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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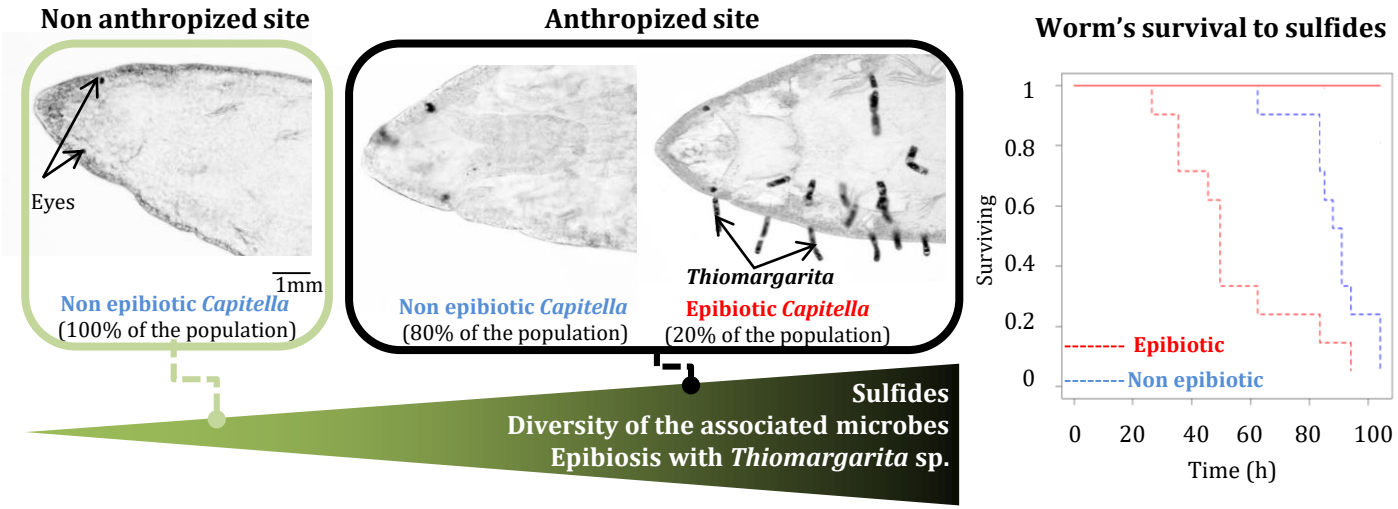
Graphical Abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

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

Abstract

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

INTRODUCTION

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

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

trochophore larva, which enters juvenile development, becoming sessile, and subsequently acquiring symbiotic bacteria through skin infection. After chemoautotrophic bacteria are established in the midgut of the juveniles, it undergoes substantial remodelling and enlargement to become the trophosome, while the remainder of the digestive tract fully disappears in adults (Stewart and Cavanaugh, 2006). Lacking a mouth and a gut and being unable to obtain organic compounds by diffusion, adults gain the latter *via* sulfur oxidation-CO_2 fixation driven by the endosymbionts confined into peculiar cells (namely bacteriocytes) of the trophosome. The tubeworm depends completely on the chemoautotrophic bacteria for the byproducts of their carbon fixation cycles needed for its growth. Reciprocally, endosymbionts rely on *R. pachyptila* for the assimilation of nutrients needed for the array of metabolic reactions they employ (Bright and Lallier, 2010).

Soon after this first description of chemoautotrophic symbiosis, additional thiotrophic symbioses were described at oxic–anoxic interfaces of more accessible coastal shallow-waters also recognized as chemosynthetic based ecosystems (Dubilier et al., 2008; Petersen et al., 2011; Stewart et al., 2005). Gutless oligochaetes' (annelids) and stilbonematids' (nematodes) symbioses are among them and constitute a remarkably well-described and interesting mode of nutrition (Bulgheresi, 2016; Dubilier et al., 2006; Polz et al., 1992). More recently, the nematode *Metoncholaimus albidus*, reported in the Roscoff Harbor (Brittany, France), has also been shown to be associated with distinct microbial communities known to be involved in sulfur metabolism (Bellec et al., 2019).

Marine worms belonging to the genus *Capitella* represent the most common component species of benthic communities in organically enriched ecosystems throughout the world (Kitamori, 1975; Pearson and Rosenberg, 1978; Reish, 1979). This so-called sediment "black zone" - previously considered to be azoic – is characterized by strongly reducing, micro- to anaerobic conditions with high concentrations of reduced sulfur species like

dissolved sulfides and polysulfides, thiols… and sulfide precipitates such as MeS (where Me can be Fe, Pb, Zn, Cd…), Fe_3S_4 and FeS_2 (Wood, 1992). Differential tolerance to sulfide has been observed between sibling species of *Capitella*, leading to the hypothesis that these ecophysiological differences were genetically fixed and that sulfidic environments could have been the driving force of such species diversification (Gamenick et al., 1998). The tolerance to sulfides in *Capitella* sp1 from North America (subsequently identified as *Capitella teleta* (Blake et al., 2009)) was evaluated through experimental exposure of the annelids to H_2S under laboratory conditions. Sulfide concentrations up to 2mM were considered as a cue for *Capitella* sp1 larval settlement (Cuomo, 1985) whereas those exceeding 10mM were detrimental to their survival (Dubilier et al 1988). The presence of sulfides up to 7mM was also shown to favor the burrowing activity of adults thus stimulating the respiratory activities of the bacteria associated with the mucus-lined burrow of the worm in soft agar microcosm (Wada et al., 2006). This was coupled with an enhanced growth and survival rates of the adults observed in sediments supplied with sulfides for 6 weeks (Tsutsumi et al., 2001). Consequently, *Capitella* species does not seem to favor organically enriched sediment with sulfides but rather prefers the environments that sulfides provide. As mentioned before, hydrogen sulfide can be exploited for the chemosynthesis of organic matter by chemoautotrophic bacteria. *Capitella* species are not gutless worms and an examination of *Capitella* sp. I for the presence of enzymes commonly associated with chemoautotrophic bacteria ~40 years ago has led to the conclusion by the authors that adults were not associated with chemoautotrophic symbionts (Cavanaugh, 1983; Cuomo, 1985).

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

whether such host-symbiont interactions (notably with chemoautotrophic bacteria) exist and to evaluate secondarily whether changing environmental conditions, and especially sulfides can affect these associations and the worm tolerance to this chemical.

For this end, *Capitella* specimens collected from two sites with differing levels of anthropic influence and sulfides were compared. Biogeochemical characteristics of the sites were documented, and microbial communities associated with specimens of *Capitella* sp. were assessed using a RNASeq-based approach. Finally, the cost *versus* benefit of the transient association with sulfur-oxidizing ectosymbionts was studied in animals exposed to lethal doses of sulfides.

1. MATERIALS AND METHODS

1.1. Specimen collection

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

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

1.2. Sediments

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

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

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

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

Where $[Me]_{sample}$ and $[Me]_{reference}$ are the concentrations of metal (Me: Cd, Co, Cu, Ni, Pb or Zn) in our samples and in the reference material, respectively (Audry et al., 2004; Davide 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 ≤ 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

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

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

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

1.4. Morphological observations of associated microorganisms

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1.8. Tolerance tests to experimental exposure to sulfides

Animals collected in July 2020 at the Roscoff Harbor were checked for filamentous epibionts under the microscope and then split into two groups: non-epibiotic *Capitella* (3 batches of 10 individuals each) and epibiotic *Capitella* (3 batches of 10 individuals each). Each batch was placed in a petri dish (35mm) containing 2 mL of artificial seawater (Instant Ocean). The 3 "non epibiotic" batches and the 3 "epibiotic" batches were separately exposed to increasing concentrations of sulfides (batch 0 mM, 1 mM and 3 mM of Na_2S $9H_2O$ (SIGMA) in artificial seawater (Instant Ocean® Sea Salt) for 4 days in a moisture chamber in the dark at 16°C. Mortality was assessed every 3 hours, dead animals were counted and immediately removed. The sulfide concentration was also measured and adjusted when required at the same intervals by using the N, N-dimethyl-p-phenylenediamine colorimetric method (Walkley and Black, 2003).

Survivorship data were analyzed through Cox proportional hazard models (Andersen and Gill, 1982), using the 'coxph' function within the 'survival' package in R programming language (Jackson, 2016). All survival data were analyzed together (same mortality 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

reduced sulfur) ranged from 141 to 978 mgS kg^{-1} and from 447 and 712 mgS kg^{-1} for the Roscoff Harbor and Le Laber sites, respectively.

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

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

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

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

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

Morphological observations – In October 2013 (RNAseq sampling date), around 20% of worms exhibited an epibiosis with long white hair-like projection at the Roscoff Harbor (Figs. 2B, 3B, D). This association was also observed in some *Capitella* worms from Le Laber during the 2015 temporal survey of the two localities, notably during the summer period. Electron microscopy and FISH hybridizations using the probe EUB338 evidenced dense assemblages of filamentous structures (Figs 3D, 4A), with small bacteria attached to larger and more visible ones strongly anchored in the tegument (Figs. 3B, 4B, C) of the epibiotic *Capitella* worms only (Fig. 4, Figs. 3F, G). The larger epibiotic microorganisms were easily observable under transmission or light microscopy (Fig. 3A-D) displaying a size reaching 50 microns from basal to apical ends with refringent cytoplasmic inclusions resembling sulfur granules typical of sulfur-oxidizing bacteria, the lack of a nucleus based on DAPI staining (Fig. 4) and the presence of a large vacuole in the center of the cells (Figs 4D, E). Large *Capitella* epibionts also displayed a larger basal bacterium with an elongated rod shape, atop of which a second, spherical-to-elongate bacterium is budding.

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

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

which could be either epibionts (tegument), part of the gut contents, or parasites. In the following analyses, we only considered contigs with abundances greater than 100 FPKM in at least one of the libraries. Some of these may correspond to different fragments of the same organism (*e.g.* fragment of 28S, another fragment of 28S, fragment of 18S, etc.).

The sequence assembly followed by quantification allowed us to identify contigs corresponding to associated organisms that are found in all three groups (1/ non epibiotic *Capitella* from Le Laber 2/ non epibiotic *Capitella* from the Roscoff Harbor and 3/ epibiotic *Capitella* from the Roscoff Harbor) but in variable abundances (Table 3), contigs that are more common at Le Laber (Table 4), and contigs that are more abundant in the group corresponding to animals with epibiotic microorganisms (Table 5). *Capitella* from the three compared groups are host to a variety of eukaryotes at intermediate occurrence (Tables 3-5).

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

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

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

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

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

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

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

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

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

Observed prevalence of *Thiomargarita* fluctuated between zero and 0.44 among sampling dates (average over the year: 0.10), with 95% of observations between zero and 0.31 and a median prevalence of 0.08. Worm size as estimated from the width at the fifth setiger varied between 0.19 and 1.56 mm (average: 0.54 mm) with a slightly fluctuating average value (between 0.43 and 0.68 mm), without any clear temporal trend. The numbers of males, females and undetermined individuals also do not vary much between sampling dates (Fig. S7). A statistical analysis of time-series was performed using the association occurrences as a quantitative variable and the sampling date, size and gender of the worm as explanatory variables. Overall, the probability of association with *Thiomargarita* increases in summer and increases with the worm's size (Fig. 6). Independently of worm's size, this probability is also higher for males and undetermined individuals than for females (Figs. S5 and S6). As many models have comparable AICc and Akaike values (Table S6), model predictions have been explored using the Akaike-weighted average of all tested models (Figs. 6, S5 and S6). The analysis of evidence ratios (ratio of Akaike weights of models incorporating or not the focal variable) of all explanatory variables (Table S7) indicates that all variables have *likely* effects, except 'site' (*implausible* effect), and 'sex:date', 'sex:date^2' and 'sex:size:date^2' interactions (only *plausible* effects) using the vocabulary of (Massol et al., 2007).

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

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

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

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

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

4. DISCUSSION

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

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

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

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

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

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

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

Unlike its close relatives *Thioploca* and *Beggiatoa*, *Thiomargarita* are not motile. They store elemental sulfur as granules at the periphery of a very large vacuole that occupies 98% of the cell volume where nitrate is stored (Schulz, 2006). Because of their lack of motility, *Thiomargarita* cells must live in an environment where they will be alternatively exposed to sulfide in the porewater and to nitrate in the overlaying seawater. Compared to previously reported *Thiomargarita* morphologies, the cells attached to *Capitella* are more elongated but the observation of budding structures are similar to those reported in *Provanna laevis* and byssal threads of *Bathymodiolus* mussels from deep-sea cold seeps (Bailey et al., 2011), and suggests that the cells are actively growing. Unlike *Thioploca*, whose populations decline at oxygen concentrations greater than 3 μM, and *Beggiatoa* mats, which thrive with oxygen concentration of 1-2.5 μM, *Thiomargarita* cells can withstand exposure to full atmospheric oxygen concentrations (Schulz, 2006). *Thiomargarita* morphotypes have also been observed attached to various debris while sorting the sediment samples, suggesting their ability to efficiently colonize a wide variety of surfaces, including *Capitella.* The presence of *Thiomargarita* can easily be viewed as a 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 thiobiont 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 CO_2 (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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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 1997; 25: 3389-3402.

Amsellem L, Brouat C, Duron O, Porter SS, Vilcinskas A, Facon B. Chapter Three - Importance of Microorganisms to Macroorganisms Invasions: Is the Essential Invisible to the Eye? (The Little Prince, A. de Saint-Exupéry, 1943). In: Bohan DA, Dumbrell AJ, Massol F, editors. Advances in Ecological Research. 57. Academic Press, 2017, pp. 99-146.

Andersen PK, Gill RD. cox's regression model for counting processes: a large sample study. 1982.

Ankley GT, Benoit DA, Hoke RA, Leonard EN, West CW, Phipps GL, et al. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. Archives of Environmental Contamination and Toxicology 1993; 25: 12-19.

Audry S, Schäfer J, Blanc G, Jouanneau J-M. Fifty-year sedimentary record of heavy metal pollution (Cd, Zn, Cu, Pb) in the Lot River reservoirs (France). Environmental Pollution 2004; 132: 413-426.

Bailey JV, Salman V, Rouse GW, Schulz-Vogt HN, Levin LA, Orphan VJ. Dimorphism in methane seep-dwelling ecotypes of the largest known bacteria. The Isme Journal 2011; 5: 1926.

Bellec L, Bonavita M-AC, Hourdez S, Jebbar M, Tasiemski A, Durand L, et al. Chemosynthetic ectosymbionts associated with a shallow-water marine nematode. Scientific Reports 2019; 9: 7019.

Bernier C, Boidin-Wichlacz C, Tasiemski A, Hautekeete N, Massol F, Cuvillier-Hot V. Transgenerational Immune Priming in the Field: Maternal Environmental Experience Leads to Differential Immune Transfer to Oocytes in the Marine Annelid *Hediste diversicolor*. Genes (Basel) 2019; 10.

734 Blake JA, Grassle JP, Eckelbarger KJ. *Capitella teleta* , a new species designation for the
735 opportunistic and experimental *Capitella* sp. I, with a review of the literature for
736 confirmed records. 2009 2009; 2: 29.
737 Boaden PJS. Anaerobiosis, Meiofauna and Early Metazoan Evolution. Zoologica Scripta
738 1975; 4: 21-24.
739 Boidin-Wichlacz C, Jollivet D, Papot C, Roisin L, Massol F, Tasiemski A. Genetic
740 diversification and life-cycle of the polychaete *Capitella* spp. from the English
741 Channel: Evidence for sympatric cryptic species and alternative reproductive
742 strategies. Under review.
743 Bright M, Lallier FH. The biology of Vestimentiferan tubeworms. Oceanography and Marine
744 Biology: An Annual Review, Volume 48. CRC Press, 2010, pp. 213-265.
745 Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR. Phylosymbiosis:
746 Relationships and Functional Effects of Microbial Communities across Host
747 Evolutionary History. PLoS biology. 15, 2017, pp. e1002587.
748 Bulgheresi S. All the microbiology nematodes can teach us. FEMS Microbiology Ecology
749 2016; 92.
750 Burnham KP, Anderson DR, Huyvaert KP. AIC model selection and multimodel inference in
751 behavioral ecology: some background, observations, and comparisons. Behavioral
752 Ecology and Sociobiology 2011; 65: 23-35.
753 Carrier TJ, Reitzel AM. The Hologenome Across Environments and the Implications of a
754 Host-Associated Microbial Repertoire. Frontiers in Microbiology 2017; 8.
755 Cavanaugh CM. Symbiotic chemoautotrophic bacteria in marine invertebrates from
756 sulphide-rich habitats. Nature 1983; 302: 58-61.
757 Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB. Prokaryotic cells in the
758 hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic
759 symbionts. Science 1981; 213: 340-342.
760 Chabrerie O, Massol F, Facon B, Thevenoux R, Hess M, Ulmer R, et al. Biological Invasion
761 Theories: Merging Perspectives from Population, Community and Ecosystem Scales.
762 Wiley, 2019.
763 Clay K. Editorial: Defensive symbiosis: a microbial perspective. Functional Ecology 2014;
764 28: 293-298.
765 Cuomo MC. Sulphide as a larval settlement cue forCapitella sp I. Biogeochemistry 1985; 1:
766 169-181.
767 Cuvillier-Hot V, Gaudron SM, Massol F, Boidin-Wichlacz C, Pennel T, Lesven L, et al. Immune
768 failure reveals vulnerability of populations exposed to pollution in the bioindicator
769 species Hediste diversicolor. Science of The Total Environment 2018; 613-614:
770 1527-1542.
771 Davide V, Pardos M, Diserens J, Ugazio G, Thomas R, Dominik J. Characterisation of bed
772 sediments and suspension of the river Po (Italy) during normal and high flow
773 conditions. Water Research 2003; 37: 2847-2864.
774 Desbruyères D, Gaill F, Laubier L, Prieur D, Rau GH. Unusual nutrition of the "Pompeii
775 worm" *Alvinella pompejana* (polychaetous annelid) from a hydrothermal vent
776 environment: SEM, TEM, 13C and 15N evidence. Marine Biology 1983; 75: 201-205.
777 Dion E, Zélé F, Simon J-C, Outreman Y. Rapid evolution of parasitoids when faced with the
778 symbiont-mediated resistance of their hosts. Journal of Evolutionary Biology 2011;
779 24: 741-750.
780 Dubilier N. H2S—A settlement cue or a toxic substance for *Capitella* sp. I larvae? The
781 Biological Bulletin 1988; 174: 30-38.

Dubilier N, Bergin C, Lott C. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat Rev Microbiol 2008; 6: 725-40.
Dubilier N, Blazejak A, Rühland C. Symbioses between Bacteria and Gutless Marine Oligochaetes. In: Overmann J, editor. Molecular Basis of Symbiosis. Springer Berlin Heidelberg, Berlin, Heidelberg, 2006, pp. 251-275.
Duperron S. Characterization of Bacterial Symbionts in Deep-Sea Fauna: Protocols for Sample Conditioning, Fluorescence In Situ Hybridization, and Image Analysis. In: McGenity TJ, Timmis KN, Nogales B, editors. Hydrocarbon and Lipid Microbiology Protocols: Field Studies. Springer Berlin Heidelberg, Berlin, Heidelberg, 2017, pp. 343-362.
Duperron S, Halary S, Lorion J, Sibuet M, Gaill F. Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel Idas sp. (Bivalvia: Mytilidae). Environmental Microbiology 2008; 10: 433-445.
Duperron S, Nadalig T, Caprais J-C, Sibuet M, Fiala-Médioni A, Amann R, et al. Dual Symbiosis in a Bathymodiolus sp. Mussel from a Methane Seep on the Gabon Continental Margin (Southeast Atlantic): 16S rRNA Phylogeny and Distribution of the Symbionts in Gills. Applied and Environmental Microbiology 2005; 71: 1694.
Felbeck H. Chemoautotrophic Potential of the Hydrothermal Vent Tube Worm, *Riftia pachyptila* Jones (Vestimentifera). Science 1981; 213: 336-8.
Fenchel TM, Riedl RJ. The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. Marine Biology 1970; 7: 255-268.
Ferrari J, Vavre F. Bacterial symbionts in insects or the story of communities affecting communities. Philos Trans R Soc Lond B Biol Sci 2011; 366: 1389-400.
Gamenick I, Vismann B, Grieshaber MK, Giere O. Ecophysiological differentiation of *Capitella capitata* (Polychaeta). Sibling species from different sulfidic habitats. Marine Ecology Progress Series 1998; 175: 155-166.
Gao Y, Lesven L, Gillan D, Sabbe K, Billon G, De Galan S, et al. Geochemical behavior of trace elements in sub-tidal marine sediments of the Belgian coast. Marine Chemistry 2009; 117: 88-96.
Gasnier-Fauchet F, Gharib A, Nardon P. Comparison of methionine metabolism in symbiotic and aposymbiotic larvae of Sitophilus oryzae L. (Coleoptera: Curculionidae)—I. Evidence for a glycine N-methyltransferase-like activity in the aposymbiotic larvae. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 1986; 85: 245-250.
Gilbert SF, Bosch TCG, Ledon-Rettig C. Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. Nature Reviews Genetics 2015; 16: 611-622.
Gouda HA. The effect of peritrich ciliates on some freshwater leeches from Assiut, Egypt. Journal of Invertebrate Pathology 2006; 93: 143-149.
Grassle J, Grassle J. Sibling species in the marine pollution indicator *Capitella* (polychaeta). Science 1976; 192: 567-569.
Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, et al. Emerging marine diseases--climate links and anthropogenic factors. Science 1999; 285: 1505-10.
Hiroaki T, Sam W, Shigeru M, Megumi S, Sagiri I, Kazuhiro K. Exploitation of a chemosynthetic food resource by the polychaete Capitella sp. I. Marine Ecology Progress Series 2001; 216: 119-127.
Huang X, Madan A. CAP3: A DNA Sequence Assembly Program. Genome Research 1999; 9: 868-877.

Jackson CH. flexsurv: A Platform for Parametric Survival Modeling in R. J Stat Softw 2016; 70.
Kaltenpoth M, Engl T. Defensive microbial symbionts in Hymenoptera. Functional Ecology 2014; 28: 315-327.
Kitamori R. Benthos as an environmental indicator (S) with special reference to indicator species. In: Problems CotJESoE, editor. Aquatic environment. 2. Kankyo to seibutsu shihyo, Kyoritsu Shuppan, Tokyo, p., 1975, pp. 265-273.
Kohl KD, Weiss RB, Cox J, Dale C, Dearing MD. Gut microbes of mammalian herbivores facilitate intake of plant toxins. Ecology Letters 2014; 17: 1238-1246.
Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 2016; 33: 1870-1874.
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007; 23: 2947-2948.
Le Bloa S, Boidin-Wichlacz C, Cueff-Gauchard V, Rosa RD, Cuvillier-Hot V, Durand L, et al. Antimicrobial Peptides and Ectosymbiotic Relationships: Involvement of a Novel Type IIa Crustin in the Life Cycle of a Deep-Sea Vent Shrimp. Front Immunol 2020; 11: 1511.
Le Bris N, Gaill F. How does the annelid Alvinella pompejana deal with an extreme hydrothermal environment? Reviews in Environmental Science and Bio/Technology 2006; 6: 197.
Lee B-G, Lee J-S, Luoma SN, Choi HJ, Koh C-H. Influence of Acid Volatile Sulfide and Metal Concentrations on Metal Bioavailability to Marine Invertebrates in Contaminated Sediments. Environmental Science & Technology 2000; 34: 4517-4523.
Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 2011; 12: 323.
Lourino-Cabana B, Billon G, Lesven L, Sabbe K, Gillan DC, Gao Y, et al. Monthly variation of trace metals in North Sea sediments. From experimental data to modeling calculations. Marine Pollution Bulletin 2014; 87: 237-246.
Macke E, Tasiemski A, Massol F, Callens M, Decaestecker E. Life history and eco-evolutionary dynamics in light of the gut microbiota. Oikos 2017; 126: 508-531.
Margulis L. Symbiogenesis and symbionticism. In: L. Margulis RFE, editor. Symbiosis as a source of evolutionary innovation: speciation and morphogenesis MIT press, Cambridge, 1991, pp. pp 1-14.
Massol F, David P, Gerdeaux D, Jarne P. The influence of trophic status and large-scale climatic change on the structure of fish communities in Perialpine lakes. The Journal of animal ecology 2007; 76: 538-551.
McFall-Ngai M. Are biologists in 'future shock'? Symbiosis integrates biology across domains. Nat Rev Microbiol 2008; 6: 789-92.
McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Loso T, Douglas AE, et al. Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci U S A 2013; 110: 3229-36.
Moran NA, Wernegreen JJ. Lifestyle evolution in symbiotic bacteria: insights from genomics. Trends in Ecology & Evolution 2000; 15: 321-326.
Morsy MR, Oswald J, He J, Tang Y, Roossinck MJ. Teasing apart a three-way symbiosis: Transcriptome analyses of *Curvularia protuberata* in response to viral infection and heat stress. Biochemical and Biophysical Research Communications 2010; 401: 225-230.

877 Nougué O, Gallet R, Chevin L-M, Lenormand T. Niche limits of symbiotic gut microbiota
878 constrain the salinity tolerance of Brine shrimp. The American Naturalist 2015; 186:
879 390-403.
880 Nygren A. Cryptic polychaete diversity: a review. Zoologica Scripta 2014; 43: 172-183.
881 Oliver KM, Smith AH, Russell JA. Defensive symbiosis in the real world – advancing
882 ecological studies of heritable, protective bacteria in aphids and beyond. Functional
883 Ecology 2014; 28: 341-355.
884 Pardo EV, Teixeira LLS, Amaral ACZ. Morphometric analysis of Capitella capitata
885 (Polychaeta, Capitellidae). Iheringia. Série Zoologia 2010; 100: 13-18.
886 Pearson TH, Rosenberg R. Macrobenthic succession in relation to organic enrichment and
887 pollution of the environment. Oceanogr. mar. Biol. A. Rev. 1978: 229-311.
888 Petersen JM, Zielinski FU, Pape T, Seifert R, Moraru C, Amann R, et al. Hydrogen is an energy
889 source for hydrothermal vent symbioses. Nature 2011; 476: 176-180.
890 Polin S, Simon J-C, Outreman Y. An ecological cost associated with protective symbionts of
891 aphids. Ecology and Evolution 2014; 4: 836-840.
892 Polz MF, Felbeck H, Novak R, Nebelsick M, Ott JA. Chemoautotrophic, sulfur-oxidizing
893 symbiotic bacteria on marine nematodes: Morphological and biochemical
894 characterization. Microbial Ecology 1992; 24: 313-329.
895 Reish DJ. Bristle worms (Annelida: Polychaeta). In: Hart CW, Fuller, S. L. H, editor. Pollution
896 ecology of estuarine invertebrates. Academic Press, New York,, 1979, pp. 78-125.
897 Richier S, Furla P, Plantivaux A, Merle P-L, Allemand D. Symbiosis-induced adaptation to
898 oxidative stress. Journal of Experimental Biology 2005; 208: 277.
899 Salman V, Amann R, Girnth A-C, Polerecky L, Bailey JV, Høgslund S, et al. A single-cell
900 sequencing approach to the classification of large, vacuolated sulfur bacteria.
901 Systematic and Applied Microbiology 2011; 34: 243-259.
902 Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets.
903 Bioinformatics 2011; 27: 863-864.
904 Schmieder R, Lim YW, Edwards R. Identification and removal of ribosomal RNA sequences
905 from metatranscriptomes. Bioinformatics 2011; 28: 433-435.
906 Schulz HN. The Genus Thiomargarita. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H,
907 Stackebrandt E, editors. The Prokaryotes: Volume 6: Proteobacteria: Gamma
908 Subclass. Springer New York, New York, NY, 2006, pp. 1156-1163.
909 Simdyanov TG, Guillou L, Diakin AY, Mikhailov KV, Schrével J, Aleoshin VV. A new view on
910 the morphology and phylogeny of eugregarines suggested by the evidence from the
911 gregarine *Ancora sagittata* (Leuckart, 1860) Labbé, 1899 (Apicomplexa:
912 Eugregarinida). PeerJ 2017; 5: e3354.
913 Sterckeman T, Douay F, Baize D, Fourrier H, Proix N, Schvartz C, et al. Trace element
914 distributions in soils developed in loess deposits from northern France. European
915 Journal of Soil Science 2006; 57: 392-410.
916 Stewart FJ, Cavanaugh CM. Symbiosis of Thioautotrophic Bacteria with *Riftia pachyptila*. In:
917 Overmann J, editor. Molecular Basis of Symbiosis. Springer Berlin Heidelberg, Berlin,
918 Heidelberg, 2006, pp. 197-225.
919 Stewart FJ, Newton ILG, Cavanaugh CM. Chemosynthetic endosymbioses: adaptations to
920 oxic–anoxic interfaces. Trends in Microbiology 2005; 13: 439-448.
921 Tasiemski A, Jung S, Boidin-Wichlacz C, Jollivet D, Cuvillier-Hot V, Pradillon F, et al.
922 Characterization and function of the first antibiotic isolated from a vent organism:
923 the extremophile metazoan *Alvinella pompejana*. PLoS One 2014; 9: e95737.
924 Tasiemski A, Massol F, Cuvillier-Hot V, Boidin-Wichlacz C, Roger E, Rodet F, et al. Reciprocal
925 immune benefit based on complementary production of antibiotics by the leech

926 *Hirudo verbana* and its gut symbiont *Aeromonas veronii*. Scientific Reports 2015; 5:
927 17498.
928 Teasdale PR, Hayward S, Davison W. In situ, High-Resolution Measurement of Dissolved
929 Sulfide Using Diffusive Gradients in Thin Films with Computer-Imaging
930 Densitometry. Analytical Chemistry 1999; 71: 2186-2191.
931 Tsutsumi H, Wainright S, Montani S, Saga M, Ichihara S, Kogure K. Exploitation of a
932 chemosynthetic food resource by the polychaete *Capitella* sp. I. Marine Ecology
933 Progress Series 2001; 216: 119-127.
934 Wada M, Wu SS, Tsutsumi H, Kita-Tsukamoto K, Hyung-Ki D, Nomura H, et al. Effects of
935 sodium sulfide on burrowing activity of *Capitella* sp. I and bacterial respiratory
936 activity in seawater soft-agar microcosms. Plankton and Benthos Research 2006; 1:
937 117-122.
938 Waldichuk M. The assessment of sublethal effects of pollutants in the sea. Review of the
939 problems. Philos Trans R Soc Lond B Biol Sci 1979; 286: 399-424.
940 Walkley A, Black C. Centre d'expertise en analyse environnementale du Québec et Ministère
941 de l'agriculture, des pêcheries et de l'alimentation du Québec. Détermination de la
942 matière organique par dosage du carbone organique dans les sols agricoles:
943 méthode Walkley-Black modifiée, MA 2003.
944 Wood A. Sulphur cycling on the continents: Wetlands, terrestrial ecosystems and
945 associated water bodies (Scope 48): edited by R.W. Howarth, J.W.B. Stewart and M.V.
946 Ivanov, John Wiley, 1992. £75.00 hbk (xx + 350 pages) ISBN 0 471 93153 5. Trends
947 in Ecology & Evolution 1992; 7: 356.
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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

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

Figure 6: Predicted probability of association with epibiotic microorganisms as a function of the time of the year (month, x-axis) and the size of the worm (in mm, y-axis), obtained from model-averaging 166 GLMs linking site, size, date, date^2 and sex to association with epibiotic microorganisms. Predictions are made for a uniform sampling of worms among the sexes (undetermined, females and males represent 1/3 of the sample each), the sizes (uniform distribution between 0 and 1.8 mm), the sampling dates and the sampling sites. The color of each square on the heatmap indicates the average predicted probability of association of all worms of that size sampled at that date, following the legend on the right.

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

Figure 8: Tolerance tests to sulfides. Kaplan-Meier plots showing the survivorship of non-epibiotic (red) *versus* epibiotic (blue) worms sampled from the Roscoff Harbor (2020) experimentally exposed to 0 (solid lines), 1 mM (dotted lines) and 3 mM (dashed lines) 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 <63µm). 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	*Paramphinome jeffreysi* 28S ribosomal RNA gene, partial sequence	Polychaete	2702	658	243
KX982503.1	0	*Ancora sagittata* 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 *Capitella*	925	48	1594
DQ779991.1	$6\ 10^{-88}$	*Gymnodinium aureolum* 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	*Eimeria leucisci* 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	*Vorticella* 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	$5\ 10^{-88}$	*Rhabditoides inermiformis* 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}	*Contracaecum spiculigerum* 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}	*Sarcocystis arctica* isolate Vl2.2 28S ribosomal RNA gene, partial sequence	Vertebrate parasite	201	0	398
KC869522.1	9 10^{-88}	*Isodictya grandis* voucher NCI439 28S ribosomal RNA gene, partial sequence	Marine sponge	380	74	394
FJ969135.1	0	*Plectus tenuis* 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}	*Fukuia kurodai* ooyagii 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	*Sphaerospora dicentrarchi* 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}	*Sphaerospora dicentrarchi* 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	*Formosa agariphila* KMM 3901, complete genome	Algal bacterial associate	287	1	19

FJ417074.1	0	*Sphaerospora* sp. M0379 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	285	8	76
AY641571.1	0	*Kudoa iwatai* isolate J small subunit ribosomal RNA gene, partial sequence	Fish parasite	276	6	102
FQ032815.1	$4\ 10^{-131}$	Uncultured *Sphingobacteria* bacterium, whole genome shotgun sequence	?	265	0	11
JQ723993.1	$5\ 10^{-180}$	*Vorticellides* 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	*Cytophaga* 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	$5\ 10^{-98}$	*Phaeodactylum tricornutum* chloroplast, complete genome	Diatom	222	0	117
DQ377695.1	0	*Sphaerospora* 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	$8\ 10^{-178}$	*Sphaerospora dicentrarchi* 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	$2\ 10^{-133}$	Eurythoe sp. AMW4444 28S ribosomal RNA gene, partial sequence	Polychaete	154	36	117
FN563149.1	0	*Rhodococcus equi* 103S chromosome	Pathogen causing pneumonia in horses	148	1	7
AB636470.1	10^{-98}	*Kudoa ogawai* 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}	*Kudoa dianae* isolate M0290 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	133	0	77

GU479649.1	0	*Tubificoides brownae* 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	$4\ 10^{-103}$	*Sphaerospora dicentrarchi* isolate M0749 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	128	18	37
JX178767.1	$2\ 10^{-108}$	*Zoothamnium* sp. 1 JG-2011 18S ribosomal RNA gene, partial sequence	Peritrich ciliate	95	49	120
CP004404.1	$7\ 10^{-169}$	*Psychromonas* 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	$7\ 10^{-77}$	*Toxoplasma gondii* strain CASTELLS chromosome Ia region 5 genomic sequence	Animal parasite	651	95	1
JN256118.1	$6\ 10^{-72}$	*Sarcocystis* sp. ex *Corvus monedula* isolate kuos1 28S ribosomal RNA gene, partial sequence	Bird parasite	582	0	1
FJ417076.1	$4\ 10^{-114}$	*Sphaerospora* sp. M0379 28S large subunit ribosomal RNA gene, partial sequence	Fish parasite	478	79	2
X75453.1	$7\ 10^{-99}$	*Toxoplasma gondii* (strain P) rDNA for 17s,5.8s,26s, and 5s ribosomal RNA	Animal parasite	468	5	0
CP001601.1	0	*Corynebacterium aurimucosum* ATCC 700975, complete genome	Mycolic acid-containing actinomycetes	436	0	2
HQ243019.1	$3\ 10^{-35}$	Uncultured *Glomus* 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	$5\ 10^{-58}$	*Sarcocystis mucosa* 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 *Thiomargarita* 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 *Alviniconcha*	19	0	2477
JQ768460.1	$7\ 10^{-130}$	*Spiroplasma* 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 *Hepatoplasma crinochetorum* 23S ribosomal RNA, complete sequence	Isopod midgut gland mollicute bacterium	4	7	1625
NR_076721.1	0	*Thioalkalivibrio* sp. K90mix strain K90mix 23S ribosomal RNA, complete sequence	Haloalkaliphilic sulfur-oxidizing bacterium	9	1	1359
FR774200.1	0	*Thiomargarita* 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	$5\ 10^{-66}$	*Mycoplasma pulmonis* 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 *Thiomargarita nelsonii* partial 16S rRNA gene and ITS1, isolate NAM071	Giant sulfur bacterium	0	0	1170
FR690946.1	10^{-109}	Candidatus *Thiomargarita nelsonii* 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}	*Oleispira antarctica* 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	*Kangiella koreensis* DSM 16069 strain DSM 16069 23S ribosomal RNA, complete sequence	Oceanospirillales free-living bacterium	3	0	589
NR_076212.1	$2\ 10^{-131}$	*Treponema denticola* 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	$4\ 10^{-116}$	*Rhabditoides inermiformis* 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}	*Marinomonas mediterranea* MMB-1 strain MMB-1 23S ribosomal RNA, complete sequence	Free-living melanogenic bacterium	0	0	460
NR_076770.1	10^{-144}	*Spirochaeta smaragdinae* 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	*Desulfuromonas acetoxidans* 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	*Spiroplasma litorale* 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	*Oleispira antarctica* strain RB-8, complete genome sequence	Hydrocarbonoclastic aerobic bacterium	0	0	262
JN018328.1	$3\ 10^{-56}$	*Damon gracilis* voucher MNHN-JAB38 28S ribosomal RNA gene, partial sequence	Whip-spider	0	0	230
NR_102551.1	0	*Thioalkalivibrio nitratireducens* 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	$3\ 10^{-61}$	*Desulfobacca acetoxidans* DSM 11109 strain DSM 11109 23S ribosomal RNA, complete sequence	Deltaproteobacterium Sulfate reducer	0	0	138
GU245692.1	$4\ 10^{-59}$	*Krefftascaris sharpiloi* 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	$2\ 10^{-77}$	*Spiroplasma chrysopicola* DF-1 strain DF-1 23S ribosomal RNA, complete sequence	Dipteran-associated gut mollicute	1	0	116

Figure 1

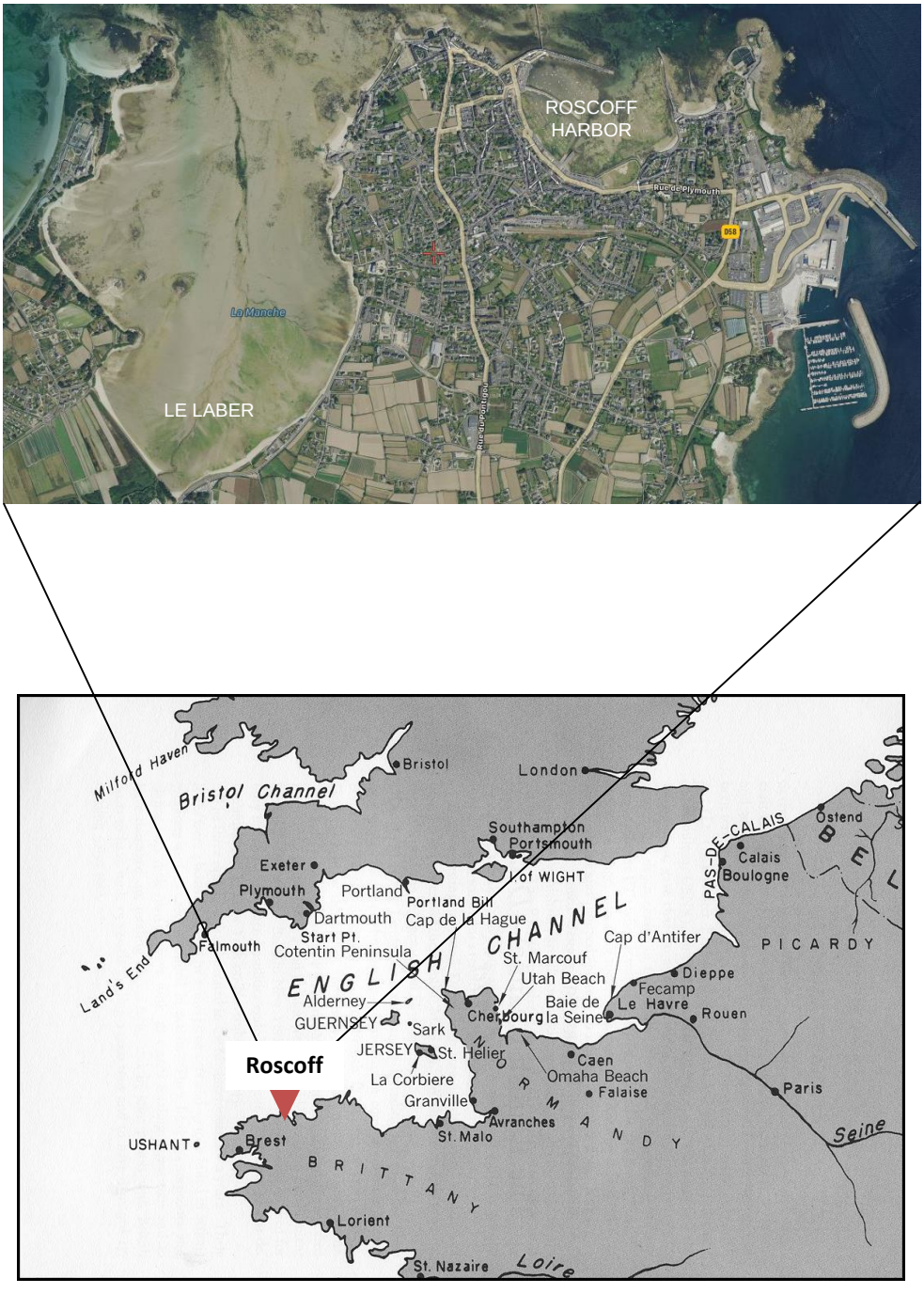
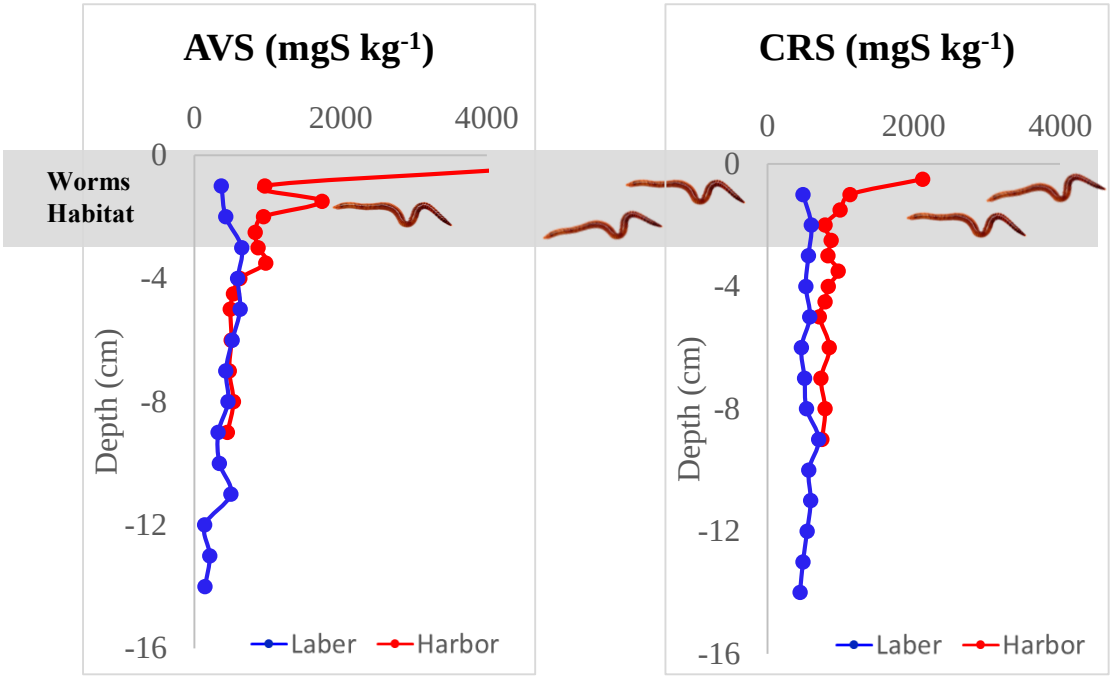


Figure 2

A


AVS (mgS kg-1)
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2000
4000
0
-4
-8
-12
-16
Depth (cm)
Worms Habitat
Laber
Harbor
CRS (mgS kg-1)
0
2000
4000
0
-4
-8
-12
-16
Depth (cm)
Laber
Harbor

B

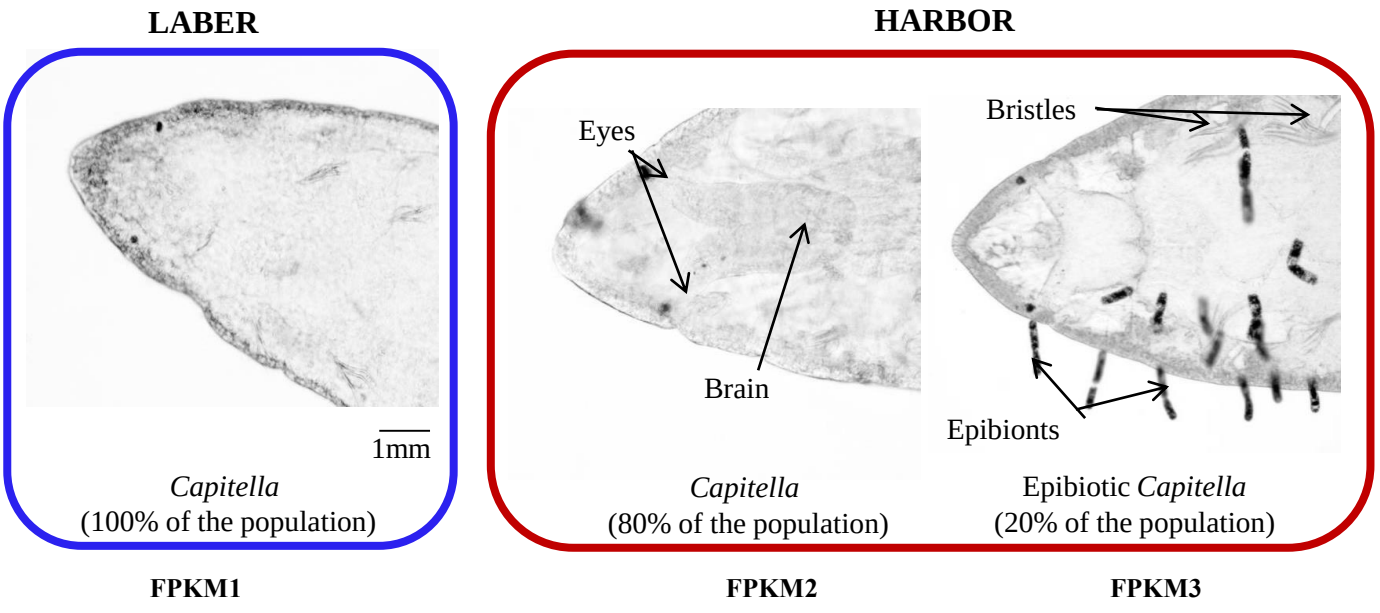
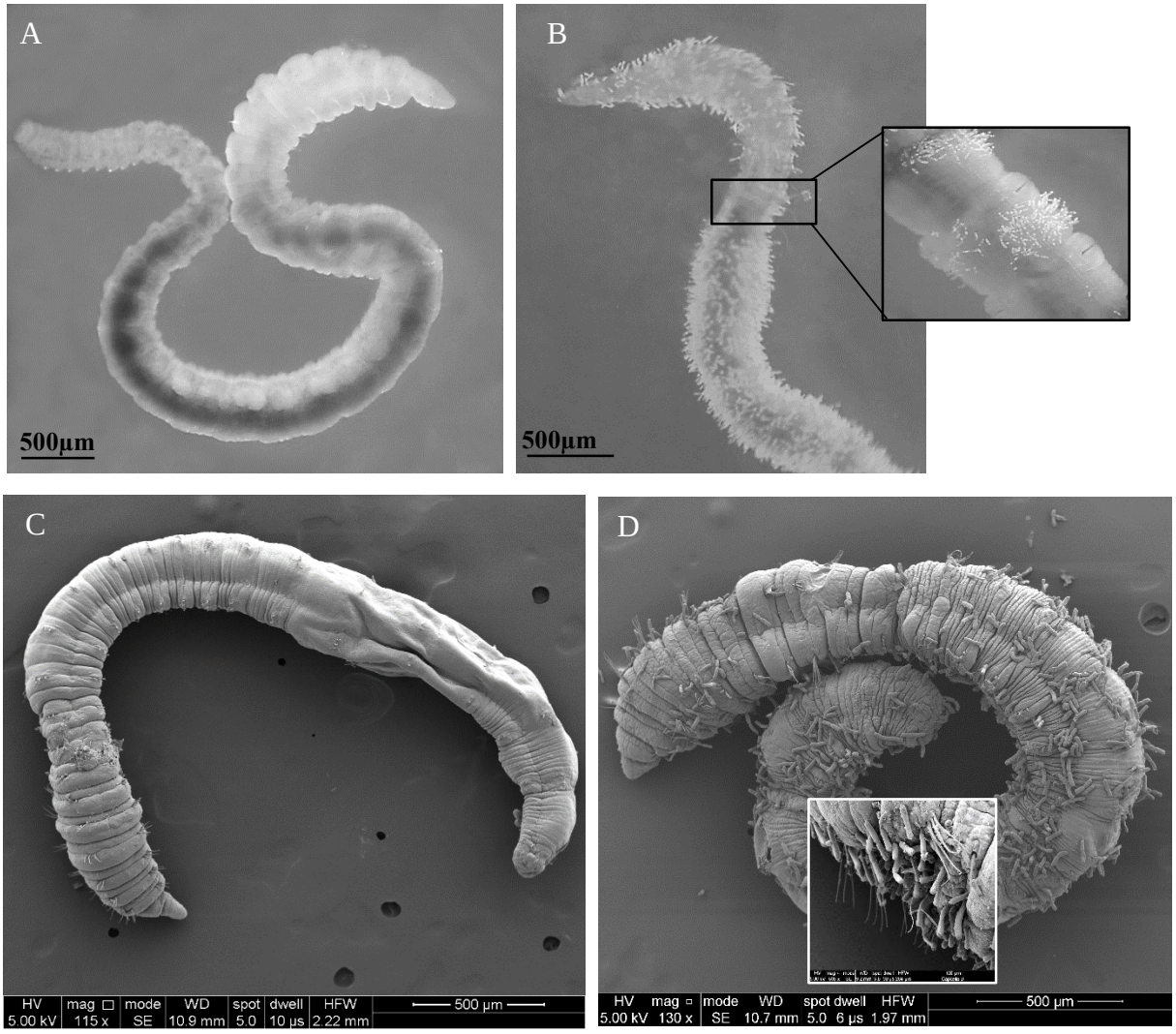

LABER
HARBOR
1mm
Eyes
Brain
Bristles
Epibionts
Capitella
(100% of the population)
Capitella
(80% of the population)
Epibiotic Capitella
(20% of the population)
FPKM1
FPKM2
FPKM3

Figure 3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500µm
B
500µm
C
HV mag mode WD spot dwell HFW
5.00 kV 115 x SE 10.9 mm 5.0 10 µs 2.22 mm
500 µm
D
HV mag mode WD spot dwell HFW
5.00 kV 130 x SE 10.7 mm 5.0 6 µs 1.97 mm
500 µm

Figure 4

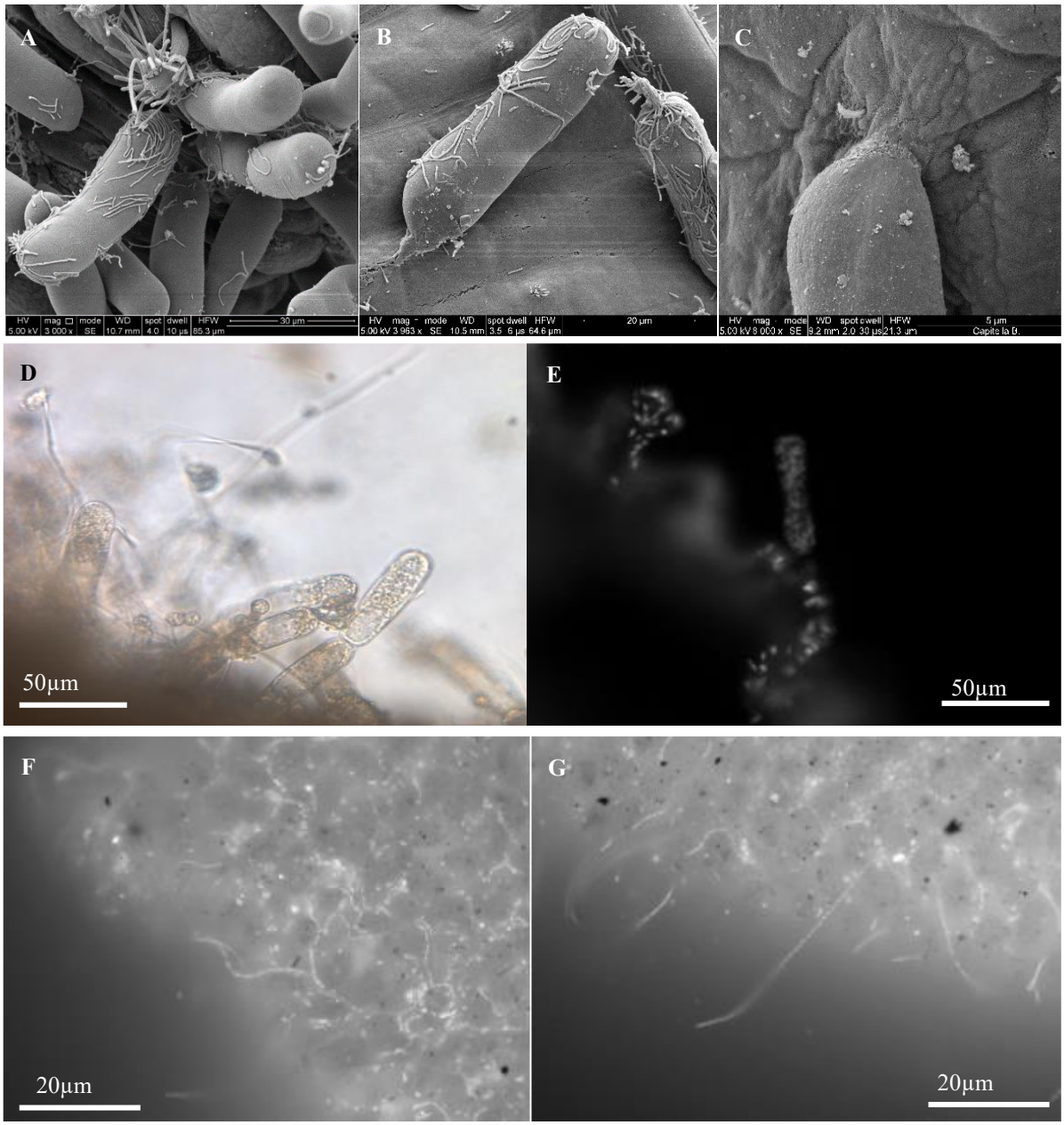
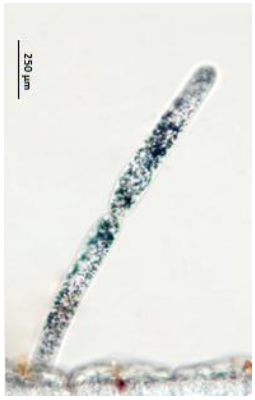


Figure 5

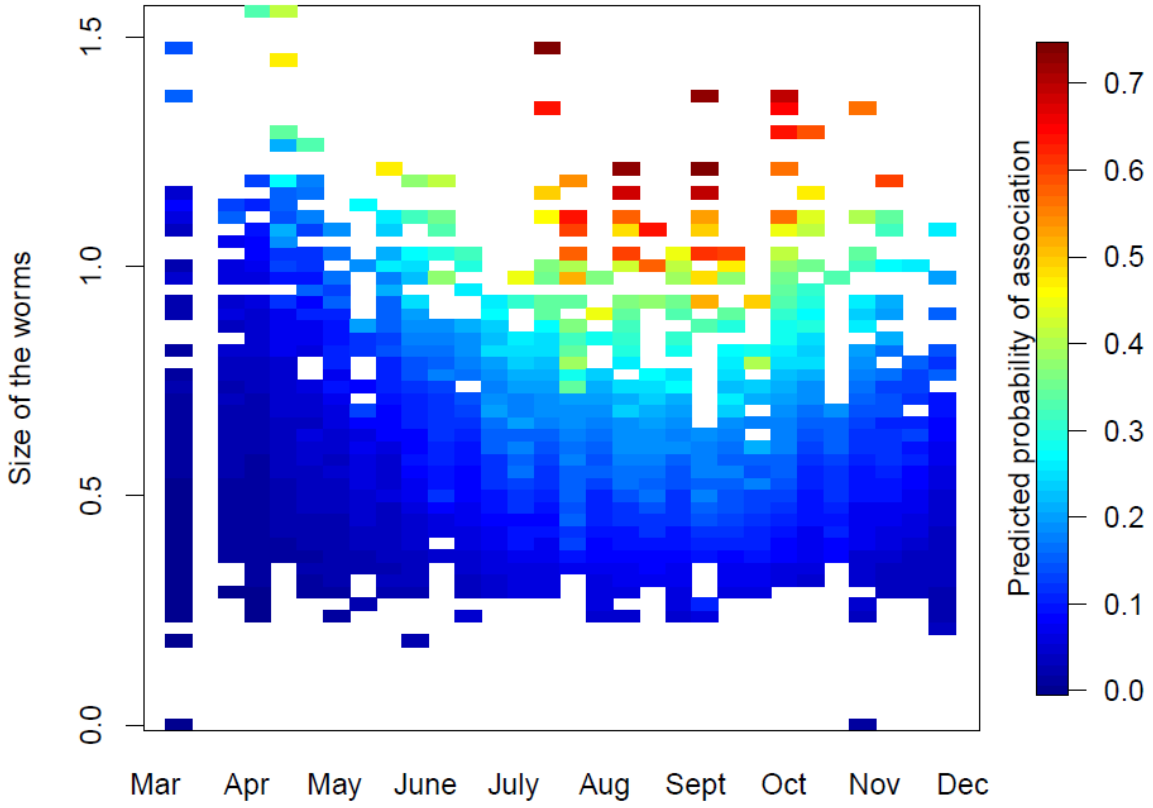


FR690957 *"Candidatus* Thiomargarita nelsonii" isolate NAM069
HF954113 Uncultured *Thiomargarita* sp. clone HYR001
FN811661 *"Candidatus* Thiomargarita nelsonii" isolate Costa COS008
FR690966 *"Candidatus* Thiomargarita nelsonii" isolate COS015
FR690959 *"Candidatus* Thiomargarita nelsonii" isolate NAM071
FR690953 *"Candidatus* Thiomargarita nelsonii" isolate NAM064
HF954103 Uncultured *Thiomargarita* sp. clone NAM094
FR690945 *"Candidatus* Thiomargarita nelsonii" isolate NAM056
FR690927 *"Candidatus* Thiomargarita nelsonii" isolate NAM037
MZ053470 "*Candidatus* Thiomargarita sp." from body surface of *Capitella* spp.
FR690879 *Thiomargarita namibiensis* isolate NAM001
FR690914 *Thiomargarita namibiensis* isolate COS006
AF532774 "*Candidatus* Parabeggiatoa communis" isolate Limfjorden L8
AF532772 "*Candidatus* Parabeggiatoa communis" isolate Limfjorden L22
FJ814745 Uncultured *Beggiatoa* sp. clone V1994 9A02
FR666858 Uncultured *Beggiatoa* sp. clone AMV1058
AF035956 *Beggiatoa* sp. Bay of Concepcion
FR847869 "*Candidatus* Halobeggiatoa sp." clone HMW-R907
FR847865 "*Candidatus* Halobeggiatoa sp." HMW-S2548
FR847884 Uncultured *Beggiatoa* sp. clone HMW-S2139
AF110277 *Beggiatoa* sp. MS-81-6
FR717278 *Beggiatoa* sp. 35Flor

71 58 100 100 61 100 100 50 96 100 100 100

0.020

Figure 6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1.0
1.5
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec
Predicted probability of association
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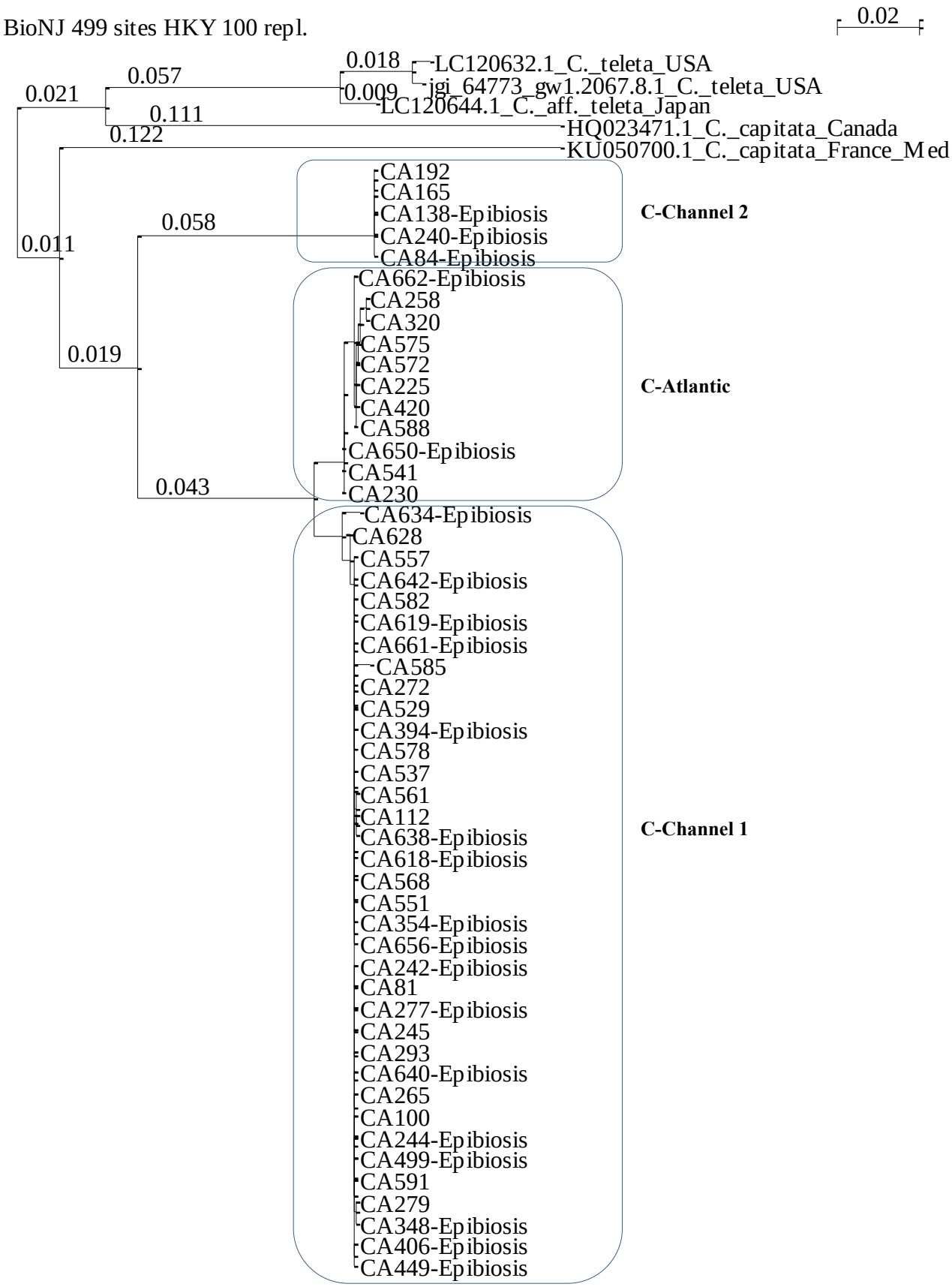
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Figure 7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CA165
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CA84-Epibiosis
C-Channel 2
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CA258
CA320
CA575
CA572
CA225
CA420
CA588
C-Atlantic
0.019
CA650-Epibiosis
CA541
CA230
0.043
CA634-Epibiosis
CA628
CA557
CA642-Epibiosis
CA582
CA619-Epibiosis
CA661-Epibiosis
CA585
CA272
CA529
CA394-Epibiosis
CA578
CA537
CA561
CA112
CA638-Epibiosis
CA618-Epibiosis
CA568
CA551
CA354-Epibiosis
CA656-Epibiosis
CA242-Epibiosis
CA81
CA277-Epibiosis
CA245
CA293
CA640-Epibiosis
CA265
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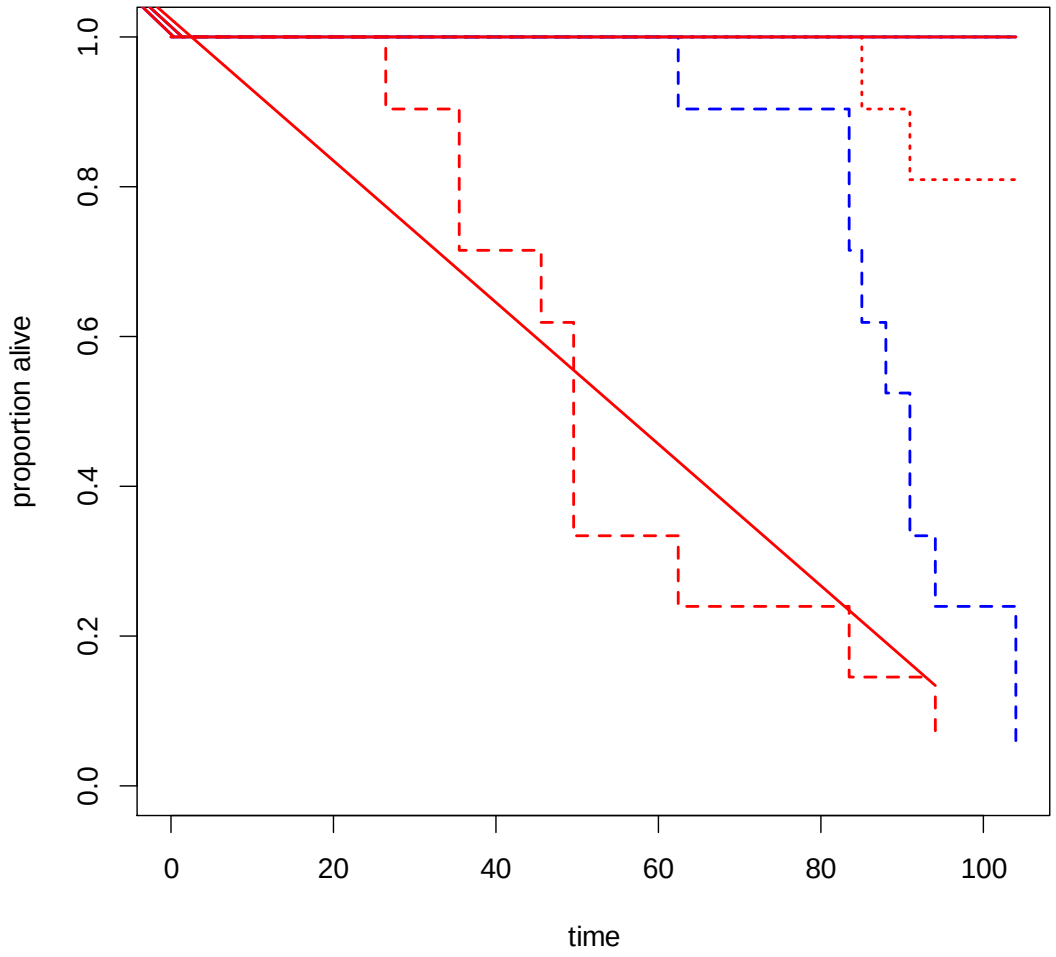
CA406-Epibiosis
CA449-Epibiosis
C-Channel 1

Figure 8



Credit author statement

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: