Supplementary Material

1. Supplementary Data

Probe sequence

As 16S rDNA gene sequences revealed four distinct phylotypes (Durand et al., 2010, 2015; Apremont et al., 2018), three probes were designed to cover the diversity of Mycoplasmatales lineages identified in *R. chacei* and *R. exoculata* foreguts, namely Myco378-1, -2 and -3. A single probe was designed for the unique Deferribacteres phylotype, namely Def1229 (Table 2). The probes designed were: Myco378-1 (**5'GTGGAAAATTCCCTACTGCTG'3**),

Myco378-2 (5'GTGAAAAATTCCCTACTGCTG'3),

Myco378-3 (5' GCGAAAAATTCCCTACTGCTG'3)

and Def1229 (**5'GCCCTCTGTATAGTCCATTG'3**) (Table 2). The location of the three probes Myco378 (nucleotide position 378-398 according to Mycoplasmatales 16S rDNA sequence) on the 16S rRNA sequence is very close to the location of the general Eubacteria probe (nucleotide position 338-358 according to *E. coli* 16S rDNA sequence) but does not overlap and therefore co-hybridization should be possible.

Determination of optimal stringency conditions for the hybridization of the probes Def1229, Myco378-1, Myco378-2 and Myco378-3.

To ensure the specificity of the probes, tests were performed on transversal sections of different organs from several adult specimens, as none of the shrimp-associated bacterial lineages have been grown in the laboratory (Zbinden and Cambon-Bonavita, 2020). The midgut tube is expected to be colonized by Deferribacteres according to previous sequencing results

(Durand et al., 2009, 2105; Apremont et al., 2018), and the foregut is expected to host Mycoplasmatales. Scaphognathites of the cephalothoracic cavity were used as a control for non-specific hybridization since Deferribacteres or Mycoplasmatales were never detected on these structures using DNA sequencing approaches.

Specificity and stringency conditions of Myco378-1, Myco378-2 and Myco378-3 hybridization probes

First, to confirm the specificity of the 3 probes, tests were performed on *R. exoculata* adult foregut sections, all along the organ from the œsophagus towards its posterior end, using hybridization buffer with 30%, 35%, 40% and 45% formamide and a hybridization temperature of 46°C (Supplementary Fig. 1A-C). On part of the foregut, many small rods were identified whatever the Myco378 probe used (with or without co-hybridization with Eub338 probe) using 30% formamide in the hybridization buffer. A higher percentage of formamide showed a better signal on the rods (intense signal with 45% formamide) (Supplementary Fig. 1C). In co-hybridization with Eub338 probe (45% formamide), these foregut rods were revealed by intense fluorescence with both probes in all experiments. These rods were never hybridized with the probes Epsy549 nor GAM42.

R. exoculata adult scaphognathite sections were used to perform the specificity tests with the Myco378 probes. The tests were first performed with Epsy549, GAM42a, or Eub338 co-hybridized with Myco378-1, Myco378-2 or Myco378-3 probes (30-35-40-45-50% formamide in buffer) (Supplementary Fig. 2, Supplementary Table 1). None of the Myco378 probes ever labelled Gammaproteobacteria that were hybridized with the GAM42a probe. Each Myco378 probe slightly hybridized with Campylobacteria cells (the largest filamentous bacteria, hybridized with Epsy549 probe). This faint hybridization signal decreased when a higher percentage of formamide (i.e. higher stringency) was applied to reduce non-specific

hybridization. These non-specific hybridizations were lower at 40% to 50% formamide for Myco378-1, at 45% to 50% formamide for Myco378-2 probes, and at 50% formamide for Myco378-3 probe. Other tests with higher hybridization and washing temperatures (48°C and 50°C respectively) to increase further stringency were performed, but no improvement was obtained. In general, the three Myco378 probes were more specific using 45%-50% formamide concentration. Campylobacteria cells showed weak and rare for Myco378-1 fluorescence (Supplementary Fig. 2, Supplementary Table 1), appearing to be non-specific hybridization signal (as entire Campylobacteria cells hybridized clearly with Epsy-549 probe). For Myco378-2 and more particularly for Myco378-3, few subunits of Campylobacteria gave a fluorescent signal for 45% and 50% formamide.

Thus, the optimal conditions retained to ensure the best specificity of these probes is a hybridization at 46°C for 3 hours with a buffer hybridization containing 45% formamide, washing at 48°C for 30 minutes (Supplementary Table 1). Because the probe Myco378-3 was the least specific (as fluorescent labelling of Campylobacteria cells appeared under all probing conditions), it was not retained for experiments. The probe Myco378-1, which showed the best results in terms of signal specificity and efficiency, was further used in the different FISH experiments on foreguts.

Specificity and stringency conditions of Def1229 probe hybridization

The Def1229 probe was tested on multiple midgut tube sections of an adult *R. exoculata*. Regardless of the section of the midgut tube, hybridization with the Def1229 probe consistently highlighted thin filamentous cells inserted between the microvilli of the midgut epithelial cells (Supplementary Fig. 3A-E). Moreover, varying formamide concentration in the hybridization buffer had no impact on the hybridization signal. In fact, regardless of the stringency condition (20% to 50% formamide), a high fluorescence signal intensity was detected with Def1229 in

the midgut tube (Supplementary Fig. 3A-E, Supplementary Table 2). On sections of adult *R. exoculata* scaphognathites used as negative control and at all stringencies (20% to 50% formamide), no fluorescence was ever detected with the Def1229 probe (Supplementary Fig. 4, Supplementary Fig. 5, Supplementary Fig. 6, Supplementary Table 2).

To further assess Def1229 specificity, co-hybridization with probes Eub338, Epsy549 and GAM42a were carried out on adult midgut tubes and scaphognathites (Supplementary Fig. 4, Supplementary Fig. 5, Supplementary Fig. 6, Supplementary Table 2). These co-hybridizations confirmed the previous results. Indeed, on the scaphognathite sections, large filamentous bacteria (Campylobacteria), thin filamentous bacteria (Gammaproteobacteria), coccoids and rods were only detected with the probes Epsy549 (Campylobacteria), GAM42a (Gammaproteobacteria) and Eub338 (every bacteria), regardless of stringency conditions (20% to 50% formamide) (Supplementary Fig. 4, Supplementary Fig. 5, Supplementary Fig. 6, Supplementary Table 2). On the contrary, bacteria attached to the midgut tube wall cells were only revealed by Eub338 and Def1229 probes (Supplementary Fig. 3A-D, Supplementary Table 2). These observations allowed us to validate the Def1229 probe and its specificity regardless of the stringency condition.

By convention, when formamide concentration has no effect on probe stringency, hybridization buffers containing 30% formamide are retained (in agreement with the optimal protocols described for universal probes: hybridization at 46°C for 3 hours and washing at 48°C for 15 minutes). The Def1229 probe is therefore specific to Deferribacteres of the shrimp *R. exoculata.*

2. Supplementary Figures and Tables

Supplementary Figures

Supplementary Figure 1: Stringency tests with Myco378-1. **(A-C)** represent sections through the œsophagus of *R. exoculata* adult hybridized with Myco378-1-Cy3 only (yellow-green) in **(A)**, orange-pink in **(B, C)**). Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 35% **(A, B)**, 45% **(C)**. White arrows are targeting some Mycoplasmatales observed on different part of the foregut. Images in were taken with Apotome®. **(A1, C1)** represent the same photographs as **(A, C)** without DAPI. Scale bars = 20 µm.

Supplementary Figure 2: Stringency tests with Myco378-1/Eub338, Myco3782-2/ Eub338 and Myco378-3/Eub338. **(A-C)** represent sections through the scaphognathites of *R.exoculata* adult hybridized with Myco378-1-Cy3 (green)/ Eub338-Cy5 (pink) **(A)** or with Myco378-2-Cy3 (green)/ Eub338-Cy5 (pink) **(B)** or with Myco378-3-Cy3 (green)/ Eub338-Cy5 (pink) **(C)**. Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 35% **(A)**, 40% **(B)**, 50% **(C)**. White arrows are targeting some Campylobacteria observed on the setae of the scaphognathites. Pictures were taken with Apotome®. Scale bars = 20 µm.

Supplementary Figure 3: Stringency tests with Def1229. **(A-E)** represent transversal sections through the midgut tube of a *R. exoculata* adult hybridized with Eub338-Cy3 (green) /Def1229-Cy5 (red) **(A-D)** or with Def1229-Cy3 (orange) only **(E)**. Double Hybridizations appear in yellow-green. Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 20% **(A)**, 30% **(B, E)**, 40% **(C)**, 50 % **(D)**. White arrows are targeting

some Deferribacteres observed on the different part of the midgut tube. Images in **(A, B, D, E)**, were taken with Apotome® and with Z-stack, and with DIC (Differential interference contrast) for **(E)** only. Scale bars = 20 µm.

Supplementary Figure 4: Stringency tests with Def1229/ Eub338. **(A-C)** represent sections through the scaphognathites of a *R. exoculata* adult hybridized with Eub338-Cy5 (pink in **(A, B)** and red in **(C)**) /Def1229-Cy3 (white) **(A-C)**. Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 20% **(A)**, 30% **(C)**, 40% **(B)**. White arrows are targeting some Campylobacteria observed on the setae of the scaphognathites Pictures were taken with Apotome®. Scale bars = 20 µm.

Supplementary Figure 5: Stringency tests with Def1229/ GAM42a. **(A-D)** represent sections through the scaphognathites of *R. exoculata* adult hybridized with GAM42a-Cy3 (white) / Def1229-Cy5 (red). Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 20% **(A)**, 30% **(B)**, 40% **(C)**, 50 % **(D)**. White arrows are targeting some Gammaproteobacteria observed on the setae of the scaphognathites Pictures **(B, C, D)** were taken with Apotome®. Scale bars = 20 µm.

Supplementary Figure 6: Stringency tests with Def1229/ Epsy549. **(A-B)** represent sections through the scaphognathites of a *R. exoculata* adult hybridized with Epsy549-Cy5 (red)/ Def1229-Cy3 (white). **(B)** represents a colony of Campylobacteria on host tissue. Tissue cell nuclei are labeled with DAPI (blue). Formamide concentration in hybridization buffer was 30% **(A)**, 40% **(B)**. White arrows are targeting some Campylobacteria observed on the setae of the scaphognathites. Picture **(A)** was taken with Apotome®. Scale bars = 20 µm.

Supplementary Figure 7: Photography of the pyloric chamber of a *R. exoculata* adult in transversal section. **(A)** Entire pyloric chamber observed by autofluorescence in orange with 548 wavelength, image mosaic from 7577 x 7577 µm sections, blue DAPI staining showing host cell nucleus, DIC, Apotome®, scale bar = 200 µm. The blue circle encloses the different plates and the white circle encloses the setae. **(B)** Zoom on the plates of the pyloric chamber. Scale bar = 50 µm. **(C)** Zoom on the setae of the pyloric chamber. Scale bar = 200 µm.

Supplementary Tables

Supplementary Table 1: Summary of the stringency tests performed with the probes Myco378-1, Myco378-2 and Myco378-3. * some segments of *Campylobacteria* gave a positive signal; ** rare segments of *Campylobacteria* gave a positive signal. Probes were hybridized at 46°C, and washed at 48°C.

Formamide %	35	40	45	50	30	35	40	45
Probe \ target tissue	**Scaphognathite**				**Foregut**			
Myco378-1	-/+*	-*	-**	-**	++	++	+++	+++
Myco378-2	+*	-/+*	-/+*	-/+**	++	++	+++	+++
Myco378-3	++*	++*	+*	-/+*	++	++	+++	+++
Eub338	+++	+++	+++	+++	+++	+++	+++	+++
Epsy549	+++	+++	+++	+++	-	-	-	-
GAM42a	+++	+++	+++	+++	-	-	-	-
Myco378-1 + Eub338	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+ Myco378-1 +++Eub338	+ Myco378-1 +++Eub338	++ Myco378-1 +++Eub338	++ Myco378-1 +++Eub338
Myco378-1 + Epsy549	-/+* Myco378-1 +++Epsy549	-* Myco378-1 +++Epsy549	-** Myco378-1 +++Epsy549	-** Myco378-1 +++Epsy549	++ Myco378-1 only	++ Myco378-1 only	+++ Myco378-1 only	+++ Myco378-1 only
Myco378-1 + GAM42a	-/+* Myco378-1+ ++GAM42a	-* Myco378-1 +++GAM42a	-** Myco378-1 +++GAM42a	-** Myco378-1 +++GAM42a	++ Myco378-1 only	++ Myco378-1 only	+++ Myco378-1 only	+++ Myco378-1 only

Myco378-2 + Eub338	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+ Myco378-2 +++Eub338	+ Myco378-2 +++Eub338	++ Myco378-2 +++Eub338	++ Myco378-2 +++Eub338
Myco378-2 + Epsy549	+* Myco378-2 +++Epsy549	-/+* Myco378-2 +++Epsy549	-/+* Myco378-2 +++Epsy549	-/+** Myco378-2 +++Epsy549	++ Myco378-2 only	++ Myco378-2 only	+++ Myco378-2 only	+++ Myco378-2 only
Myco378-2 + GAM42a	+* Myco378-2 +++GAM42a	-/+* Myco378-2 +++GAM42a	-/+* Myco378-2 +++GAM42a	-/+** Myco378-2 +++GAM42a	++ Myco378-2 only	++ Myco378-2 only	+++ Myco378-2 only	+++ Myco378-2 only
Myco378-3 + Eub338	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+ Myco378-3 +++Eub338	+ Myco378-3 +++Eub338	++ Myco378-3 +++Eub338	++ Myco378-3 +++Eub338
Myco378-3 + Epsy549	++* Myco378-3 +++Epsy549	++* Myco378-3 +++Epsy549	+* Myco378-3 +++Epsy549	-/+* Myco378-3 +++Epsy549	++ Myco378-3 only	++ Myco378-3 only	+++ Myco378-3 only	+++ Myco378-3 only
Myco378-3 + GAM42a	++* Myco378-3 +++GAM42a	++* Myco378-3 +++GAM42a	+* Myco378-3 +++GAM42a	-/+* Myco378-3 +++GAM42a	++ Myco378-3 only	++ Myco378-3 only	+++ Myco378-3 only	+++ Myco378-3 only

Supplementary Table 2: Summary of the stringency tests performed with the probe Def1229.

Probes were hybridized at 46°C, and washed at 48°C.

Formamide %	20	30	40	50	20	30	40	50
Probes\target tissue	**Scaphognathite**				**Midgut tube**			
Def1229	-	-	-	-	+++	+++	+++	+++
Eub338	+++	+++	+++	+++	+++	+++	+++	+++
Epsy549	+++	+++	+++	+++	-	-	-	-
GAM42a	+++	+++	+++	+++	-	-	-	-
Def1229 + Eub338	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+++ Eub338 only	+++ both probes	+++ both probes	+++ both probes	+++ both probes
Def1229 + Epsy549	+++ Epsy549 only	+++ Epsy549 only	+++ Epsy549 only	+++ Epsy549 only	+++ Def1229 only	+++ Def1229 only	+++ Def1229 only	+++ Def1229 only
Def1229 + GAM42a	+++ GAM42a only	+++ GAM42a only	+++ GAM42a only	+++ GAM42a only	+++ Def1229 only	+++ Def1229 only	+++ Def1229 only	+++ Def1229 only