrence of the three different
478 mitochondrial lineages (C-Channel1, C-Channel2 and C-Atlantic) in our set of epibiotic and
479 non-epibiotic worms. The two most closely related species (C-Channel1 and C-Atlantic: see
480 (Boidin-Wichlacz et al., Under review)) dominate the assemblage and correspond to about
481 90% of the sampling. The epibiosis with the *Thiomargarita*-like epsilon proteobacteria was
482 checked and is present in all of the mitochondrial lineages examined, including the rarer C-
483 Channel2 one.

484

485 **3.5. Sulfide tolerance of non-epibiotic versus epibiotic *Capitella* spp.**

486 A tolerance assay was performed on adult worms from the Roscoff Harbor presenting the
487 epibiotic and non-epibiotic phenotypes, exposed to 0,1 and 3 mM of sulfides. As shown in
488 figure 8, both phenotypes survive to a 3 mM exposure for 1 day (23h30). After this delay,
489 non-epibiotic worms (NE) start immediately to die reaching a 50% mortality after 48h. On
490 the contrary, epibiotic worms (E) first die after an additional 36h delay (first death
491 observed at 58h30) and reached the 50% mortality following a 88h post exposure to 3 mM.
492 In both cases, NE and E all die following a post exposure to 3 mM of 88h and 91h,
493 respectively while non-exposed individuals (0 mM) remain alive until the end of the
494 experiment (104h). A dose-dependent effect was observable, with a shift of the 1 mM
495 mortality curve in NE when compared to the 3 mM curve showing a better survival of this

496 later group to a 1mM than to a 3 mM exposure. No mortality was observed in E exposed to
497 1 mM during the allotted time.

498

499 4. DISCUSSION

500 **The appearance of animals exhibiting an epibiosis is concomitant with a higher level** 501 **of sulfides**

502 *Capitella* worms from the English Channel, which also represent three distinct
503 mitochondrial lineages (Boidin-Wichlacz et al., Under review) are opportunistic species
504 that occupy the top 5 cm of sediment of estuaries and polluted harbors: a black zone
505 (named thiobiome) rich in organic matter especially in the muddy sediments. The surveyed
506 sites are enriched in silts, with a high concentration of organic carbon in the Roscoff Harbor.
507 Concentrations, availability and lability of metals estimated through SEM were greater in
508 the Roscoff Harbor than in the Laber site (excepted for Cr) without reaching levels of
509 contamination as high as those reported in industrialized harbor of the Northern France
510 (e.g. Boulogne Harbor (Table S4)(Cuvillier-Hot et al., 2018) (Fig. S2)). Although
511 concentrations of ETM slightly varied during the monitoring period, the sediments from
512 both sites never reached the threshold of the toxicity index (calculated at a macroscopic
513 scale from about 1 g of sediments) classically used to investigate polluted environments.
514 By contrast, the two sites colonized by the worms strongly exhibited spatial and/or
515 temporal differences in AVS concentrations reaching highly toxic levels for most organisms
516 including other *Capitella* species from different locations (higher than 10mM) (Dubilier,
517 1988). This could be explained by differences in the hydrologic conditions and the
518 anthropogenic contamination between the two sites over the year. The seasonal survey
519 shows that sulfide production takes place throughout the year in the Roscoff Harbor while
520 it mostly occurs in the summer period at Le Laber. In the Harbor sediments, the important

521 input of organic matter linked to anthropogenic activities and anthropization processes
522 results in the production of high quantities of AVS through the bioreduction of sulfates by
523 the Sulfate-Reducing Bacteria (SRB) (<https://doi.org/10.1016/j.scitotenv.2018.08.278> ;
524 [https://doi.org/10.1016/S0967-0637\(02\)00092-4](https://doi.org/10.1016/S0967-0637(02)00092-4)). The confinement of the Roscoff
525 Harbor added to the accumulation of cadavers of crabs due to fishing offloading activities
526 in this zone, promotes green algal proliferation and a high retention of organic matter (with
527 enrichments in TOC and nitrogen contents), and, as a consequence, a greater production of
528 sulfide due to microbial degradation over the year when compared with Le Laber. By
529 contrast, although not affected by off falls, the site of the Laber is subjected to a short and
530 local eutrophication due a river input that favors intense proliferation of benthic algae at
531 the surface of the sediment in this area during the summer period. By being open to the
532 ocean, tidal currents renew twice a day the oxygenation of the water sediment interface of
533 the Le Laber site, promoting the quick reoxidation of AVS
534 (<https://doi.org/10.1016/j.oceano.2018.03.003> ; doi 10.1007/s10498-005-4574-2).
535 These differential sediment compositions qualitatively and quantitatively are likely to
536 change the community structure of micro- and macroorganisms co-inhabiting with
537 *Capitella*. Concomitantly with these geochemistry differences over the year and space, we
538 observed two distinct phenotypes of *Capitella* worms from the English Channel, which are
539 co-occurring independently between at least three genetic lineages of the worm: one
540 characterized by a tegument covered by a consortium of large filamentous bacteria and
541 another one with an epidermis perfectly clean of any microorganisms as checked by
542 electron microscopy and confirmed by PCR and RNASeq. Epibiosis with the large
543 filamentous bacteria were only observed in sediments where the sulfide concentrations
544 reach levels known to be toxic for other *Capitella* species (Cuomo, 1985). Under these
545 conditions, the prevalence of the epibiotic association is around 20-30% and mostly affects

546 larger individuals. Trace metals do not seem to affect the epibiosis, since during our survey
547 over the year 2015, the appearance of animals exhibiting an epibiosis increased
548 concomitantly with the level of sulfides in the site Le Laber.

549

550 **Distinct prokaryotic and eukaryotic associations with the host phenotypes**

551 We assessed the diversity of microorganisms associated with the worm using a RNAseq
552 approach on animals with and without epibionts in the two distinct nearby habitats. First
553 assignments of contig sequences shown that these small worms are associated with a wide
554 variety of prokaryotes and eukaryotes. The composition of the associated communities
555 clearly varies according to the environmental setting. All animals used for the RNAseq
556 study were collected at the same time of the year. Although in the three groups (*i.e.* Le Laber
557 worms without epibionts, Roscoff Harbor worms with and without epibionts), the
558 apicomplexan fish parasites are very common, bacterial associates and vorticellid ciliates
559 were quite distinct. We did not observe any lethal effects of ciliates on *Capitella* maintained
560 in the laboratory (unpublished data) by contrast to the enhanced mortality reported for
561 freshwater leeches covered by vorticellid ciliates (Gouda, 2006).

562 Even though *Capitella* with and without epibionts were found in the same sediment sample
563 at the Roscoff Harbor, associated bacterial communities from epibiotic animals were quite
564 distinct from non-epibiotic *Capitella*. Assuming the animals were exposed to the same
565 environmental conditions in the Roscoff Harbor, this observation suggests that the two
566 groups are characterized either by physiological or genetic differences. As previously
567 shown, the barcoding effort revealed that up to three lineages are present in Roscoff, all of
568 which can be the host to the large epibiotic filamentous bacteria. As a consequence,
569 intraspecific genetic differences do not explain the presence of epibiosis and the
570 polymorphic physiological response of the worms seems to represent the best explanation.

571 Pollution, even at sub-lethal levels can affect the physiology of organisms and affect their
572 relationships with other organisms. Several studies have shown that, when they are not
573 directly lethal, thermal and/or chemical modifications of the environment often induce
574 endocrine and behavioral changes in marine organisms, as well as alterations of their
575 energetic metabolism and immunity (Harvell et al., 1999; Waldichuk, 1979). Cuvillier-Hot
576 *et al.* (2018) showed that heavy metals and phthalates, even at concentrations below the
577 toxicity index, alter the immune response as well as the trans-generational immune
578 priming of natural populations of the coastal annelid *Hediste diversicolor* and make them
579 less resistant to an experimental infection by the environmental bacterium *Bacillus*
580 *hwajinpoensis* SW-72 isolated from the burrow of the worm (Bernier et al., 2019; Cuvillier-
581 Hot et al., 2018). These observations clearly show the impact of changing environmental
582 conditions on host-bacteria interactions in marine invertebrates.

583

584 **Worm epibiosis is characterized by a tegumental association with the giant sulfur**
585 **oxidizing bacterium *Thiomargarita* sp.**

586 The combined analyses of the RNAseq data, the targeted bacterial 16S amplification results
587 and microscopic observations, allowed the estimation of the abundance and the phylotype
588 diversity of the epibiotic bacteria associated with the *Capitella* worms in the Roscoff
589 Harbor. Most abundant bacteria fall into three groups: (i) sulfur-oxidizing bacteria (mostly
590 *Thiomargarita* but also *Thiotrix*, *Thioalkalivibrio*, and *Sulfuromonas*), (ii) mollicutes
591 (including *Spiroplasma*), typically found in invertebrate guts, and (iii) spirochaetes. We
592 identified the largest and most visible epibiont as being a large gammaproteobacterium
593 belonging to genus *Thiomargarita*, closely related to *Candidatus* ‘*Thiomargarita nelsonii*’.
594 This is the first report of *Thiomargarita* in a coastal ecosystem. This giant
595 chemolithotrophic bacterium was often encountered as a free-living species associated

596 with deep-sea microbial mats. *Thiomargarita* were also found attached to the byssus of a
597 mussel at deep-sea hydrothermal vents (Schulz, 2006), the shell of gastropod *Provanna*
598 *laevis* at deep-sea methane cold seeps, and on the integument of other seep fauna (Bailey
599 et al., 2011). The ecological behavior of the gastropod *Provanna laevis* was shown to be
600 modified by the presence of *Thiomargarita*, the snail orienting its shell downward to allow
601 its *Thiomargarita* epibionts to be exposed to sulfide-rich water while the animal had access
602 to the oxygen-rich overlaying water, leaving its head partially exposed (Bailey et al., 2011).
603 The fluctuating sulfide-driven chemosynthetic environment appears as an obvious shared
604 characteristic between the *Capitella* and the seep fauna habitats.

605 Unlike its close relatives *Thioploca* and *Beggiatoa*, *Thiomargarita* are not motile. They store
606 elemental sulfur as granules at the periphery of a very large vacuole that occupies 98% of
607 the cell volume where nitrate is stored (Schulz, 2006). Because of their lack of motility,
608 *Thiomargarita* cells must live in an environment where they will be alternatively exposed
609 to sulfide in the porewater and to nitrate in the overlaying seawater. Compared to
610 previously reported *Thiomargarita* morphologies, the cells attached to *Capitella* are more
611 elongated but the observation of budding structures are similar to those reported in
612 *Provanna laevis* and byssal threads of *Bathymodiolus* mussels from deep-sea cold seeps
613 (Bailey et al., 2011), and suggests that the cells are actively growing. Unlike *Thioploca*,
614 whose populations decline at oxygen concentrations greater than 3 μM , and *Beggiatoa*
615 mats, which thrive with oxygen concentration of 1-2.5 μM , *Thiomargarita* cells can
616 withstand exposure to full atmospheric oxygen concentrations (Schulz, 2006).
617 *Thiomargarita* morphotypes have also been observed attached to various debris while
618 sorting the sediment samples, suggesting their ability to efficiently colonize a wide variety
619 of surfaces, including *Capitella*. The presence of *Thiomargarita* can easily be viewed as a
620 form of biofouling. Their density was, however, higher on the worms, suggesting that these

621 animals offer a more suitable environment. Moreover, we found that *Thiomargarita* was
622 present on the tegument of the three genetic lineages, cryptic species of *Capitella*, but at a
623 higher prevalence on large worms during the summer period, irrespectively of gender,
624 although more frequently encountered on males and indeterminate individuals.

625

626 **Is thionobiont epibiosis a facultative mutualistic association to face transient**
627 **concentrations of sulfide?**

628 The complex of *Capitella* species living in the English Channel is exposed to high
629 concentrations of sulfide in the sediment while pumping overlaying oxygenated water by
630 peristalsis in their burrow. Since *Thiomargarita* is a non-motile, facultative anaerobic
631 sulfur-oxidizing bacterium, the association with the animal could thus represent an
632 opportunistic strategy from the bacterial viewpoint, bridging the oxic-anoxic gap and
633 allowing bacteria access to both electron donors and acceptors. On the other hand, sulfide
634 uptake might be a way to detoxify the environment of *Capitella* and a positive by-product
635 of the bacterium's activity, although this hypothesis needs to be tested. Other sulfur
636 bacteria detected could interact as a consortium of smaller filamentous bacteria working
637 at the surface of *Thiomargarita* cells, as already shown in Namibia sediments (Bailey et al.,
638 2011) but also found in association with the hydrothermal-vent species *Alvinella*
639 *pompejana* (Le Bris and Gaill, 2006). During the survey of epibiosis over nearly a year, we
640 found a greater abundance of worms with *Thiomargarita* in during the summer on the
641 largest animals from both sampled sites. Summer is the period of the year when
642 temperatures are the highest and thus during which bacterial degradation of organic
643 matter, producing sulfide, is likely to be at its highest in the sediment. The prevalence of
644 the association depends on the presence of free bacteria in the mud what remains to be
645 seasonally surveyed. One might assume that *Thiomargarita* which oxidizes dissolved

646 sulfide in the pore water grow better during the summer period (Schulz, 2006).

647 *Capitella* is a typical member of the 'sulfide system'. Fenchel & Riedl (Fenchel and Riedl,
648 1970) coined this term to describe life under these hostile conditions (later called
649 'thiobiome' or 'thiobios' by Boaden (Boaden, 1975)). Although the thiobiome allows less
650 competitive stress, specific physical and structural adaptations are needed for the survival
651 and thriving of this complex and specific biome. Our observations suggest that at highly
652 "toxic" levels of hydrogen sulfide, physiological adaptations of *Capitella* alone could not be
653 sufficient to detoxify the reduced sulfur compounds and that a facultative epidermal
654 association with *Thiomargarita* and other sulfur oxidizing bacteria available in sediment
655 may constitute a vital additional strategy. The tolerance assay provided here evidenced that
656 the observed epibiosis is beneficial to the host when subjected to highly sulfide-rich
657 environments. Besides detoxication, sulfur-oxidizing epibionts may provide nutrients to
658 the host as suggested for deep sea hydrothermal annelids (Desbruyères et al., 1983).
659 *Capitella* has been shown to feed on free-living autotrophs that use sulfide oxidation to fix
660 CO₂ (Hiroaki et al., 2001). Thiobionts might supply *Capitella* in nutrients presumably
661 explaining why epibiotic specimens are larger than the non-epibiotic ones.

662 There is ample empirical evidence of symbioses providing protection against specific
663 natural enemies, e.g. in aphids facing parasitoids and predators (Dion et al., 2011; Oliver et
664 al., 2014; Polin et al., 2014) or pathogens (Clay, 2014; Tasiemski et al., 2015). Such
665 symbioses have also been suggested as potential means to explain the success of some
666 invasive species in new habitats (Amsellem et al., 2017; Chabrerie et al., 2019; Macke et al.,
667 2017). While many of the aforementioned symbioses involved obligatory endosymbionts,
668 the present data bring to light an adaptive advantage of a facultative ectosymbiosis to face
669 changing habitats.

670 A derived question was to know if this *Capitella-Thiomargarita* association was species-

671 specific; to find a specific niche may allow to avoid competition with congeneric species.
672 *Capitella teleta* and *C. capitata* which form a cryptic species complex (Grassle and Grassle,
673 1976; Nygren, 2014). Even if the populations of *Capitella* inhabiting Roscoff constitute an
674 assemblage of cryptic species (Boidin-Wichlacz et al. under review), barcode analyses
675 performed on the main lineages showed that the epibiotic association is not completely
676 genetically determined (e.g. an intraspecific polymorphism of the immune genes involved
677 in the control of the association might exist). The facultative association is likely due to
678 physiological differences between individuals, more or less correlated to their size and
679 possibly micro-environments at the scale of the worm itself. The observation could also
680 mean that *Thiomargarita* and other epibiotic bacteria correspond to biofouling/parasitic
681 agents capable of colonizing a range of invertebrates, including *Capitella* from different
682 species, when they are under high sulfidic stresses.

683 **Conclusion**

684 Our data provide clear evidences of the impact of sediment microgeochemistry on
685 associations between *Capitella* and its surrounding microorganisms with the peculiar
686 development of a transient beneficial epibiosis in worms exposed to high sulfide
687 concentrations. Occurrence and maintenance of an epibiotic community depend on the
688 host's ability to control the epibiont's colonization and proliferation through its immune
689 actors. Such defense is probably influenced by variable environmental conditions.
690 Consequently, the next step will be to investigate how and if the immune system of *Capitella*
691 can become permissive to the establishment of this facultative epibiosis as observed for the
692 hydrothermal vent worm, *Alvinella pompejana* and shrimp *Rimicaris exoculata* (Le Bloa et
693 al., 2020; Tasiemski et al., 2014). Regardless of future findings, this emphasizes the
694 importance of investigating symbiotic associations in their proper environmental context.

695

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705

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951 **Figure legends**

952 **Figure 1:** Location of the study (Roscoff, France) with the sampling sites. Sites coordinates
953 are the following: Le Laber: 48°42'47.67"N 4° 0'5.17"O- 48°42'45.92"N 4° 0'3.85"O-
954 48°42'45.12"N 4° 0'3.60"O, Roscoff Harbor: 48°43'35.46"N 3°58'52.05"O -48°43'34.49"N
955 3°58'51.64"O- 48°43'34.20"N 3°58'50.53"O and 48°43'34.03"N 3°58'49.15"O

956 **Figure 2:** (A) Sedimentary AVS and CRS concentration profiles (mgS kg⁻¹ sed) in Roscoff
957 Harbor (blue line) and Le Laber (red line) performed in November 2014 (corresponding to
958 the sampling of the animals for NGS sequencing). (B) Semi-thin sections of *Capitella*
959 sampled for the NGS sequencing: not colonized (in Le Laber, FPKM1 and in the Roscoff
960 Harbor, FPKM2) and colonized by the epibiotic community (in the Roscoff Harbor only,
961 FPKM3).

962 **Figure-3:** Visible (top) and electron microscopy (bottom) showing non epibiotic (A, C) and
963 epibiotic *Capitella* (B, D). Squares show a zoom on the microbial epibiotic community.

964 **Figure 4:** Epibionts of *Capitella* spp. (A) Electron microscopy of the *Thiomargarita* like
965 bacteria (C) Notice that *Thiomargarita*-like bacteria are strongly anchored on the tegument
966 and (A, B) themselves host epibiotic communities most likely consisting of bacteria some
967 displaying filamentous morphologies. (D) Several *Thiomargarita*-like structures and other
968 microbial morphotypes. (E) DAPI staining of a *Thiomargarita*-like structure (in the center)
969 attached to the tegument of *Capitella*. (F, G) FISH hybridization on the tegument of an
970 epibiotic *Capitella* specimen using the generalist probe EUB338. Notice the abundance and
971 diversity of bacterial morphologies including rods, cocci and filamentous bacteria.

972 **Figure 5:** Phylogenetic reconstruction of the position of the *Thiomargarita* sp. sequence
973 obtained from 16rRNA clone libraries obtained from epibiont-covered *Capitella* annelids.
974 See material and methods for detail (FYI: Maximum likelihood using a General Time
975 Reversible Model using MEGA7. Heterogeneity in rates of evolution was accounted by using

976 Gamma distributed rates (5 categories and invariants). 1140 nucleotide positions were
977 analyzed. Scale bar corresponds to 2 % sequence variation. Bootstrap values at nodes were
978 obtained based on 100 ML replications (>50 shown).

979 **Figure 6:** Predicted probability of association with epibiotic microorganisms as a function
980 of the time of the year (month, x-axis) and the size of the worm (in mm, y-axis), obtained
981 from model-averaging 166 GLMs linking site, size, date, date² and sex to association with
982 epibiotic microorganisms. Predictions are made for a uniform sampling of worms among
983 the sexes (undetermined, females and males represent 1/3 of the sample each), the sizes
984 (uniform distribution between 0 and 1.8 mm), the sampling dates and the sampling sites.
985 The color of each square on the heatmap indicates the average predicted probability of
986 association of all worms of that size sampled at that date, following the legend on the right.

987 **Figure 7:** Neighbor-joining tree reconstruction of epibiotic and non-epibiotic *Capitella* spp.
988 individuals barcoded using the mitochondrial marker *Cox-1*. Distances between individuals
989 were calculated according to the substitution model HKY.

990 **Figure 8:** Tolerance tests to sulfides. Kaplan-Meier plots showing the survivorship of non-
991 epibiotic (red) *versus* epibiotic (blue) worms sampled from the Roscoff Harbor (2020)
992 experimentally exposed to 0 (solid lines), 1 mM (dotted lines) and 3 mM (dashed lines)
993 concentrations of sodium sulfides. Time in hours.

Table 1: Total and HCl 1M-extracted metals concentrations in the first 5 cm depth sediments of Le Laber and the Roscoff harbor (fraction <63 μ m). For HCl 1M extraction, an average has been calculated from results obtained between July and December 2015. See table S3 for discrete values and table S4 for a comparison with sediments from other similar North Atlantic French stations (Boulogne, Gravelines and Authie).

		Concentration (mg kg ⁻¹)								Concentration (g kg ⁻¹)			
		Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Ca	Fe	Mg	Al
Laber	Total	0.1	6.5	51	6.0	165	6.9	19.4	42	14	10.2	4.3	38.5
	HCl 1M	0.1	ND	2.2	1.6	15	0.9	4.1	11.1	5.6	2.1	0.8	-
	Lability (%)	76.0	-	4.3	17.9	9.0	13.7	21.3	26.6	39.2	20.9	18.7	-
Roscoff	Total	0.4	10.1	54	37.6	264	13.3	30	111	69	21.2	10.7	51.2
Harbor	HCl 1M	0.1	0.0	4.8	6.7	32	1.6	8.6	27.8	15	3.8	0.9	-
	Lability (%)	15.1	0.1	8.7	17.9	12.1	11.7	28.7	25.1	21.2	18.1	8.5	-
Ratio of total :Roscoff Harbor/Laber		4	1.55	1.06	6.27	1.60	1.93	1.55	2.64	4.93	2.08	2.49	1.33

ND: Not detected

Table 2: Dissolved sulfide concentrations (mg L⁻¹). Averaged values for 0-3, 3-15 and 0-15 cm sedimentary horizons from Le Laber and Roscoff Harbor sites (in 2015). In bold, the concentration values where the worms live.

		Concentration (mg/L)							
		11/8	12/8	18/8	21/8	1/9	9/9	15/9	24/9
Le Laber	0-3 cm		0.15	0.01		<0.005	-	<0.005	0.27
	3-15 cm		0.68	3.1		3.1		5.9	6.8
	0-15 cm		0.58	2.5		2.5		4.8	5.5
Roscoff Harbor	0-3 cm	5.8			1.1		2.8		
	3-15 cm	13			10		12		
	0-15 cm	12			8.2		9.8		

Table 3: Contigs with intermediate FPKM values (ratios between 30 and 0.03). Le Laber sample (FPKM1), Roscoff Harbor sample without (FPKM2) or with (FPKM3) epibiotic microorganisms. Only hits for FPKM values greater than 100 are represented. Contigs ranked in decreasing order of the greatest FPKM value (shaded in grey).

Accession number	E-value	Genbank description	Biology	FPKM1	FPKM2	FPKM3
AY838865.1	0	<i>Paramphinome jeffreysi</i> 28S ribosomal RNA gene, partial sequence	Polychaete	2702	658	243
KX982503.1	0	<i>Ancora sagittata</i> isolate Ancora2011 external transcribed spacer, partial sequence; 18S rRNA gene, ITS 1, 5.8S rRNA gene, ITS 2, and 28S rRNA gene, complete sequence; and external transcribed spacer, partial sequence	Gregarine of <i>Capitella</i>	925	48	1594
DQ779991.1	6 10 ⁻⁸⁸	<i>Gymnodinium aureolum</i> strain GrAr01 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, ITS 2, and large subunit ribosomal RNA gene, complete sequence; external transcribed spacer, partial sequence	Dinoflagellate algae	1042	19	1550
EF100367.1	10 ⁻¹¹⁴	Uncultured eukaryote clone D5P10A10 18S ribosomal RNA gene, partial sequence	Apicomplexan?	509	33	1134
EF100398.1	0	Uncultured eukaryote clone D2P03E11 18S ribosomal RNA gene, partial sequence	?	875	31	1086
GU479649.1	0	<i>Eimeria leucisci</i> isolate BLI637-#637 18S ribosomal RNA gene, partial sequence	Coccidian apicomplexa fish parasite	936	43	106
AY179976.1	9 10 ⁻¹⁷⁷	Uncultured eukaryote clone CCI31 18S small subunit ribosomal RNA gene, partial sequence	Apicomplexan?	530	258	869
KC558064.1	5 10 ⁻⁸⁹	Uncultured fungus clone NTS_28S_047E_2_f6 28S ribosomal RNA gene, partial sequence	?	121	0	863
JX178933.1	0	<i>Vorticella</i> sp. 4 JG-2011 clone 33 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, and ITS 2, complete sequence; 28S ribosomal RNA gene, partial sequence	Peritrich ciliate	150	170	840
GU927604.1	4 10 ⁻⁹⁴	Uncultured eukaryote clone F5K2Q4C04IDZ7B 28S ribosomal RNA gene, partial sequence	Ciliate	166	16	636
EF990727.1	5 10 ⁻⁸⁸	<i>Rhabditoides inermiformis</i> strain SB328 28S large subunit ribosomal RNA gene, partial sequence	Free-living nematodes	634	25	542

AY256244.1	2 10 ⁻¹¹⁹	Uncultured eukaryote isolate E6 small subunit ribosomal RNA gene, partial sequence	?	369	152	586
GU927618.1	4 10 ⁻⁹⁴	Uncultured eukaryote clone F5K2Q4C04IVOMC 28S ribosomal RNA gene, partial sequence	?	88	0	578
AY835682.2	0	Uncultured peritrich clone IAFDv27 18S ribosomal RNA gene, partial sequence	Peritrich ciliate	125	23	555
GU927271.1	4 10 ⁻²⁵	Uncultured eukaryote clone F5K2Q4C04H81PH 28S ribosomal RNA gene, partial sequence	?	252	56	430
AB189984.1	7 10 ⁻⁸³	<i>Contraecaecum spiculigerum</i> gene for 28S ribosomal RNA, partial sequence	Bird parasite	46	446	128
KF147653.1	0	Nematoda environmental sample clone NEMAK34 18S ribosomal RNA gene, partial sequence	?	156	7	414
KF601317.1	4 10 ⁻⁵⁴	<i>Sarcocystis arctica</i> isolate VI2.2 28S ribosomal RNA gene, partial sequence	Vertebrate parasite	201	0	398
KC869522.1	9 10 ⁻⁸⁸	<i>Isodictya grandis</i> voucher NCI439 28S ribosomal RNA gene, partial sequence	Marine sponge	380	74	394
FJ969135.1	0	<i>Plectus tenuis</i> strain ChGaSp5 small subunit ribosomal RNA gene, partial sequence	Free-living nematodes	17	22	371
EF100367.1	3 10 ⁻¹⁰⁵	Uncultured eukaryote clone D5P10A10 18S ribosomal RNA gene, partial sequence	?	366	153	60
AB611781.1	3 10 ⁻⁶⁴	<i>Fukuia kurodai ooyagii</i> gene for 28S ribosomal RNA, partial sequence, specimen_voucher: personal:Kameda Y.:5609	Gastropod	343	0	240
EF100367.1	3 10 ⁻¹²⁵	Uncultured eukaryote clone D5P10A10 18S ribosomal RNA gene, partial sequence	Apicomplexan?	173	0	323
FJ417074.1	0	<i>Sphaerospora dicentrarchi</i> isolate M0749 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	320	3	65
KF147653.1	0	Nematoda environmental sample clone NEMAK34 18S ribosomal RNA gene, partial sequence	?	11	38	319
FJ417074.1	10 ⁻¹⁴⁰	<i>Sphaerospora dicentrarchi</i> isolate M0749 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	306	0	69
JX391808.1	0	Uncultured bacterium clone NS093 16S ribosomal RNA gene, partial sequence	?	302	0	23
HG315671.1	0	<i>Formosa agariphila</i> KMM 3901, complete genome	Algal bacterial associate	287	1	19

FJ417074.1	0	<i>Sphaerospora</i> sp. M0379 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	285	8	76
AY641571.1	0	<i>Kudoa iwatai</i> isolate J small subunit ribosomal RNA gene, partial sequence	Fish parasite	276	6	102
FQ032815.1	4 10 ⁻¹³¹	Uncultured <i>Sphingobacteria</i> bacterium, whole genome shotgun sequence	?	265	0	11
JQ723993.1	5 10 ⁻¹⁸⁰	<i>Vorticellides</i> sp. 2 MD-2012 small subunit ribosomal RNA gene, partial sequence; macronuclear	Peritrich ciliate	58	29	257
AY179976.1	3 10 ⁻¹⁷⁷	Uncultured eukaryote clone CCI31 18S small subunit ribosomal RNA gene, partial sequence	?	246	67	76
HM031979.1	0	<i>Cytophaga</i> sp. UDC385 16S ribosomal RNA gene, partial sequence	Free-living bacterium	244	2	15
JX391440.1	5 10 ⁻¹⁵⁰	Uncultured bacterium clone N0004 16S ribosomal RNA gene, partial sequence	?	233	0	18
EF067920.1	5 10 ⁻⁹⁸	<i>Phaeodactylum tricornutum</i> chloroplast, complete genome	Diatom	222	0	117
DQ377695.1	0	<i>Sphaerospora</i> sp. IF-2006 from Mugil curema small subunit ribosomal RNA gene, partial sequence	Fish parasite	218	0	75
EF100398.1	0	Uncultured eukaryote clone D2P03E11 18S ribosomal RNA gene, partial sequence	?	191	15	155
FJ417074.1	8 10 ⁻¹⁷⁸	<i>Sphaerospora dicentrarchi</i> isolate M0749 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	170	0	81
GU928472.1	6 10 ⁻⁶⁸	Uncultured eukaryote clone F5K2Q4C04H5ZBN 28S ribosomal RNA gene, partial sequence	?	33	0	169
AF185190.1	2 10 ⁻¹³³	<i>Eurythoe</i> sp. AMW4444 28S ribosomal RNA gene, partial sequence	Polychaete	154	36	117
FN563149.1	0	<i>Rhodococcus equi</i> 103S chromosome	Pathogen causing pneumonia in horses	148	1	7
AB636470.1	10 ⁻⁹⁸	<i>Kudoa ogawai</i> gene for 18S ribosomal RNA, partial sequence	Fish parasite	142	0	81
FJ557946.1	0	Uncultured bacterium clone ET_G_4f03 16S ribosomal RNA gene, partial sequence	?	135	0	6
FJ417058.1	10 ⁻⁶⁹	<i>Kudoa diana</i> e isolate M0290 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	133	0	77

GU479649.1	0	<i>Tubificoides brownae</i> isolate CE3387 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, and ITS 2, complete sequence; 28S ribosomal RNA gene, partial sequence	Free-living oligochaete	131	0	13
FJ417074.1	4 10 ⁻¹⁰³	<i>Sphaerospora dicentrarchi</i> isolate M0749 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	128	18	37
JX178767.1	2 10 ⁻¹⁰⁸	<i>Zoothamnium</i> sp. 1 JG-2011 18S ribosomal RNA gene, partial sequence	Peritrich ciliate	95	49	120
CP004404.1	7 10 ⁻¹⁶⁹	<i>Psychromonas</i> sp. CNPT3, complete genome	Facultative anaerobic free-living	21	0	113
JQ743689.1	6 10 ⁻¹³⁴	Uncultured peritrich ciliate clone GDH_F10 18S ribosomal RNA gene, partial sequence	Peritrich ciliate	46	113	33
KF077586.1	0	Uncultured bacterium clone nck74g02c1 16S ribosomal RNA gene, partial sequence		103	0	3

Table 4: Contigs found with greater FPKM values in Le Laber sample (FPKM1) compared to the Roscoff harbor without (FPKM2) or with (FPKM3) epibiotic microorganisms' samples. Only hits for FPKM values greater than 100 are represented. Contigs ranked according to decreasing values of FPKM1.

Accession number	E-value	Genbank description	Biology	FPKM1	FPKM2	FPKM3
KC816721.1	8 10 ⁻¹¹³	Apicomplexa sp. type N clone N66 clone 2 18S ribosomal RNA gene, partial sequence	Coral parasite	675	0	0
JX044549.1	7 10 ⁻⁷⁷	<i>Toxoplasma gondii</i> strain CASTELLS chromosome Ia region 5 genomic sequence	Animal parasite	651	95	1
JN256118.1	6 10 ⁻⁷²	<i>Sarcocystis</i> sp. ex <i>Corvus monedula</i> isolate kuos1 28S ribosomal RNA gene, partial sequence	Bird parasite	582	0	1
FJ417076.1	4 10 ⁻¹¹⁴	<i>Sphaerospora</i> sp. M0379 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	478	79	2
X75453.1	7 10 ⁻⁹⁹	<i>Toxoplasma gondii</i> (strain P) rDNA for 17s,5.8s,26s, and 5s ribosomal RNA	Animal parasite	468	5	0
CP001601.1	0	<i>Corynebacterium aurimucosum</i> ATCC 700975, complete genome	Mycolic acid-containing actinomycetes	436	0	2
HQ243019.1	3 10 ⁻³⁵	Uncultured <i>Glomus</i> clone ZHwq2-227 18S rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and 28S rRNA gene, partial sequence	Bivalve	355	0	0
AF109679.1	5 10 ⁻⁵⁸	<i>Sarcocystis mucosa</i> small subunit ribosomal RNA gene, partial sequence	Mammal parasite	211	16	0

Table 5: Contigs found with FPKM values at 50 times greater in animals with (FPKM3) and without (FPKM2) epibiotic organisms compared with animals from Le Laber (FPKM1). Only hits for FPKM values greater than 100 are represented. Contigs ranked according to decreasing values of FPKM3.

Accession number	E-value	Genbank description	Biology	FPKM1	FPKM2	FPKM3
HF954103.1	0	Uncultured <i>Thiomargarita</i> sp. partial 16S rRNA gene, clone NAM094	Giant sulfur bacterium	2	0	2708
JX198551.1	0	Uncultured bacterium clone Tui57 16S ribosomal RNA gene, partial sequence	Oceanospirillales symbiotic with vent snail <i>Alviniconcha</i>	19	0	2477
JQ768460.1	7 10 ⁻¹³⁰	<i>Spiroplasma</i> sp. crk 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, 23S ribosomal RNA gene, and 23S-5S ribosomal RNA intergenic spacer, complete sequence; and 5S ribosomal RNA gene, partial sequence	Field cricket gut mollicute	5	0	1897
NR_121985.1	0	Candidatus <i>Hepatoplasma crinochetorum</i> 23S ribosomal RNA, complete sequence	Isopod midgut gland mollicute bacterium	4	7	1625
NR_076721.1	0	<i>Thioalkalivibrio</i> sp. K90mix strain K90mix 23S ribosomal RNA, complete sequence	Haloalkaliphilic sulfur-oxidizing bacterium	9	1	1359
FR774200.1	0	<i>Thiomargarita</i> sp. NAM092 partial 23S rRNA gene and ITS1, clone NAM092	Giant sulfur bacterium	3	0	1257
FJ654610.1	0	Uncultured gamma proteobacterium clone 005_D02_06-017371_low_week_1 16S ribosomal RNA gene, partial sequence	Cnidarian-associated	20	0	1239
JN935865.1	5 10 ⁻⁶⁶	<i>Mycoplasma pulmonis</i> strain Ash (PG34) 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence	Animal parasite	0	0	1219
FR690959.1	3 10 ⁻¹²¹	Candidatus <i>Thiomargarita nelsonii</i> partial 16S rRNA gene and ITS1, isolate NAM071	Giant sulfur bacterium	0	0	1170
FR690946.1	10 ⁻¹⁰⁹	Candidatus <i>Thiomargarita nelsonii</i> partial 16S rRNA gene, isolate NAM057	Giant sulfur bacterium	0	0	1167
EU795103.1	0	Uncultured bacterium ARCTIC45_G_10 genomic sequence	?	7	0	1004

FO203512.1	10 ⁻⁷⁴	<i>Oleispira antarctica</i> strain RB-8, complete genome sequence	Hydrocarbonoclastic aerobic bacterium	0	0	957
HQ153940.1	0	Uncultured bacterium clone V1SC07b35 16S ribosomal RNA gene, partial sequence	Hydrothermal vent microbial mats	8	0	819
EU101262.1	0	Uncultured bacterium clone RS06101_B70 16S ribosomal RNA gene, partial sequence	Sulfur-oxidizing	0	0	778
NR_076671.1	0	<i>Kangiella koreensis</i> DSM 16069 strain DSM 16069 23S ribosomal RNA, complete sequence	Oceanospirillales free-living bacterium	3	0	589
NR_076212.1	2 10 ⁻¹³¹	<i>Treponema denticola</i> ATCC 35405 strain ATCC 35405 23S ribosomal RNA, complete sequence	Periodontal disease associate	5	0	546
GU567978.1	6 10 ⁻⁹³	Uncultured gamma proteobacterium HF0200_34B07 genomic sequence	?	0	0	522
GU928698.1	9 10 ⁻⁹⁰	Uncultured eukaryote clone F5K2Q4C04JA1DB 28S ribosomal RNA gene, partial sequence	?	1	0	521
FJ202296.1	0	Uncultured bacterium clone SGUS1039 16S ribosomal RNA gene, partial sequence	?	1	0	521
HE610322.1	10 ⁻¹⁴⁰	Uncultured Mycoplasmataceae bacterium partial 16S rRNA gene, clone 3-B9	Mud-crab intestinal mollicute	4	0	476
EF990727.1	4 10 ⁻¹¹⁶	<i>Rhabditoides inermiformis</i> strain SB328 28S large subunit ribosomal RNA gene, partial sequence	Nematodes living on vegetation debris	0	0	466
HE610322.1	0	Uncultured Mycoplasmataceae bacterium partial 16S rRNA gene, clone 3-B9	Mud-crab intestinal mollicute	3	0	464
NR_076858.1	10 ⁻⁵⁸	<i>Marinomonas mediterranea</i> MMB-1 strain MMB-1 23S ribosomal RNA, complete sequence	Free-living melanogenic bacterium	0	0	460
NR_076770.1	10 ⁻¹⁴⁴	<i>Spirochaeta smaragdinae</i> DSM 11293 strain DSM 11293 23S ribosomal RNA, complete sequence	Free-living thiosulfate and sulfur reducer	3	0	432
EU101262.1	2 10 ⁻¹⁶⁹	Uncultured bacterium clone RS06101_B70 16S ribosomal RNA gene, partial sequence	Sulfur-oxidizing	1	0	381
NR_121913.1	0	<i>Desulfuromonas acetoxidans</i> strain DSM 684 23S ribosomal RNA, complete sequence	Anaerobic sulfur reducer	3	0	380
EU101262.1	0	Uncultured bacterium clone RS06101_B70 16S ribosomal RNA gene, partial sequence	Sulfur-oxidizing	1	0	314
GU908489.1	0	<i>Spiroplasma litorale</i> 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer,	Dipteran-associated gut mollicute	4	1	311

		23S ribosomal RNA gene, and 23S-5S ribosomal RNA intergenic spacer, complete sequence; and 5S ribosomal RNA gene, partial sequence				
HM103460.1	4 10 ⁻⁹³	Uncultured metazoan clone Ma29_1E_24 18S ribosomal RNA gene, partial sequence	?	0	0	290
FO203512.1	0	<i>Oleispira antarctica</i> strain RB-8, complete genome sequence	Hydrocarbonoclastic aerobic bacterium	0	0	262
JN018328.1	3 10 ⁻⁵⁶	<i>Damon gracilis</i> voucher MNHN-JAB38 28S ribosomal RNA gene, partial sequence	Whip-spider	0	0	230
NR_102551.1	0	<i>Thioalkalivibrio nitratireducens</i> DSM 14787 strain DSM 14787 23S ribosomal RNA, complete sequence	Haloalkaliphilic sulfur-oxidizing bacterium	1	2	208
DQ174761.1	2 10 ⁻⁷³	Uncultured spirochete clone HaTB8 large subunit ribosomal RNA gene, partial sequence	Coral protistan agal symbiont	2	0	205
AJ879862.1	2 10 ⁻¹⁰³	Uncultured organism 28S rRNA gene, clone ASt-53	?	0	0	176
JN145195.1	2 10 ⁻⁸¹	Uncultured eukaryote clone NZAS-293 18S ribosomal RNA gene, partial sequence	?	0	20	167
NR_076865.1	3 10 ⁻⁶¹	<i>Desulfobacca acetoxidans</i> DSM 11109 strain DSM 11109 23S ribosomal RNA, complete sequence	Deltaproteobacterium Sulfate reducer	0	0	138
GU245692.1	4 10 ⁻⁵⁹	<i>Krefftascaris sharpiloi</i> isolate 2 18S rRNA gene, partial sequence; ITS 1, 5.8S rRNA gene, and ITS 2, complete sequence; and 28S rRNA gene, partial sequence	Turtle parasite	0	0	137
NR_103985.1	2 10 ⁻⁷⁷	<i>Spiroplasma chrysopicola</i> DF-1 strain DF-1 23S ribosomal RNA, complete sequence	Dipteran-associated gut mollicute	1	0	116

Figure 1

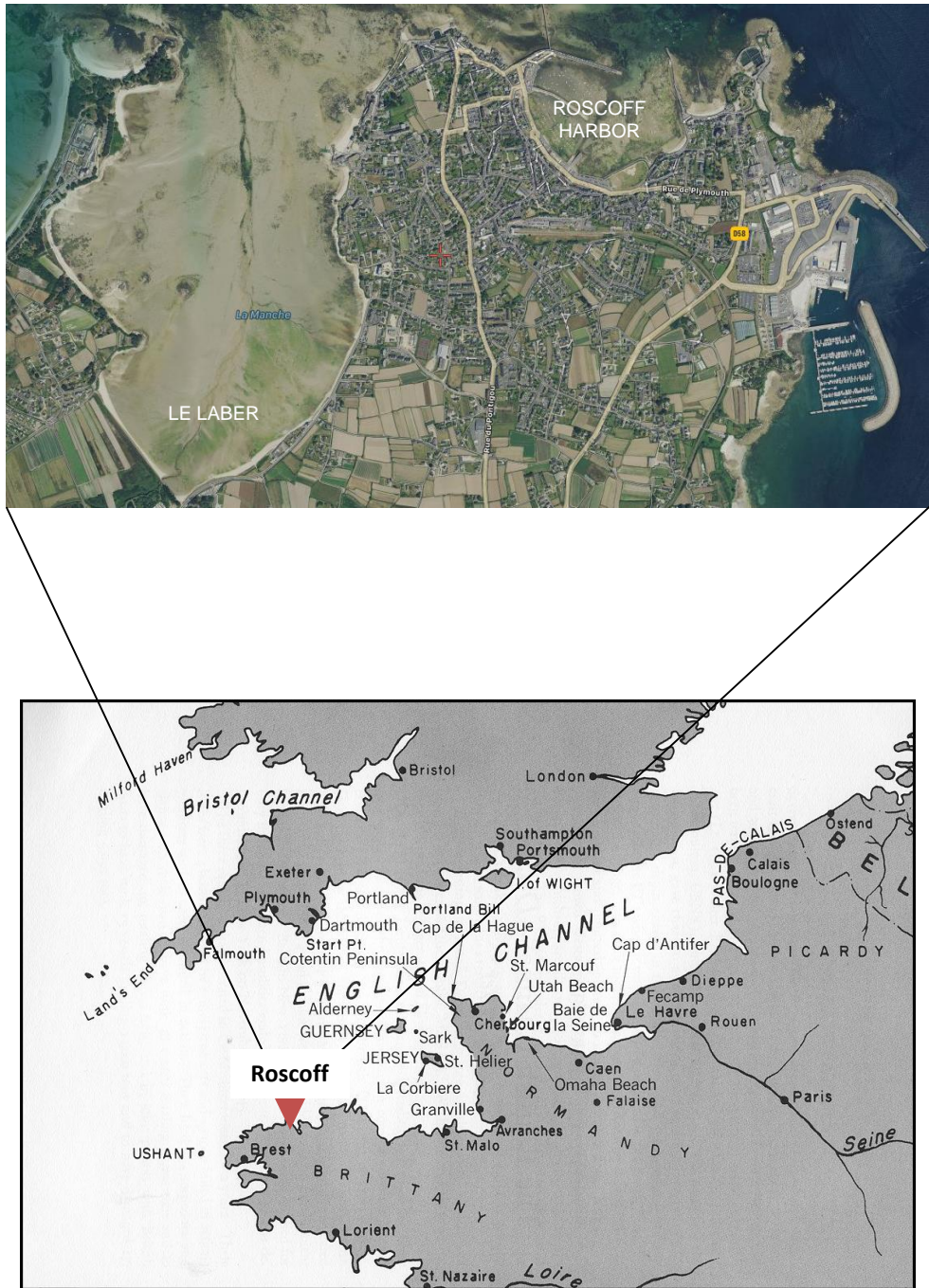


Figure 2

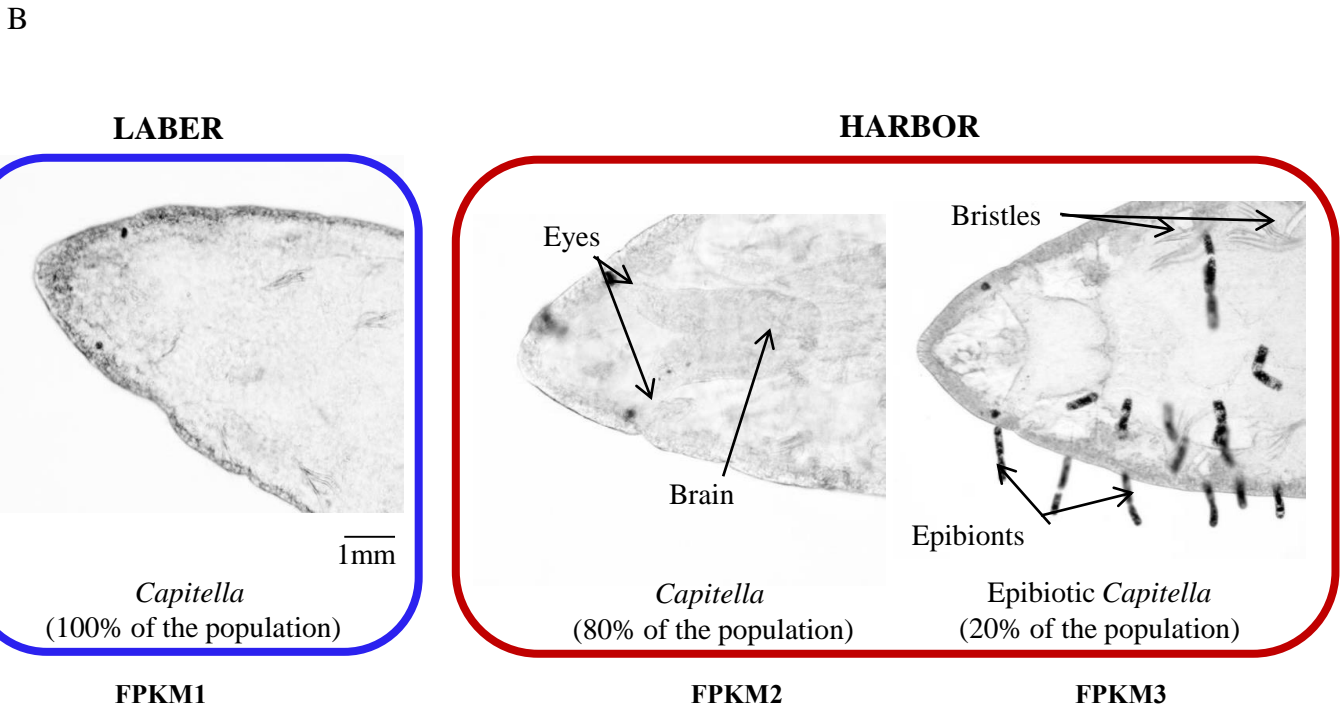
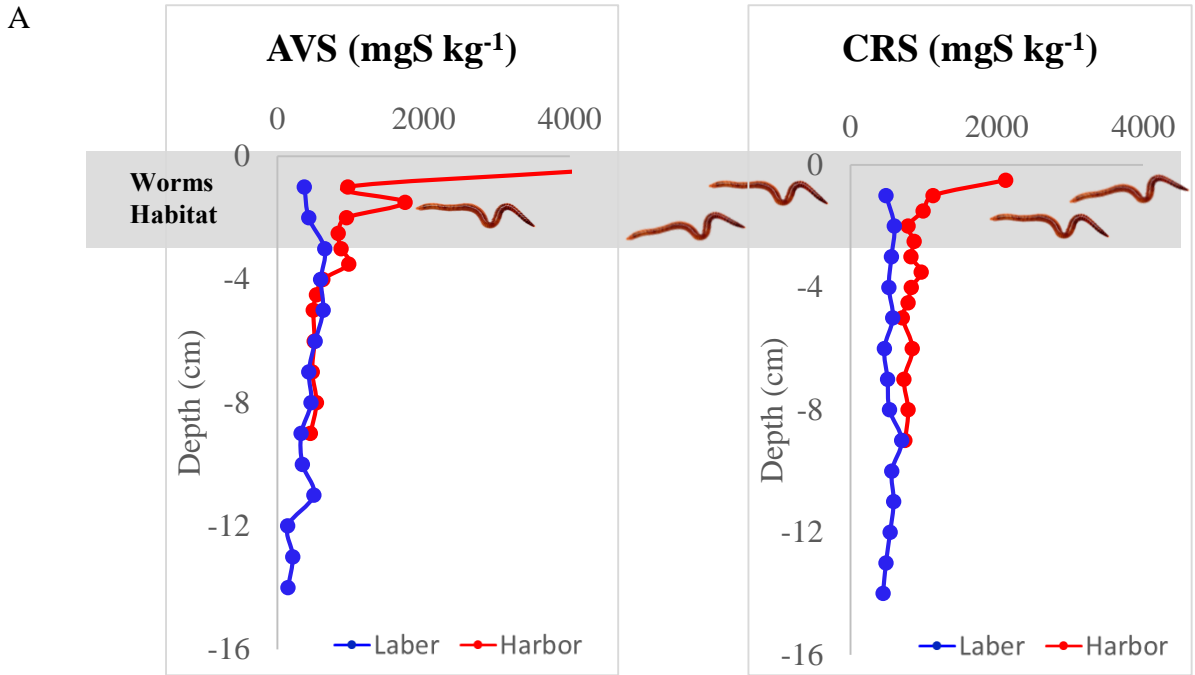


Figure 3

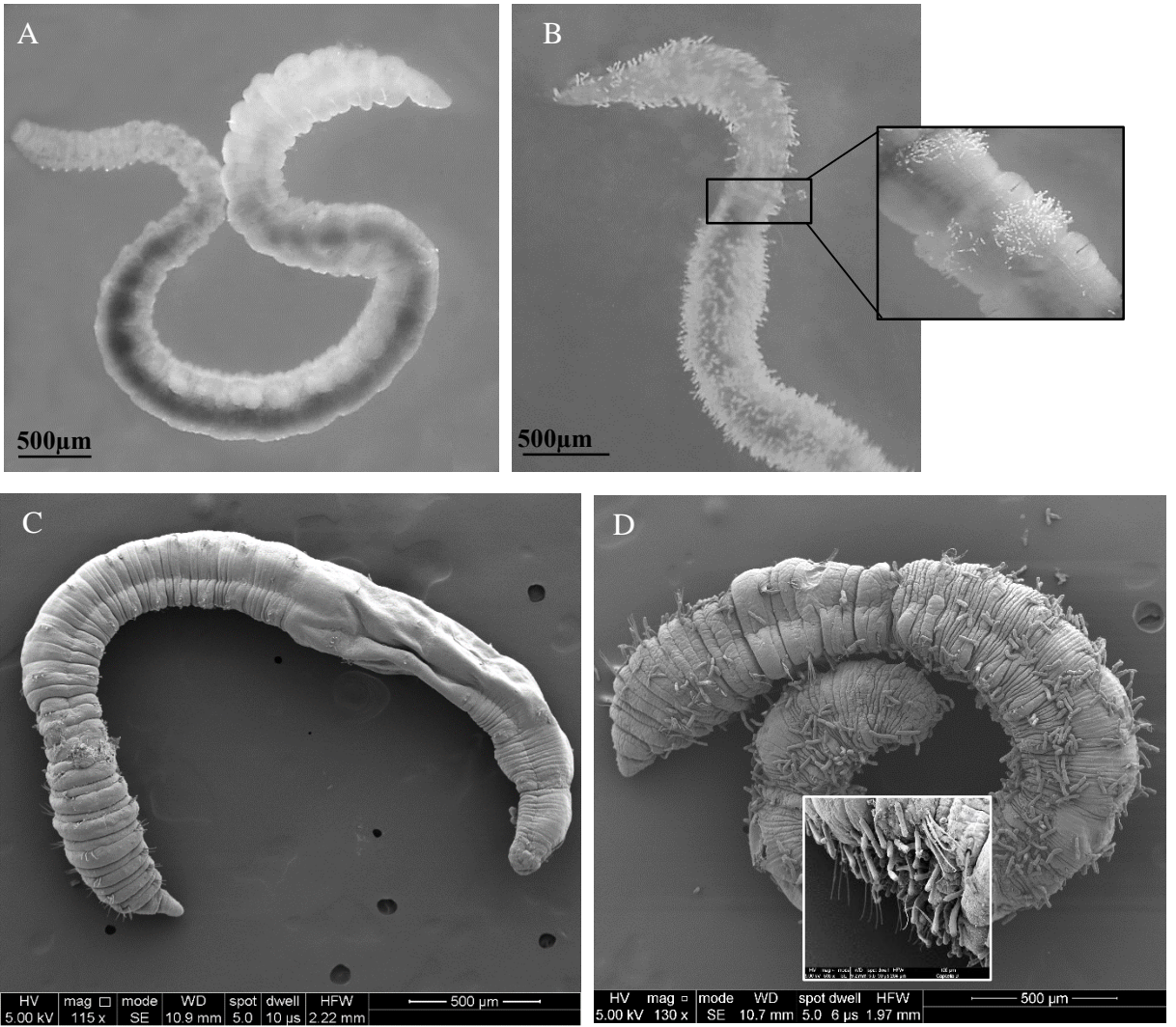


Figure 4

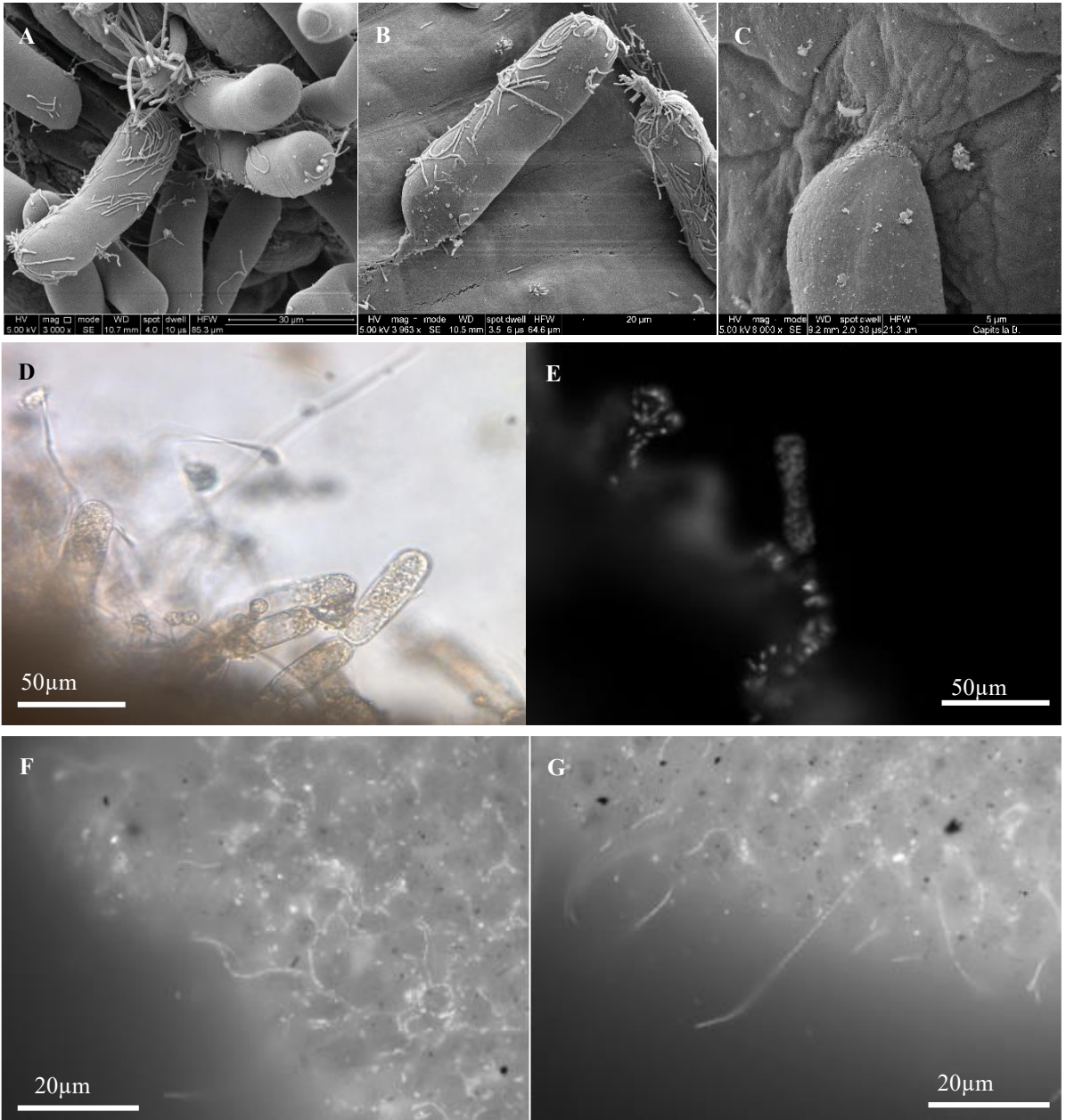


Figure 5

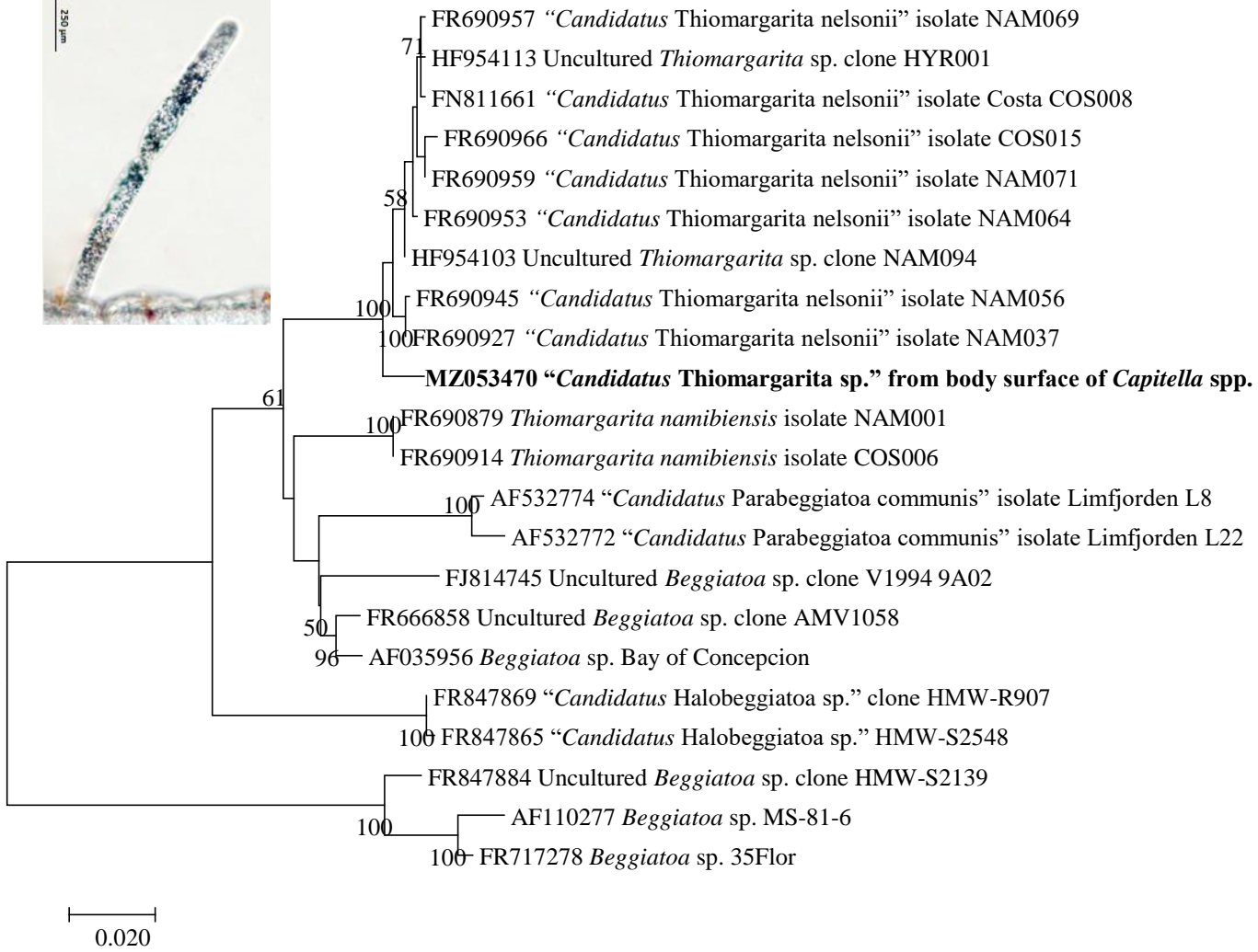
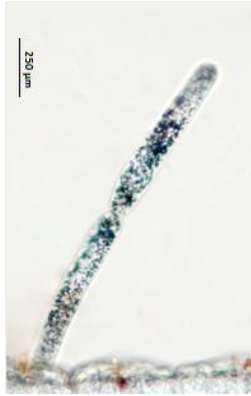


Figure 6

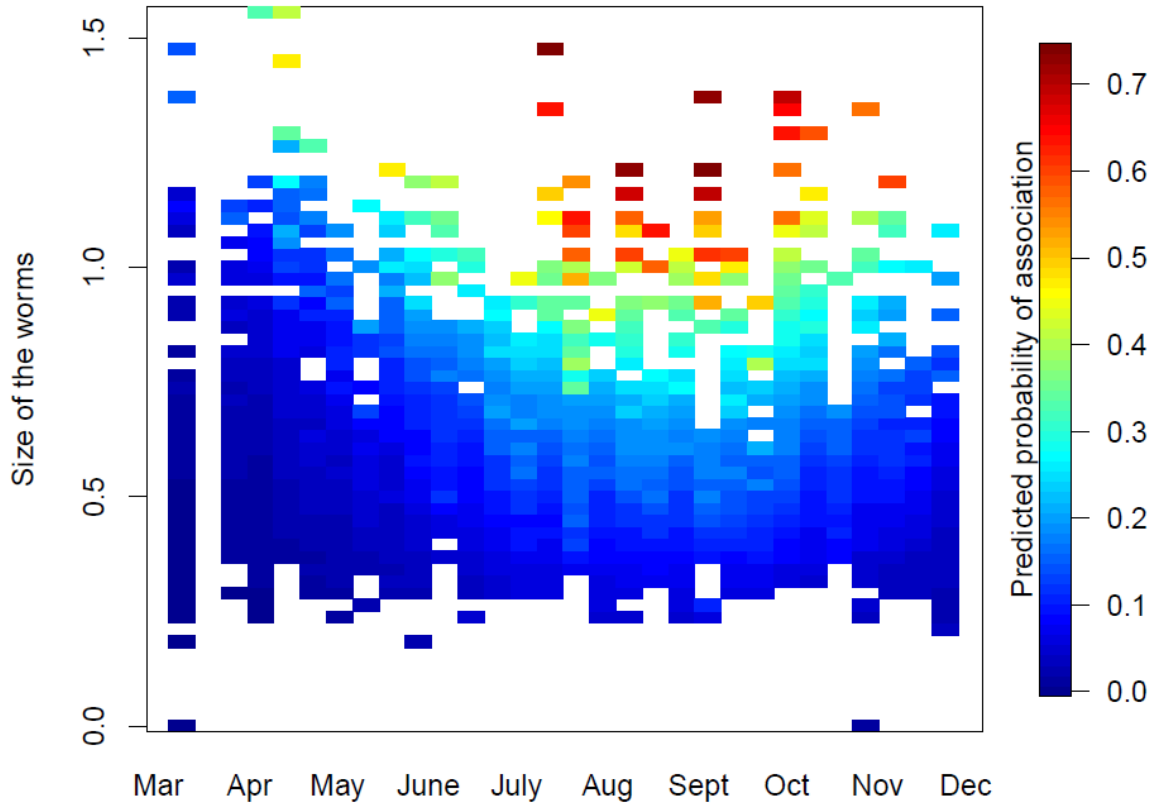


Figure 7

BioNJ 499 sites HKY 100 repl.

0.02

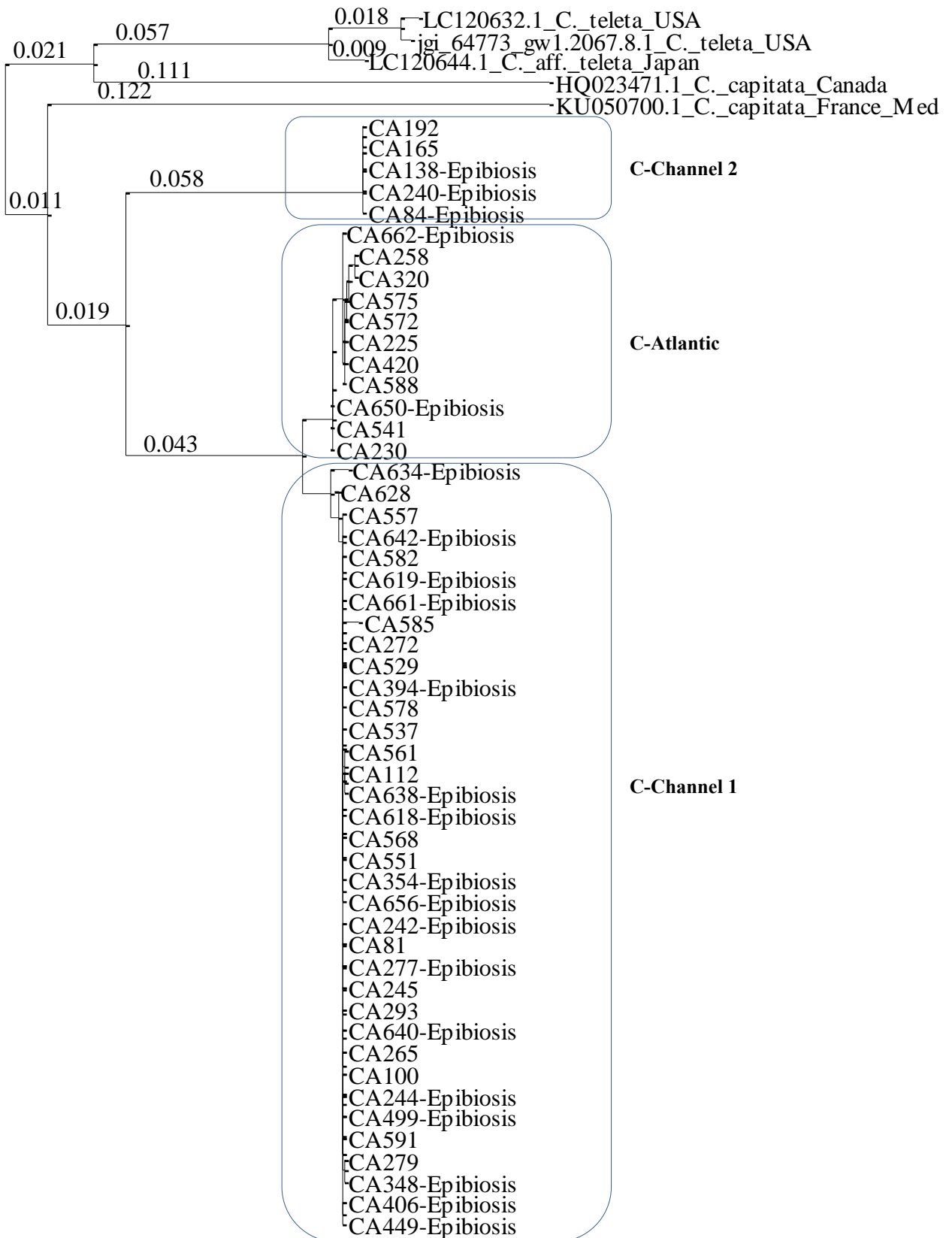
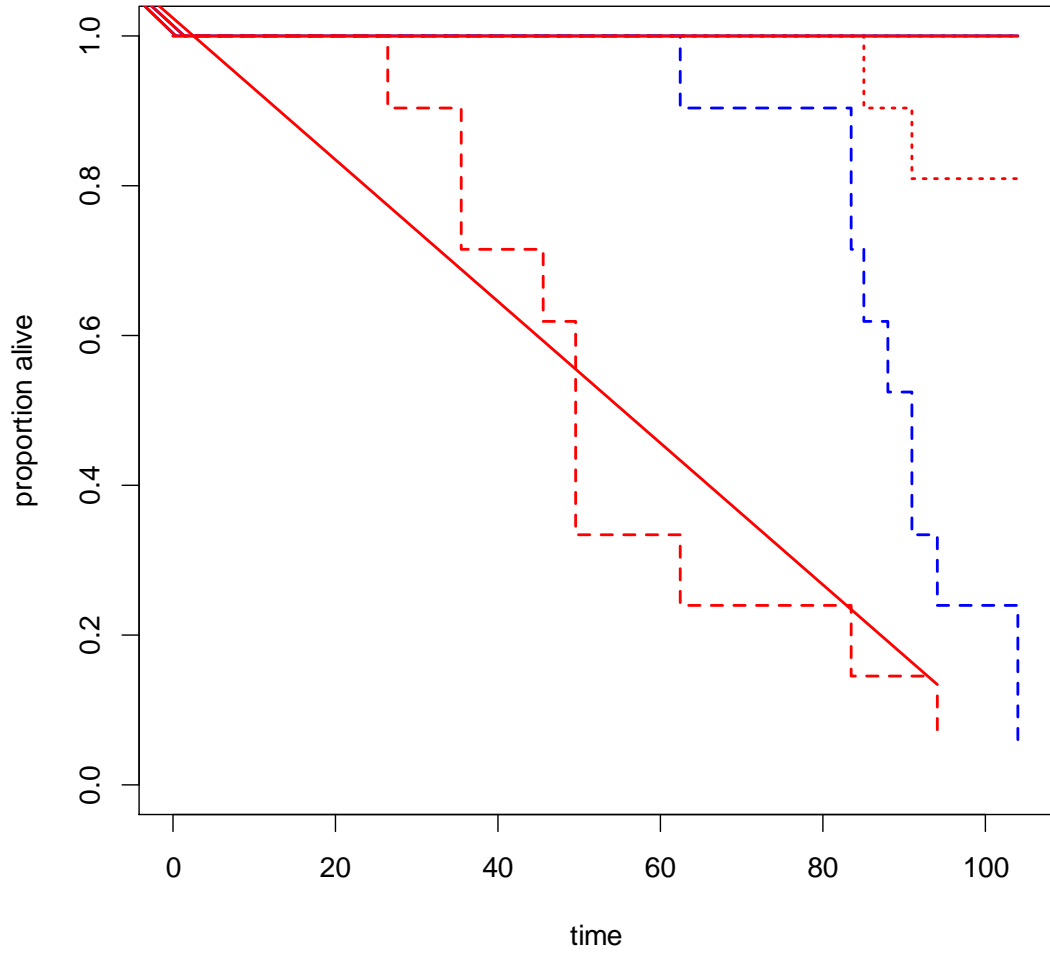


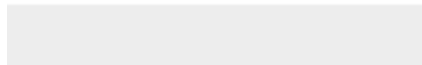
Figure 8





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Stéphane Hourdez: Provision of biological materials, data curation (RNAseq), writing the initial draft, data presentation, investigation, conceptualization. Céline Boidin-Wichlacz: Provision of biological materials, design of methodology, performing the experiments, data presentation, writing the initial draft and editing. Didier Jollivet: Genetic analyses, conceptualization, writing the initial draft, editing, and review of the published work. François Massol: Statistical analyses, creation of models, writing the initial draft, review of the published work. Maria Claudia Rayol: Provision of biological materials, performing the experiments, data collection. Renato Bruno: Provision of biological materials. Daniela Zeppilli: Provision of biological materials, critical review. Frederic Thomas: Critical review, commentary. Ludovic Lesven: Design of methodology, performing the experiments, data presentation, writing the initial draft. Gabriel Billon: conceptualization, writing the initial draft, editing, and review of the published work. Sebastien Duperron: Design of methodology, conceptualization, performing the experiments, data presentation, writing the initial draft, review and editing of the published work- Aurélie Tasiemski: Provision of biological materials, performing the experiments, data presentation, visualization, writing the initial draft, review and final editing of the published work, conceptualization, supervision and project administration.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: