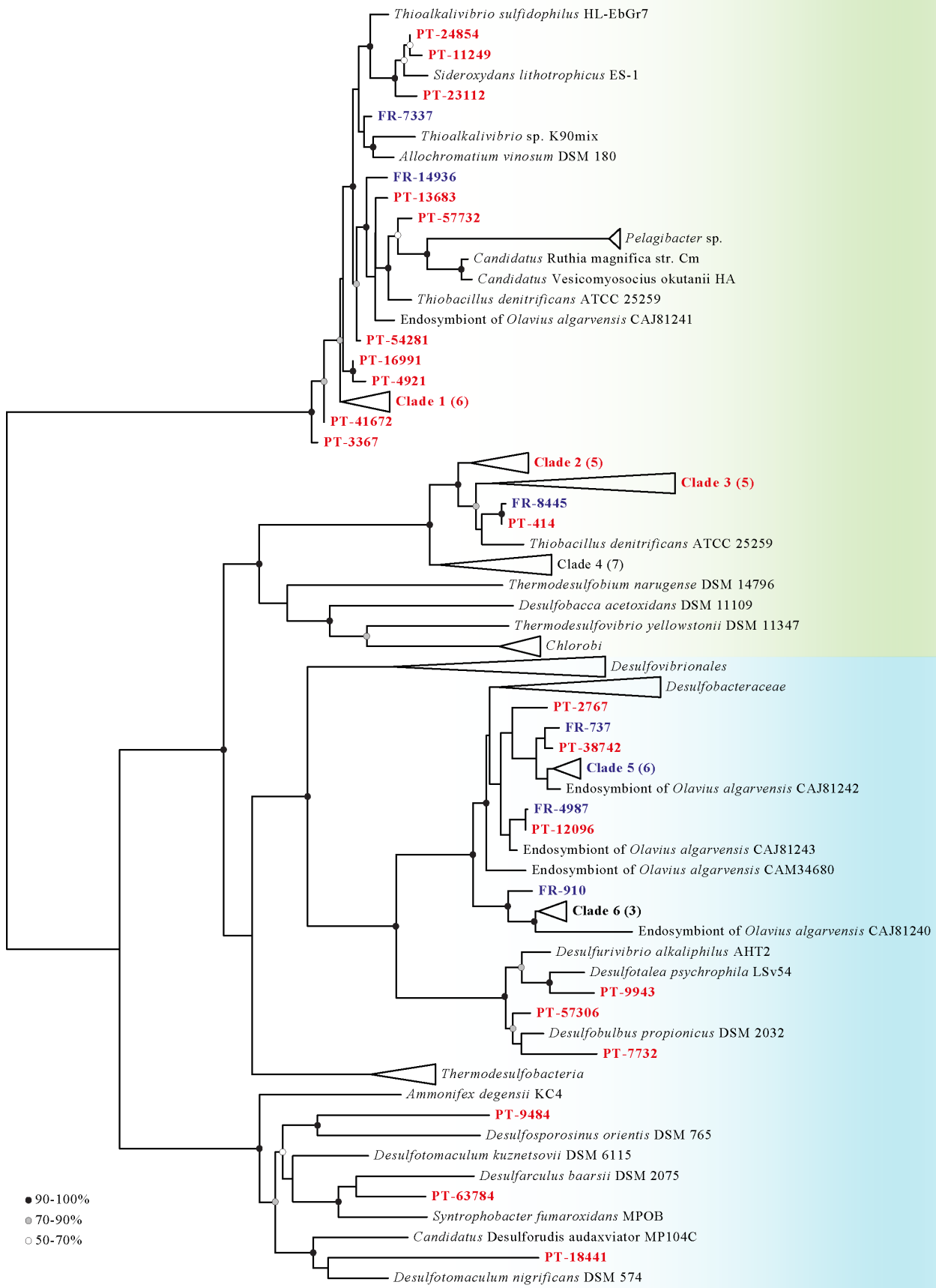


*Supplementary Material*

**Metagenomic analysis shows the presence of free-living forms of sulfur-oxidizing bacterial symbionts in the rhizosphere of the seagrass *Zostera marina***

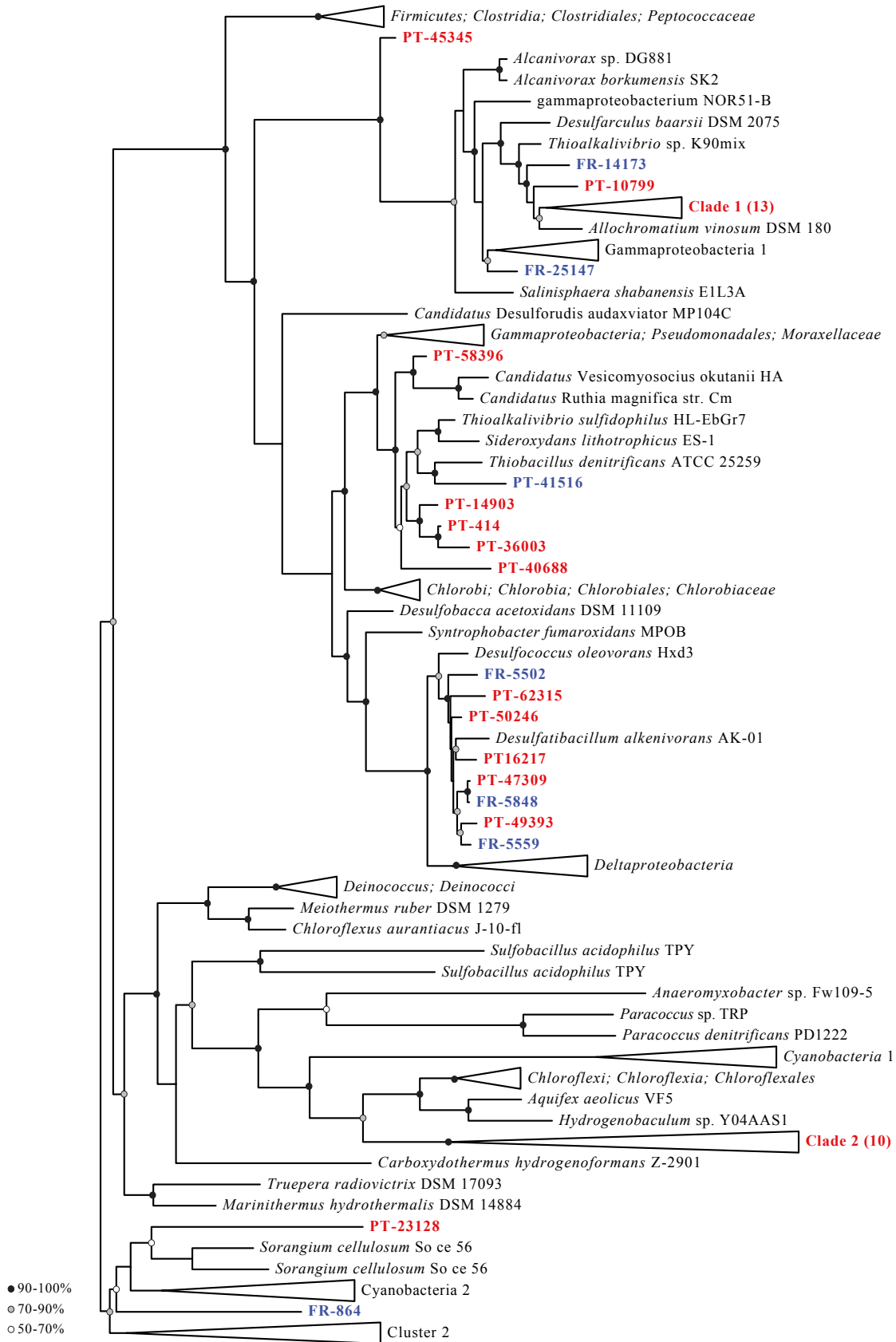
Catarina Cúcio<sup>1</sup>, Lex Overmars<sup>1</sup>, Aschwin H. Engelen<sup>2</sup>, Gerard Muyzer<sup>1\*</sup>

\*Correspondence: Gerard Muyzer: [g.muijzer@uva.nl](mailto:g.muijzer@uva.nl)

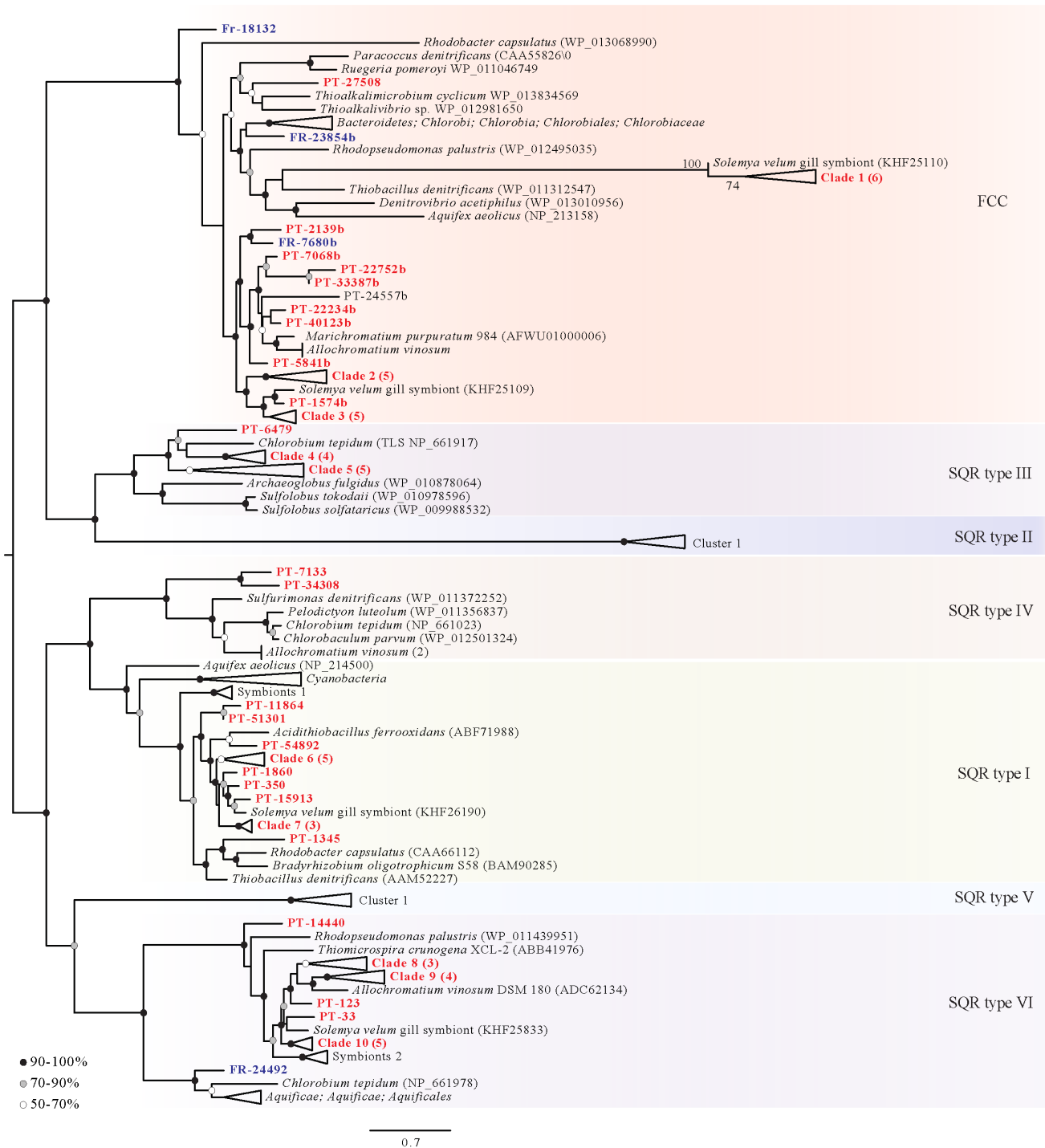


0.8

**Supplementary Figure 1** | Diversity of dissimilatory adenosine-5'-phosphosulfate reductase (apr) genes. Phylogenetic tree of aprA marker genes present in the rhizobiome of *Zostera marina* from Portugal (red, prefix PT) and France (blue, prefix FR). Contig sequences were aligned with Custal Omega to sequences of a custom database comprising aprA genes from the EggNOG 4.5 database (Huerta-Cepas et al., 2016), and sequences of *Olavius algarvensis* endosymbionts obtained from the NCBI. The green box indicates sequences involved in oxidative processes, the blue box sequences involved in reductive processes. The phylogenetic tree was inferred using the approximate maximum-likelihood method in FastTree2 (Price et al. 2010). Bootstrap values are indicated. Scale bar indicates percentage sequence difference.



**Supplementary Figure 2** | Diversity of ATP sulfurylase (sat) genes. Phylogenetic tree of sat marker genes present in the rhizobiome of *Zostera marina* from Portugal (red, prefix PT) and France (blue, prefix FR). Contig sequences were aligned with Custal Omega to sequences of a custom database comprising sat genes from the EggNOG 4.5 database (Huerta-Cepas et al., 2016), and sequences of *Olavius algarvensis* endosymbionts obtained from the NCBI. The phylogenetic tree was inferred using the approximate maximum-likelihood method in FastTree2 (Price et al. 2010). Bootstrap values are indicated. Scale bar indicates percentage sequence difference. Scale bar indicates percentage sequence difference.



**Supplementary Figure 3 | Diversity of sulfide oxidation genes *sqr* and *fcc*.** Phylogenetic tree of *sqr* marker genes present in the rhizobiome of *Zostera marina* from Portugal (red, prefix PT) and France (blue, prefix FR). Contig sequences were aligned with Custal Omega to sequences of a custom database comprising *sqr* genes from the NCBI, the EggNOG 4.5 database (Huerta-Cepas et al., 2016), and from the studies of Marcia et al. (2009), and Han and Perner (2015). The phylogenetic tree was inferred

using the approximate maximum-likelihood method in FastTree2 (Price et al. 2010). Bootstrap values are indicated. Scale bar indicates percentage sequence difference.

**Supplementary Table 1** | Relative abundance of bacterial classes present in the rhizobiome of *Zostera marina* from Portugal (ZmPt) and France (ZmFr).

<b>Class</b>	<b>ZmPt (%)</b>	<b>ZmFr (%)</b>
<i>Gammaproteobacteria</i>	25.318	21.566
<i>Deltaproteobacteria</i>	20.048	16.281
<i>Alphaproteobacteria</i>	7.859	12.210
<i>Actinobacteria</i>	5.469	4.672
<i>Betaproteobacteria</i>	4.885	4.438
<i>Bacteroidia</i>	4.485	5.454
<i>Clostridia</i>	3.348	3.109
<i>Flavobacteriia</i>	3.150	8.883
<i>Anaerolineae</i>	2.833	0.732
<i>Planctomycetia</i>	2.817	3.351
<i>Bacilli</i>	2.467	2.254
<i>Spirochaetia</i>	2.340	1.818
<i>Cytophagia</i>	2.144	2.829
<i>Phycisphaerae</i>	1.260	0.746
<i>Nitrospira</i>	1.111	0.678
<i>Gemmatimonadetes</i>	1.059	0.584
<i>Sphingobacteriia</i>	1.049	1.677
<i>Verrucomicrobiae</i>	0.641	0.836

<i>Epsilonproteobacteria</i>	0.607	1.357
<i>Methanomicrobia</i>	0.500	0.372
<i>Acidobacteriia</i>	0.434	0.305
<i>Chloroflexia</i>	0.401	0.174
<i>Deinococci</i>	0.363	0.240
<i>Caldilineae</i>	0.359	0.115
<i>Acidithiobacillia</i>	0.356	0.253
<i>Opitutae</i>	0.346	0.525
<i>Acidimicrobiia</i>	0.305	0.988
<i>Negativicutes</i>	0.281	0.242
<i>Dehalococcoidia</i>	0.266	0.107
<i>Chlorobia</i>	0.226	0.212
<i>Solibacteres</i>	0.203	0.145
<i>Synergistia</i>	0.194	0.140
<i>Thermotogae</i>	0.172	0.136
<i>Ardenticatenia</i>	0.169	0.052
<i>Ignavibacteria</i>	0.147	0.150
<i>Thermoleophilia</i>	0.135	0.100
<i>Thermomicrobia</i>	0.134	0.057
<i>Ktedonobacteria</i>	0.129	0.061

<i>Aquificae</i>	0.116	0.098
<i>Deferribacteres</i>	0.106	0.099
<i>Spartobacteria</i>	0.105	0.129
<i>Coriobacteriia</i>	0.105	0.083
<i>Lentisphaeria</i>	0.098	0.179
<i>Blastocatellia</i>	0.097	0.061
<i>Fusobacteriia</i>	0.093	0.161
<i>Chlamydiia</i>	0.092	0.115
<i>Thermodesulfobacteria</i>	0.088	0.067
<i>Holophagae</i>	0.083	0.063
<i>Zetaproteobacteria</i>	0.082	0.065
<i>Chitinivibrionia</i>	0.077	0.118
<i>Methanobacteria</i>	0.075	0.061
<i>Tissierellia</i>	0.075	0.078
<i>Mollicutes</i>	0.063	0.295
<i>Thermococci</i>	0.061	0.044
<i>Rubrobacteria</i>	0.061	0.039
<i>Nitrospina</i>	0.059	0.052
<i>Erysipelotrichia</i>	0.050	0.059
<i>Gloeobacteria</i>	0.044	0.034

<i>Chrysiogenetes</i>	0.042	0.040
<i>Limnochordia</i>	0.036	0.018
<i>Fimbriimonadia</i>	0.033	0.026
<i>Methanococci</i>	0.032	0.030
<i>Nitriliruptoria</i>	0.031	0.036
<i>Chthonomonadetes</i>	0.031	0.020
<i>Dictyoglomia</i>	0.019	0.013
<i>Fibrobacteria</i>	0.015	0.023
<i>Endomicrobia</i>	0.008	0.008
<i>Candidatus Peribacteria</i>	0.008	0.011
<i>Caldisericia</i>	0.008	0.007
<i>Elusimicrobia</i>	0.007	0.007
<i>Thermoflexia</i>	0.001	2.69E-4

**Supplementary Table 2** | Relative abundance of bacterial orders present in the rhizobiome of *Zostera marina* from Portugal (ZmPt) and France (ZmFr).

<b>Order</b>	<b>ZmPt (%)</b>	<b>ZmFr (%)</b>
<i>Desulfobacterales</i>	11.821	11.713
<i>Chromatiales</i>	8.307	4.860
<i>Bacteroidales</i>	5.721	8.013
<i>Flavobacteriales</i>	3.948	12.855
<i>Clostridiales</i>	3.569	3.966
<i>Burkholderiales</i>	3.464	3.793
<i>Rhizobiales</i>	3.444	4.597
<i>Planctomycetales</i>	3.265	4.616
<i>Rhodobacterales</i>	3.219	7.995
<i>Spirochaetales</i>	2.784	2.416
<i>Cytophagales</i>	2.734	4.155
<i>Myxococcales</i>	2.680	2.801
<i>Bacillales</i>	2.663	2.702
<i>Cellvibrionales</i>	2.556	3.429
<i>Alteromonadales</i>	2.508	3.657
<i>Thiotrichales</i>	2.247	2.268
<i>Oceanospirillales</i>	2.164	2.711
<i>Desulfovibrionales</i>	1.948	2.007

<i>Streptomycetales</i>	1.811	1.623
<i>Anaerolineales</i>	1.764	0.632
<i>Pseudomonadales</i>	1.544	1.795
<i>Methylococcales</i>	1.482	1.430
<i>Desulfuromonadales</i>	1.474	1.477
<i>Rhodospirillales</i>	1.366	2.338
<i>Gemmatimonadales</i>	1.351	0.858
<i>Sphingobacteriales</i>	1.337	2.460
<i>Xanthomonadales</i>	1.291	1.240
<i>Corynebacteriales</i>	1.192	1.229
<i>Micrococcales</i>	1.145	1.105
<i>Syntrophobacterales</i>	0.967	0.710
<i>Vibrionales</i>	0.915	1.624
<i>Oscillatoriales</i>	0.871	1.400
<i>Sphingomonadales</i>	0.865	1.093
<i>Enterobacteriales</i>	0.846	1.033
<i>Verrucomicrobiales</i>	0.818	1.228
<i>Chroococcales</i>	0.726	1.511
<i>Rhodocyclales</i>	0.600	0.572
<i>Acidobacteriales</i>	0.554	0.449

<i>Pseudonocardiales</i>	0.517	0.500
<i>Legionellales</i>	0.516	0.557
<i>Nostocales</i>	0.506	0.859
<i>Caldilineales</i>	0.458	0.170
<i>Acidithiobacillales</i>	0.454	0.371
<i>Desulfarculales</i>	0.453	0.338
<i>Lactobacillales</i>	0.447	0.567
<i>Thermoanaerobacterales</i>	0.428	0.342
<i>Nitrospirales</i>	0.418	0.387
<i>Chloroflexales</i>	0.406	0.200
<i>Neisseriales</i>	0.405	0.439
<i>Propionibacteriales</i>	0.382	0.372
<i>Acidimicrobiales</i>	0.373	1.411
<i>Campylobacterales</i>	0.365	0.903
<i>Streptosporangiales</i>	0.360	0.326
<i>Selenomonadales</i>	0.358	0.356
<i>Micromonosporales</i>	0.347	0.322
<i>Nevskiales</i>	0.328	0.359
<i>Candidatus Brocadiales</i>	0.326	0.306
<i>Opitutales</i>	0.291	0.447

<i>Chlorobiales</i>	0.288	0.311
<i>Bacteroidetes Order II. Incertae sedis</i>	0.277	0.248
<i>Caulobacterales</i>	0.264	0.330
<i>Nitrosomonadales</i>	0.260	0.281
<i>Solibacterales</i>	0.258	0.213
<i>Synergistales</i>	0.248	0.206
<i>Deinococcales</i>	0.238	0.188
<i>Thermales</i>	0.222	0.163
<i>Aeromonadales</i>	0.221	0.248
<i>Ardenticatenales</i>	0.216	0.076
<i>Frankiales</i>	0.206	0.199
<i>Methylophilales</i>	0.197	0.239
<i>Ignavibacteriales</i>	0.186	0.217
<i>Hydrogenophilales</i>	0.179	0.146
<i>Pleurocapsales</i>	0.174	2.486
<i>Solirubrobacterales</i>	0.172	0.147
<i>Halanaerobiales</i>	0.149	0.147
<i>Puniceicoccales</i>	0.145	0.313
<i>Gallionellales</i>	0.135	0.124
<i>Deferribacterales</i>	0.135	0.146

<i>Stigonematales</i>	0.135	0.251
<i>Pasteurellales</i>	0.133	0.160
<i>Bdellovibrionales</i>	0.130	0.245
<i>Chthoniobacterales</i>	0.126	0.177
<i>Lentisphaerales</i>	0.125	0.263
<i>Fusobacteriales</i>	0.118	0.237
<i>Ktedonobacterales</i>	0.118	0.065
<i>Sulfuricellales</i>	0.117	0.098
<i>Chlamydiales</i>	0.117	0.168
<i>Aquificales</i>	0.115	0.113
<i>Thermotogales</i>	0.113	0.100
<i>Actinomycetales</i>	0.113	0.115
<i>Thermodesulfobacteriales</i>	0.113	0.098
<i>Holophagales</i>	0.105	0.092
<i>Herpetosiphonales</i>	0.104	0.055
<i>Sphaerobacterales</i>	0.099	0.049
<i>Geodermatophilales</i>	0.089	0.085
<i>Rickettsiales</i>	0.087	0.174
<i>Salinisphaerales</i>	0.080	0.084
<i>Rubrobacterales</i>	0.078	0.057

<i>Nitrospinales</i>	0.075	0.076
<i>Magnetococcales</i>	0.074	0.074
<i>Mariprofundales</i>	0.072	0.063
<i>Coriobacteriales</i>	0.070	0.067
<i>Bifidobacteriales</i>	0.065	0.068
<i>Erysipelotrichales</i>	0.064	0.087
<i>Eggerthellales</i>	0.061	0.054
<i>Prochlorales</i>	0.061	0.085
<i>Kiloniellales</i>	0.061	0.162
<i>Tissierellales</i>	0.058	0.074
<i>Gloeobacterales</i>	0.056	0.050
<i>Kosmotogales</i>	0.055	0.047
<i>Chrysiogenales</i>	0.054	0.058
<i>Jiangellales</i>	0.050	0.036
<i>Ferrovales</i>	0.049	0.046
<i>Phycisphaerales</i>	0.049	0.063
<i>Desulfurellales</i>	0.047	0.045
<i>Limnochordales</i>	0.046	0.027
<i>Pelagibacterales</i>	0.046	0.071
<i>Thermogemmatisporales</i>	0.045	0.022

<i>Fimbriimonadales</i>	0.042	0.039
<i>Nitriliruptorales</i>	0.040	0.052
<i>Chthonomonadales</i>	0.039	0.029
<i>Glycomycetales</i>	0.039	0.038
<i>Petrotogales</i>	0.036	0.039
<i>Thermomicrobiales</i>	0.033	0.016
<i>Dehalococcoidales</i>	0.033	0.020
<i>Desulfurobacteriales</i>	0.031	0.030
<i>Kordiimonadales</i>	0.031	0.049
<i>Mycoplasmatales</i>	0.030	0.052
<i>Catenulisporales</i>	0.030	0.028
<i>Kineosporiales</i>	0.029	0.028
<i>Parvularculales</i>	0.027	0.032
<i>Brachyspirales</i>	0.026	0.041
<i>Nakamurellales</i>	0.026	0.023
<i>Chitinivibrionales</i>	0.026	0.045
<i>Dictyoglomales</i>	0.025	0.019
<i>Bacteroidetes Order III. Incertae sedis</i>	0.025	0.035
<i>Methylacidiphilales</i>	0.022	0.025
<i>Acholeplasmatales</i>	0.021	0.045

<i>Orbales</i>	0.021	0.027
<i>Sneathiellales</i>	0.020	0.041
<i>Fibrobacterales</i>	0.019	0.034
<i>Natranaerobiales</i>	0.017	0.014
<i>Cardiobacteriales</i>	0.017	0.017
<i>Nautiliales</i>	0.016	0.022
<i>Actinopolysporales</i>	0.015	0.015
<i>Candidatus Actinomarinales</i>	0.013	0.020
<i>Mesoaciditogales</i>	0.012	0.011
<i>Acidothermales</i>	0.011	0.008
<i>Endomicrobiales</i>	0.010	0.012
<i>Caldisericales</i>	0.010	0.010
<i>Elusimicrobiales</i>	0.009	0.010
<i>Entomoplasmatales</i>	0.009	0.014
<i>Haloplasmatales</i>	0.008	0.015
<i>Candidatus Peribacterales</i>	0.007	0.011
<i>Thermoflexales</i>	0.001	3.95E-4
<i>Rhodothalassiales</i>	1.45E-5	2.03E-5
<i>Acidiferrobacterales</i>	0.000	1.01E-5