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

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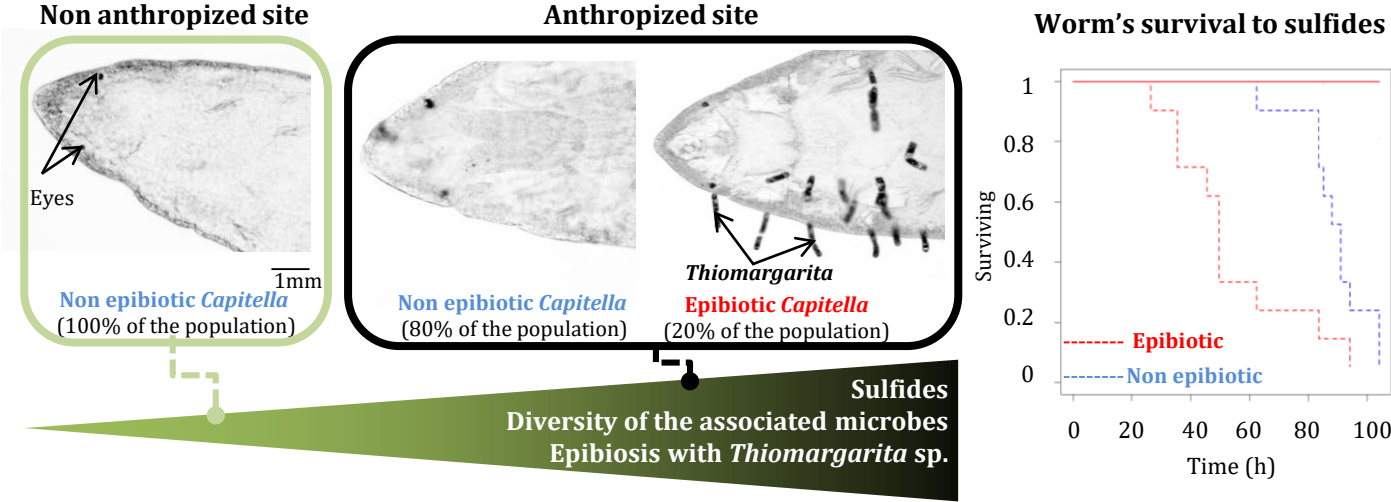
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Highlights

- 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 transient 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

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

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

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

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

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

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

1.3. Microbial communities associated with the worms

worms sampling –For the RNAseq, animals collected in 2013, were checked for 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 Na₂S 9H₂O (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

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

2. RESULTS

3.1. Geochemical characterization of sediments in both sites

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

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

396 which could be either epibionts (tegument), part of the gut contents, or parasites. In the
 397 following analyses, we only considered contigs with abundances greater than 100 FPKM in
 398 at least one of the libraries. Some of these may correspond to different fragments of the
 399 same organism (*e.g.* fragment of 28S, another fragment of 28S, fragment of 18S, etc.).
 400 The sequence assembly followed by quantification allowed us to identify contigs
 401 corresponding to associated organisms that are found in all three groups (1/ non epibiotic
 402 *Capitella* from Le Laber 2/ non epibiotic *Capitella* from the Roscoff Harbor and 3/ epibiotic
 403 *Capitella* from the Roscoff Harbor) but in variable abundances (Table 3), contigs that are
 404 more common at Le Laber (Table 4), and contigs that are more abundant in the group
 405 corresponding to animals with epibiotic microorganisms (Table 5). *Capitella* from the three
 406 compared groups are host to a variety of eukaryotes at intermediate occurrence (Tables 3-
 407 5).
 408 Among the organisms found in all three groups in variable proportions, there is a total of
 409 51 contigs (Table 3). The great majority of these organisms are eukaryotes (86.3%), in
 410 particular apicomplexan fish parasites (31.8%) for which *Capitella* could be an
 411 intermediary host (*e.g.* *Eimeria leucisci*, *Sphaerospora dicentrarchi*, *Kudoa iwatai*,
 412 *Sarcocystis* sp). The contig that ranks second in Table 3 corresponds to the known parasitic
 413 gut gregarine *Ancora sagittata* (Apicomplexa, Ancoridae) (Simdyanov et al., 2017)
 414 specifically associated to *Capitella* (Fig. 4). Other abundant types of organisms are ciliates
 415 (*e.g.* *Vorticella* sp.), nematodes, and annelids that could be part of the gut contents
 416 (*Paramphinoe jeffreysi*, *Tubificoides brownie*; Fig. 3). Bacteria occupy lower ranks in this
 417 list (ranks 28, 31, 34, 35, 44, 49, and 51), which could reflect their lesser abundance and
 418 also the fact they are single-celled (as opposed to most eukaryotes mentioned earlier). The
 419 15 top-ranking contigs are usually more abundant in the epibiotic animals from the Roscoff
 420 Harbor, with the exception of ranks 1 (a polychaete, possibly from the gut contents), 6 and

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:date²' and 'sex:size:date²' 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](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

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

Distinct prokaryotic and eukaryotic associations with the host phenotypes

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

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

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

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

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

Is thiobiont epibiosis a facultative mutualistic association to face transient concentrations of sulfide?

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

646 sulfide in the pore water grow better during the summer period (Schulz, 2006).
 647 *Capitella* is a typical member of the ‘sulfide system’. Fenchel & Riedl (Fenchel and Riedl,
 648 1970) coined this term to describe life under these hostile conditions (later called
 649 ‘thiobiome’ or ‘thiobios’ by Boaden (Boaden, 1975)). Although the thiobiome allows less
 650 competitive stress, specific physical and structural adaptations are needed for the survival
 651 and thriving of this complex and specific biome. Our observations suggest that at highly
 652 “toxic” levels of hydrogen sulfide, physiological adaptations of *Capitella* alone could not be
 653 sufficient to detoxify the reduced sulfur compounds and that a facultative epidermal
 654 association with *Thiomargarita* and other sulfur oxidizing bacteria available in sediment
 655 may constitute a vital additional strategy. The tolerance assay provided here evidenced that
 656 the observed epibiosis is beneficial to the host when subjected to highly sulfide-rich
 657 environments. Besides detoxication, sulfur-oxidizing epibionts may provide nutrients to
 658 the host as suggested for deep sea hydrothermal annelids (Desbruyères et al., 1983).
 659 *Capitella* has been shown to feed on free-living autotrophs that use sulfide oxidation to fix
 660 CO₂ (Hiroaki et al., 2001). Thiobionts might supply *Capitella* in nutrients presumably
 661 explaining why epibiotic specimens are larger than the non-epibiotic ones.
 662 There is ample empirical evidence of symbioses providing protection against specific
 663 natural enemies, e.g. in aphids facing parasitoids and predators (Dion et al., 2011; Oliver et
 664 al., 2014; Polin et al., 2014) or pathogens (Clay, 2014; Tasiemski et al., 2015). Such
 665 symbioses have also been suggested as potential means to explain the success of some
 666 invasive species in new habitats (Amsellem et al., 2017; Chabrerie et al., 2019; Macke et al.,
 667 2017). While many of the aforementioned symbioses involved obligatory endosymbionts,
 668 the present data bring to light an adaptive advantage of a facultative ectosymbiosis to face
 669 changing habitats.
 670 A derived question was to know if this *Capitella-Thiomargarita* association was species-

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

Conclusion

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

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Figure legends

Figure 1: Location of the study (Roscoff, France) with the sampling sites. Sites coordinates 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- 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 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

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

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

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

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

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

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

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

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

Table 1: Total and HCl 1M-extracted metals concentrations in the first 5 cm depth sediments of Le Laber and the Roscoff harbor (fraction <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	<i>Paramphinoe jeffreysi</i> 28S ribosomal RNA gene, partial sequence	Polychaete	2702	658	243
KX982503.1	0	<i>Ancora sagittata</i> isolate Ancora2011 external transcribed spacer, partial sequence; 18S rRNA gene, ITS 1, 5.8S rRNA gene, ITS 2, and 28S rRNA gene, complete sequence; and external transcribed spacer, partial sequence	Gregarine of <i>Capitella</i>	925	48	1594
DQ779991.1	6 10 ⁻⁸⁸	<i>Gymnodinium aureolum</i> strain GrAr01 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, ITS 2, and large subunit ribosomal RNA gene, complete sequence; external transcribed spacer, partial sequence	Dinoflagellate algae	1042	19	1550
EF100367.1	10 ⁻¹¹⁴	Uncultured eukaryote clone D5P10A10 18S ribosomal RNA gene, partial sequence	Apicomplexan?	509	33	1134
EF100398.1	0	Uncultured eukaryote clone D2P03E11 18S ribosomal RNA gene, partial sequence	?	875	31	1086
GU479649.1	0	<i>Eimeria leucisci</i> isolate BLI637-#637 18S ribosomal RNA gene, partial sequence	Coccidian apicomplexa fish parasite	936	43	106
AY179976.1	9 10 ⁻¹⁷⁷	Uncultured eukaryote clone CCI31 18S small subunit ribosomal RNA gene, partial sequence	Apicomplexan?	530	258	869
KC558064.1	5 10 ⁻⁸⁹	Uncultured fungus clone NTS_28S_047E_2_f6 28S ribosomal RNA gene, partial sequence	?	121	0	863
JX178933.1	0	<i>Vorticella</i> sp. 4 JG-2011 clone 33 18S ribosomal RNA gene, partial sequence; ITS 1, 5.8S ribosomal RNA gene, and ITS 2, complete sequence; 28S ribosomal RNA gene, partial sequence	Peritrich ciliate	150	170	840
GU927604.1	4 10 ⁻⁹⁴	Uncultured eukaryote clone F5K2Q4C04IDZ7B 28S ribosomal RNA gene, partial sequence	Ciliate	166	16	636
EF990727.1	5 10 ⁻⁸⁸	<i>Rhabditoides inermiformis</i> strain SB328 28S large subunit ribosomal RNA gene, partial sequence	Free-living nematodes	634	25	542

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

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

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

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

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

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

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

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

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

Figure 1

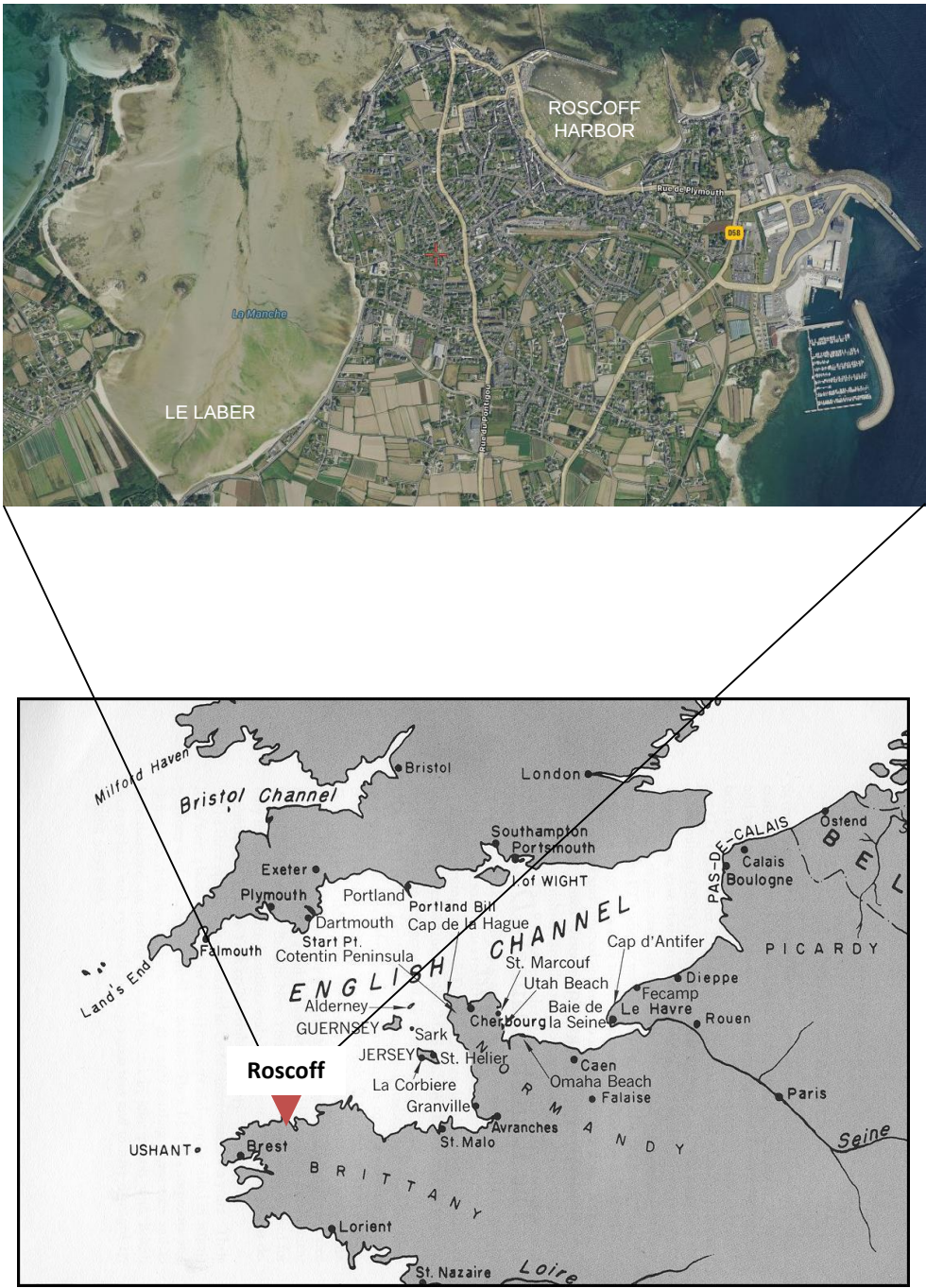
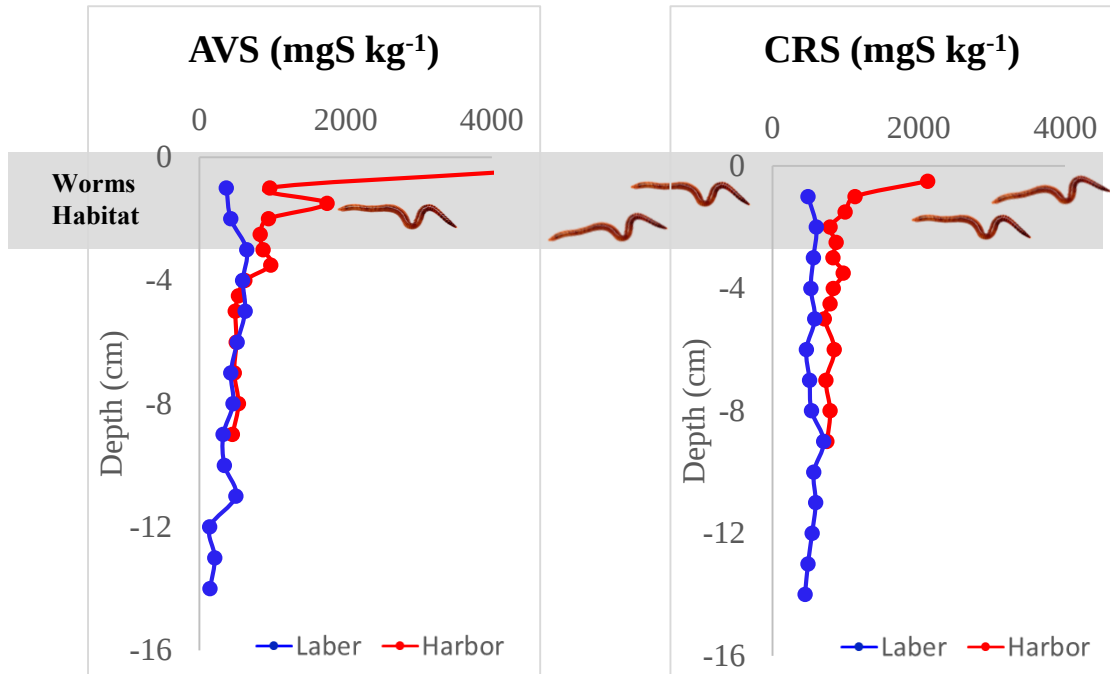


Figure 2

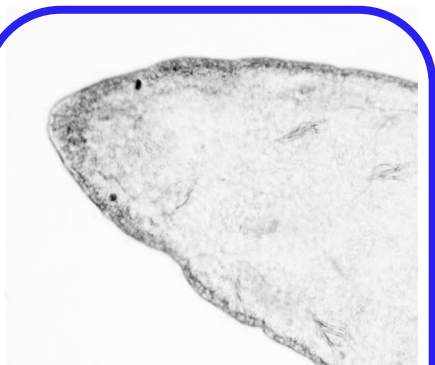
A



B

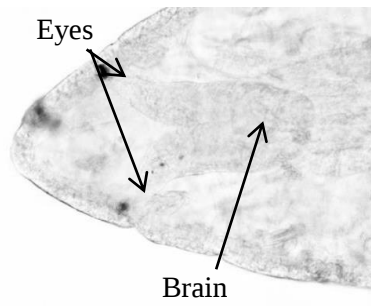
LABER

HARBOR



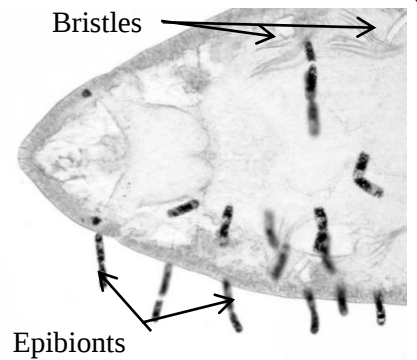
Capitella
(100% of the population)

FPKM1



Capitella
(80% of the population)

FPKM2



Epibiotic Capitella
(20% of the population)

FPKM3

Figure 3

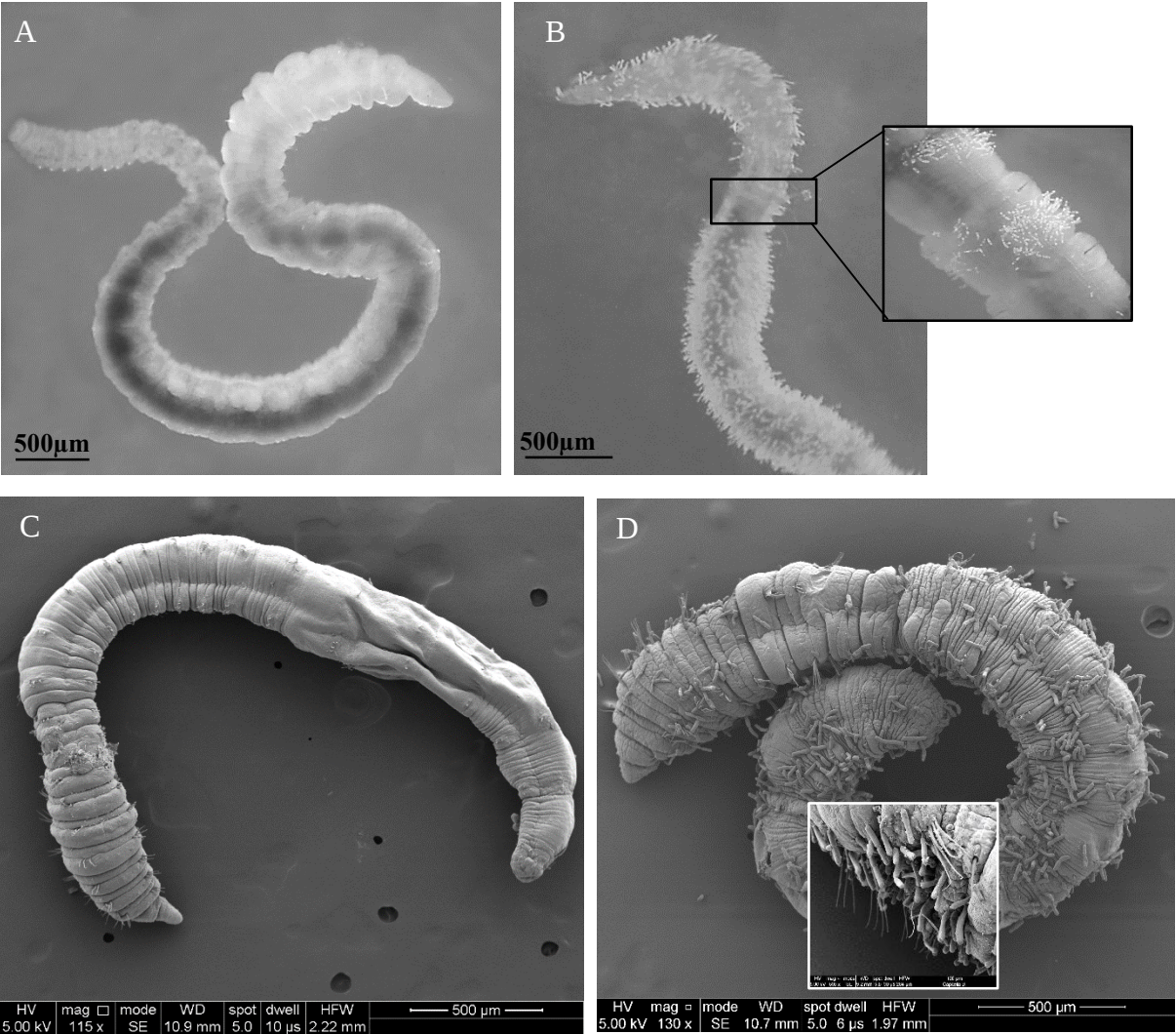


Figure 4

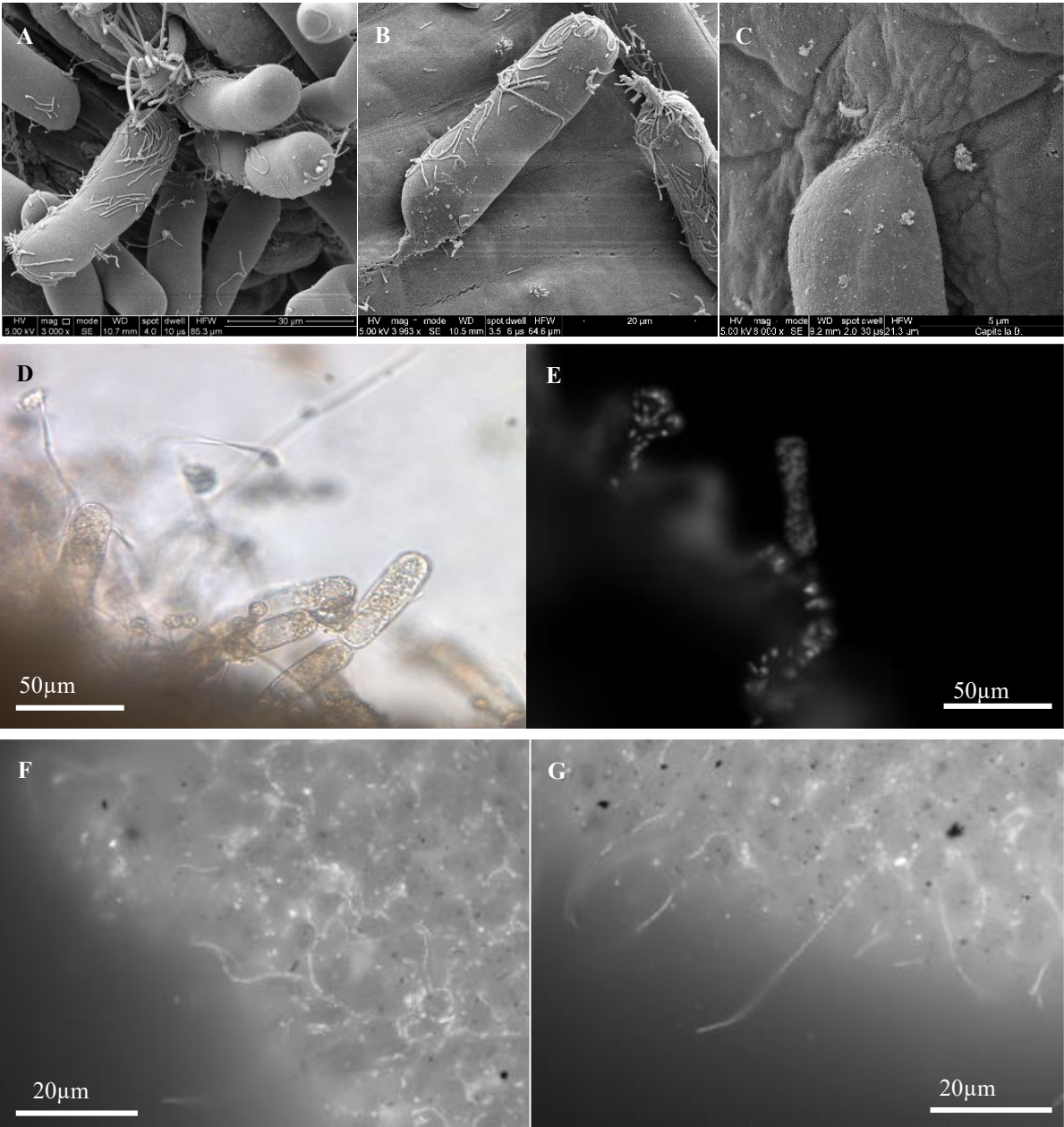


Figure 5

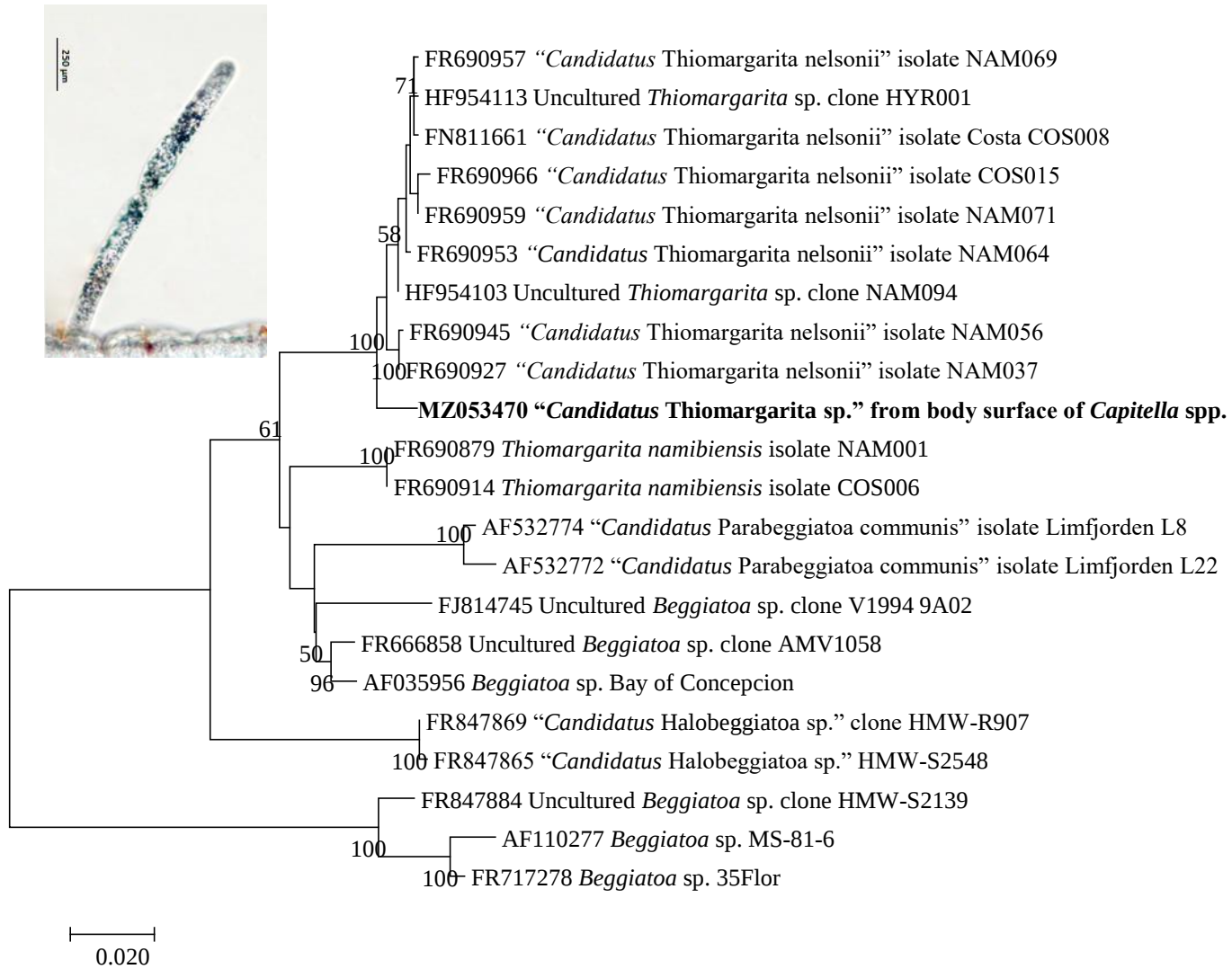


Figure 6

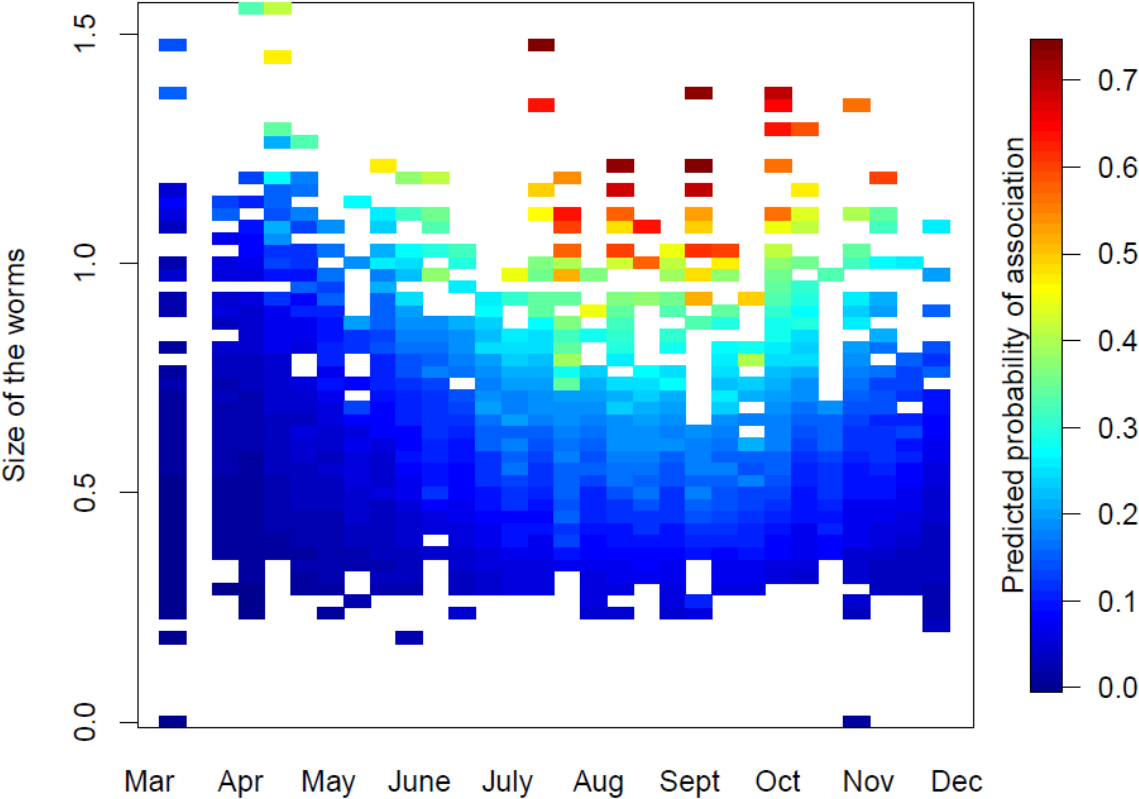


Figure 7

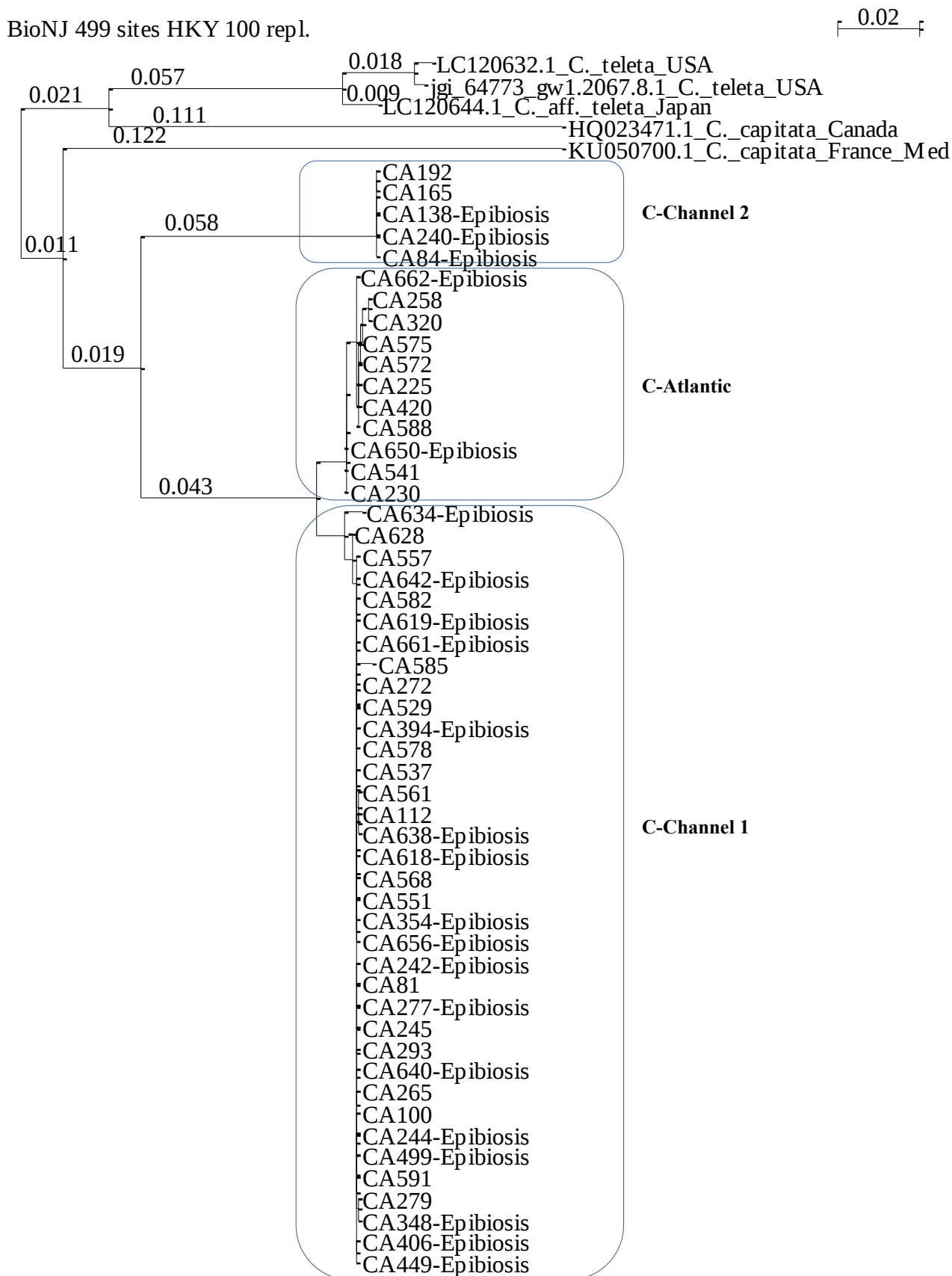
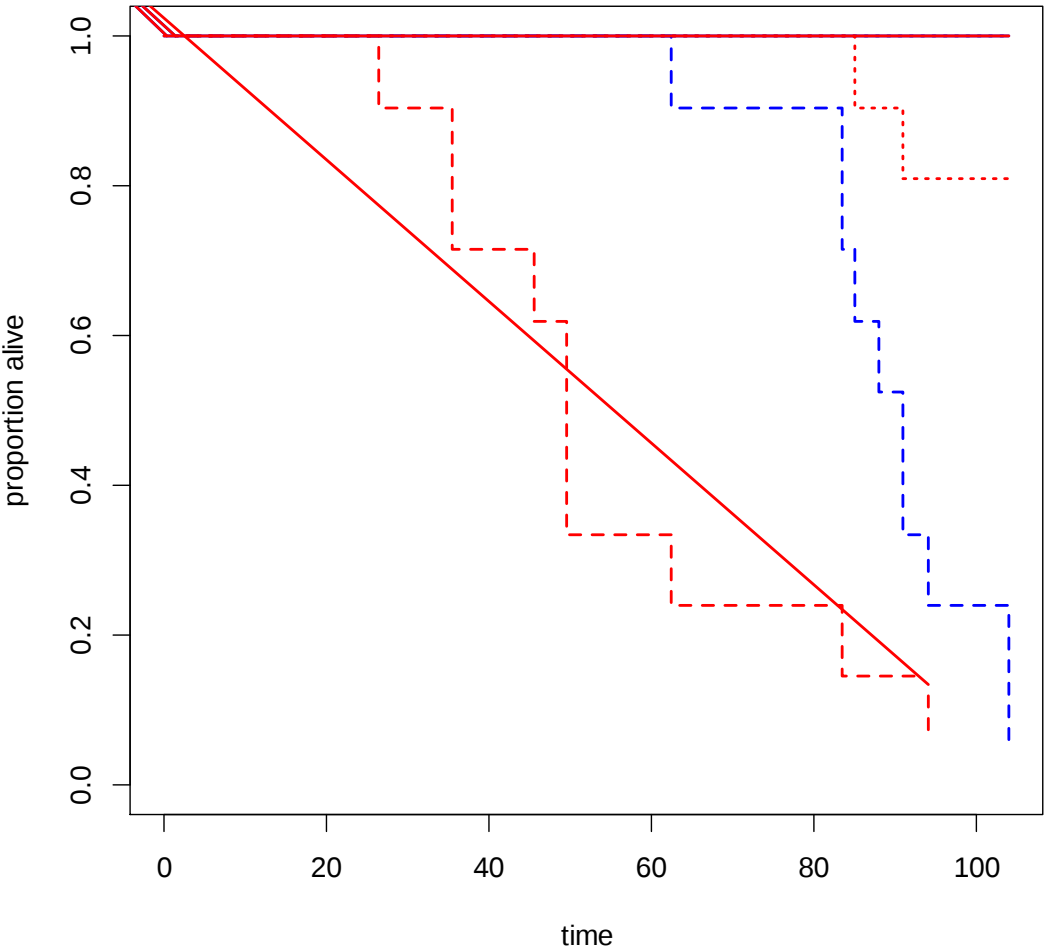
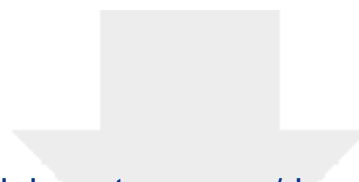


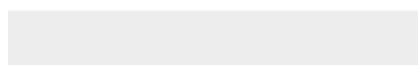
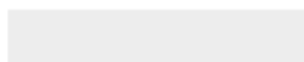
Figure 8





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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: