

Title: Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability

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Supplemental information:

Appendix S1: Field distribution of four cryptic *Litoditis marina* species (Pm1, Pm2, Pm3 and Pm4) in the Scheldt estuary in The Netherlands in four consecutive seasons. Figure adapted from Derycke et al. (2006).

Appendix S2: Primer sequences used to amplify the 16S rRNA gene of 18 *Litoditis marina* specimens in two runs on 1/8th of a plate of the 454 GS FLX Titanium system. Adaptor, midtag and primer sequences for the forward and reverse datasets are given.

Appendix S3: Summary of the sequence data from the two 454 runs performed on 18 field specimens

Appendix S4: Summary of the analyses to investigate variability between the technical replicates.

Appendix S5: Figures related to alpha diversity and beta diversity measurements of the forward dataset generated using the 454 platform of the field specimens.

Appendix S6: Rank abundance plots of the forward and reverse datasets generated with the 454 platform of the field specimens.

Appendix S7: The core OTUs of the forward and reverse datasets that are present in 100% of the specimens of species Pm1, Pm2 and Pm3 from the field. OTUs identified with LeFSe are indicated in bold. For the reverse dataset, OTUs were generated using 97% and 99% similarity.

Appendix S8: Summary of the sequence data from the MiSeq run on the specimens of the food experiment. Summary of the sequence data from a separate MiSeq run containing three biological replicas of the *E. coli* suspension are also provided.

Appendix S9: Detailed description of the taxonomic composition at the phylum, class and family level for the specimens of the food experiment.

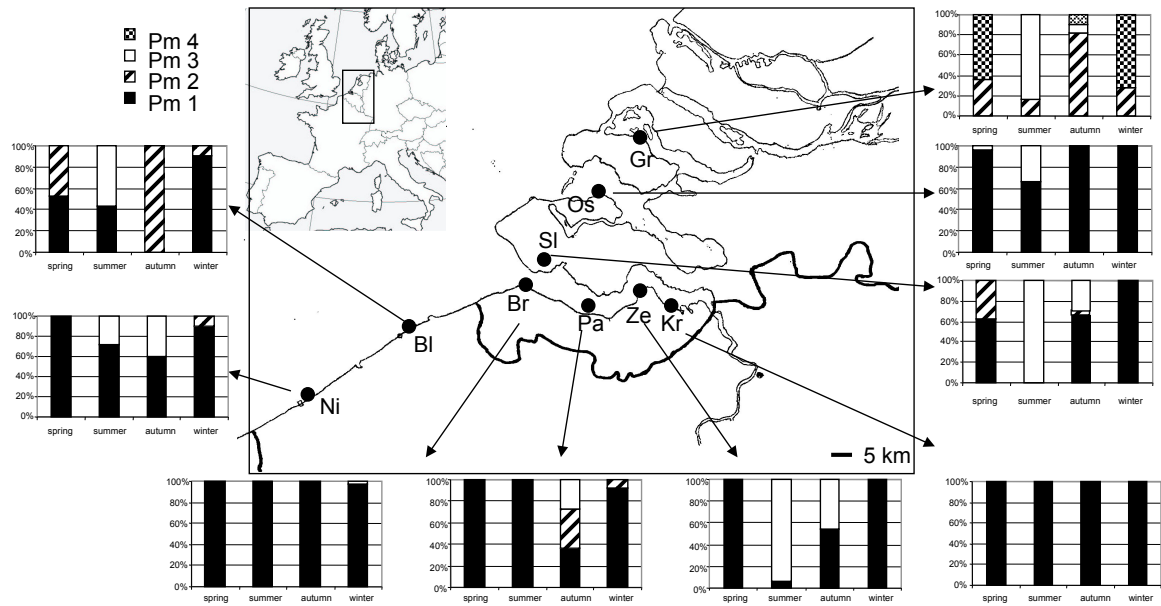
Appendix S10: Graphs of alpha diversity (rarefaction curves of number of OTUs and Shannon Index, rank abundance plots) of the specimens of the food experiment.

Appendix S11: List of biomarker taxa identified by LeFSe for Pm1 and Pm3 from the food experiment. OTU ID and taxonomic assignment using Greengenes are included.

Appendix S12: SEM pictures of nematode specimens from Pm1, Pm2 and Pm3 with bacteria attached to the cuticula.

Appendix S13: Number of shared OTUs between each nematode specimen and each replica of the bacterial mix.

Appendix S1



Litodites marina. Distribution of the 4 lineages (Pm1, Pm2, Pm3 and Pm4) inferred from mitochondrial COI data along the Belgian coastline and the Scheldt estuary in The Netherlands. For each location, a stacked column graph indicates the percentage of each lineage occurring in spring 2003, summer 2003, autumn 2003 and winter 2004. Note the small proportion of Pm3 during winter 2004 in Br. Sample abbreviations: Ni = Nieuwpoort, BI = Blankenberge, Br = Breskens, Pa = Paulina, Ze = Zeedorp, Kr = Kruispolderhaven, SI = Sloehaven, Os = Oosterschelde, Gr = Grevelingen. Figure taken from Derycke et al. 2006.

Appendix S2: Primer sequences used to amplify the 16S rRNA gene of 18 *Litodites marina* specimens in two runs on 1/8th of a plate of the 454 GS FLX Titanium system. Adaptor, midtag and primer sequences for the forward

and reverse datasets are given

Run1: 9 samples were sequenced on 1/8th of a plate using the 454 platform

DNA sample	PCR code	Forward primer name	Key	MID	Primer	Reverse primer name	Key	MID	Primer
PP2085	B143	RL17_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	AGTCGTACACT	AACGCGAAGAACCTTAC	RL17_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTGTAGGACT	CGGTGTGTACAAGGCCCGGGAACG
PP2086	B145	RL18_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	AGTGTAGTAGT	AACGCGAAGAACCTTAC	RL18_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACTACTAGACT	CGGTGTGTACAAGGCCCGGGAACG
PP2087	B147	RL19_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	ATAGTATACGT	AACGCGAAGAACCTTAC	RL19_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGTATAGTAT	CGGTGTGTACAAGGCCCGGGAACG
PP2059	B149	RL20_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CAGTACGTACT	AACGCGAAGAACCTTAC	RL20_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTACGTGCTG	CGGTGTGTACAAGGCCCGGGAACG
PP2060	B151	RL21_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGACGACGCGT	AACGCGAAGAACCTTAC	RL21_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGCGTGGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2061	B153	RL22_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGACGAGTACT	AACGCGAAGAACCTTAC	RL22_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTACTGGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2074	B155	RL23_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGATACTACGT	AACGCGAAGAACCTTAC	RL23_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGTAGTGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2067	B157	RL24_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGTACGTGAT	AACGCGAAGAACCTTAC	RL24_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ATCGACGGACG	CGGTGTGTACAAGGCCCGGGAACG
PP2095	B165	RL25_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CTACTCGTAGT	AACGCGAAGAACCTTAC	RL25_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACTACGGTAG	CGGTGTGTACAAGGCCCGGGAACG

Run2: 9 samples were sequenced on 1/8th of a plate using the 454 platform using the same primers as for the first run

PP2054	B212	RL17_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	AGTCGTACACT	AACGCGAAGAACCTTAC	RL17_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTGTAGGACT	CGGTGTGTACAAGGCCCGGGAACG
PP2092	B196	RL18_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	AGTGTAGTAGT	AACGCGAAGAACCTTAC	RL18_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACTACTAGACT	CGGTGTGTACAAGGCCCGGGAACG
PP2103	B198	RL19_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	ATAGTATACGT	AACGCGAAGAACCTTAC	RL19_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGTATAGTAT	CGGTGTGTACAAGGCCCGGGAACG
PP2058	B200	RL20_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CAGTACGTACT	AACGCGAAGAACCTTAC	RL20_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTACGTGCTG	CGGTGTGTACAAGGCCCGGGAACG
PP2076	B214	RL21_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGACGACGCGT	AACGCGAAGAACCTTAC	RL21_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGCGTGGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2098	B204	RL22_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGACGAGTACT	AACGCGAAGAACCTTAC	RL22_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	AGTACTGGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2077	B206	RL23_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGATACTACGT	AACGCGAAGAACCTTAC	RL23_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACGTAGTGTGC	CGGTGTGTACAAGGCCCGGGAACG
PP2093	B208	RL24_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CGTACGTGAT	AACGCGAAGAACCTTAC	RL24_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ATCGACGGACG	CGGTGTGTACAAGGCCCGGGAACG
PP2096	B210	RL25_A_16S_968F	CGTATCGCCTCCCTCGGCCATCAG	CTACTCGTAGT	AACGCGAAGAACCTTAC	RL25_B_16S_1401R	CTATGCGCCTTGCCAGCCCGCTCAG	ACTACGGTAG	CGGTGTGTACAAGGCCCGGGAACG

Appendix S3: Summary of the number of sequences and OTUs obtained after quality filtering using the open reference OTU picking workflow for each nematode specimen ('Samples'). Forward and Reverse sequences were analysed separately. 'Raw sequences': number of sequences obtained without any filtering. 'Sequences after denoising': number of sequences retained in the dataset after filtering and denoising with FlowClus and removal of chimera sequences. '#OTU_>1': the number of OTUs that had more than one sequence in the whole dataset. 'Good's coverage' is calculated per sample. Top 9 specimens are from the first sequencing run, the bottom 9 specimens are from the second sequencing run.

Samples	Forward sequences				Reverse sequences			
	raw sequences	sequences after denoising	#OTU_>1	Good's coverage	raw sequences	sequences after denoising	#OTU_>1	Good's coverage
Pm1_143	998	937	144	0.91	959	891	159	0.91
Pm1_145	14004	12995	352	0.92	13896	13238	473	0.93
Pm1_147	8285	7492	357	0.88	8039	7304	398	0.88
Pm2_149	1524	1420	169	0.94	1459	1374	193	0.90
Pm2_151	699	626	99	0.95	1072	1011	162	0.91
Pm2_153	4345	4134	321	0.86	3998	3803	370	0.85
Pm3_155	2697	2567	263	0.86	2581	2467	265	0.86
Pm3_157	10024	9700	240	0.93	11039	10829	275	0.92
Pm3_159	839	802	85	0.95	682	643	97	0.95
	43415	40673	1098		43725	41560	1295	
Pm1_212	22891	20564	401	0.92	27557	25068	513	0.92
Pm1_196	4755	4497	291	0.88	5436	5125	297	0.89
Pm1_198	3909	3734	89	0.97	4660	4473	140	0.96
Pm2_200	3687	3430	248	0.90	3502	3254	297	0.90
Pm2_214	2044	1958	116	0.94	7174	6990	270	0.91
Pm2_204	3423	3014	223	0.90	4085	3676	277	0.88
Pm3_206	1022	973	111	0.93	2028	1927	183	0.92
Pm3_208	2005	1932	109	0.95	2883	2782	160	0.93
Pm3_210	2566	2419	213	0.90	2348	2252	224	0.91
	46302	42521	1036		59673	55547	1354	

Appendix S4: Summary of the analyses to investigate variability between the technical replicates.

To explore the extent of PCR bias, we have labeled the technical replicates from three randomly chosen samples from the food experiment with different barcodes. Ideally, we would have liked to see:

a/ a similar number of OTU's between the three technical replica's of the same sample. This was the case (see column Total # OTUs in Table 1).

b/ a large proportion (if not all) of OTU's to be shared between technical replicates. Only a small fraction (generally < 23%) of the OTUs appeared in only one replicate, except for replica Pm3Ea, where 40% of the OTUs were uniquely found in that replica. Most OTUs were thus shared between at least two replicates (Figure 1).

c/ if unique OTUs are found, they should be there in very low frequency so that they will not have strong impacts on the final dataset. This was the case: the relative frequencies observed for each of the unique OTUs ranged between 0,01 and 0,21% confirming that they received only a very small fraction of the sequence reads (Table 2).

d/ the variation between technical replicates to be lower compared to the variation between biological replicates. We calculated Generalized Unifrac distances between the technical and biological replicates and found indeed that the variation among the technical replicates was lower than that compared to the biological replicates (Figure 2).

Table 1: Total number of reads and OTUs for each of the three technical replicates for treatment Pm1B, Pm3E and Pm3bact. Replicates are indicated with letters a, b and c. "Unique OTUs" are OTUs that were only found in that particular replica. "OTUs_Shared_in_2_Repl" are OTUs that were shared between two replicates of the treatment; "OTUs_Shared_in_3_Repl" are OTUs that were present in all three replicates of the treatment.

	Total # reads	Total # OTUs	Unique OTUs	OTUs_Shared_ in 2 Repl	OTUs_Shared_ in 3 Repl
Pm1Ba	52894	581	87	222	272
Pm1Bb	56379	609	99	238	272
Pm1Bc	68788	651	137	242	272
Pm3Ea	136469	697	280	247	170
Pm3Eb	107838	497	66	261	170
Pm3Ec	125507	436	102	164	170
Pm3bacta	42930	419	81	173	165
Pm3bactb	68610	455	77	213	165
Pm3bactc	44634	323	28	130	165

Table 2: Calculation of the maximum relative frequency of unique OTUs per replica. For each replica, the frequency of the most abundant OTU is calculated (“Max_sequence count”) and divided by the total number of sequence reads obtained for that replica (‘Total reads’) to yield the relative frequency in each OTU.

	PM3bactc1a	PM3bactc1b	PM3bactc1c	PM1B1b	PM1B1a	PM1B1c	PM3E10a	PM3E10c	PM3E10b
Max_sequence count	70	7	4	110	76	22	271	33	73
Total reads	41982	59285	42921	51587	54788	66762	133859	106476	123950
Relative frequency	0,17	0,01	0,01	0,21	0,14	0,03	0,20	0,03	0,06

Figure 1: Percentage of OTUs uniquely found in each replica, shared between two replica’s and shared between three replica’s.

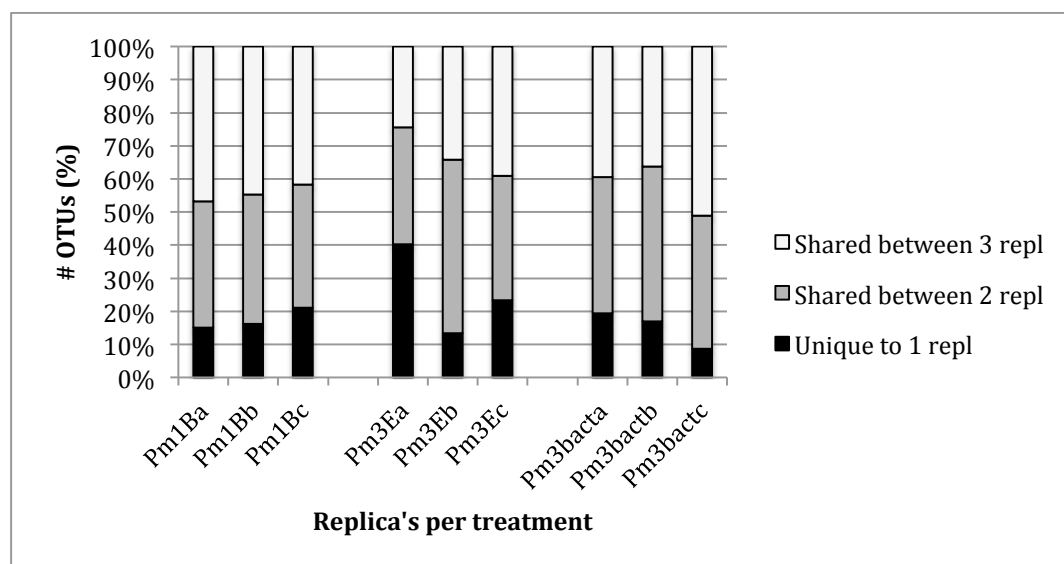
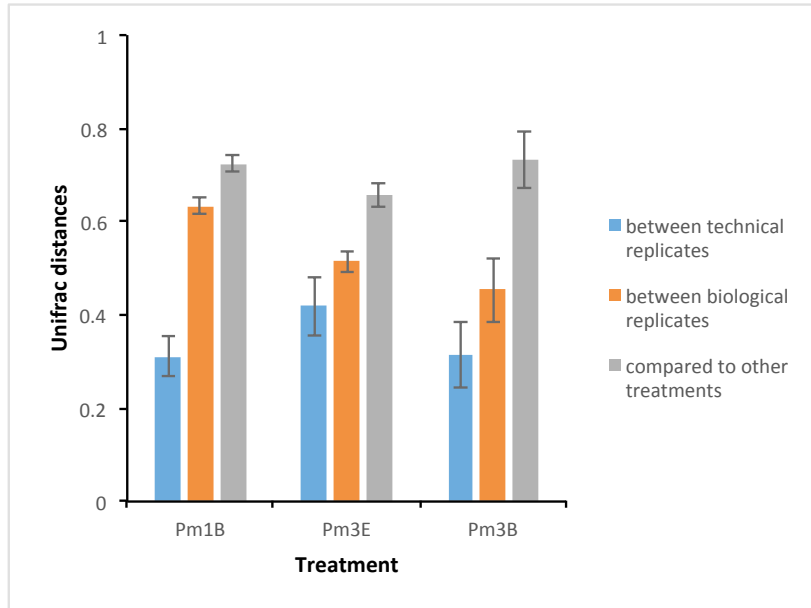


Figure 2: Average Unifrac distances between technical replicates and biological replicates of each of three treatments (Pm1B, Pm3E and Pm3B).



Appendix S5: Figures related to alpha diversity and beta diversity measurements of the forward dataset generated using the 454 platform of the field specimens.

Figure 1: Taxonomic assignments at the phylum level of representative sequences of each OTU from the Forward dataset. Relative frequency of reads below 0.025 are pooled in a ‘Low frequency Class’. Bars represent average values of six specimens for species Pm1 (black), Pm2 (grey) and Pm3 (white). Error bars are standard deviations based on six replicates from each species.

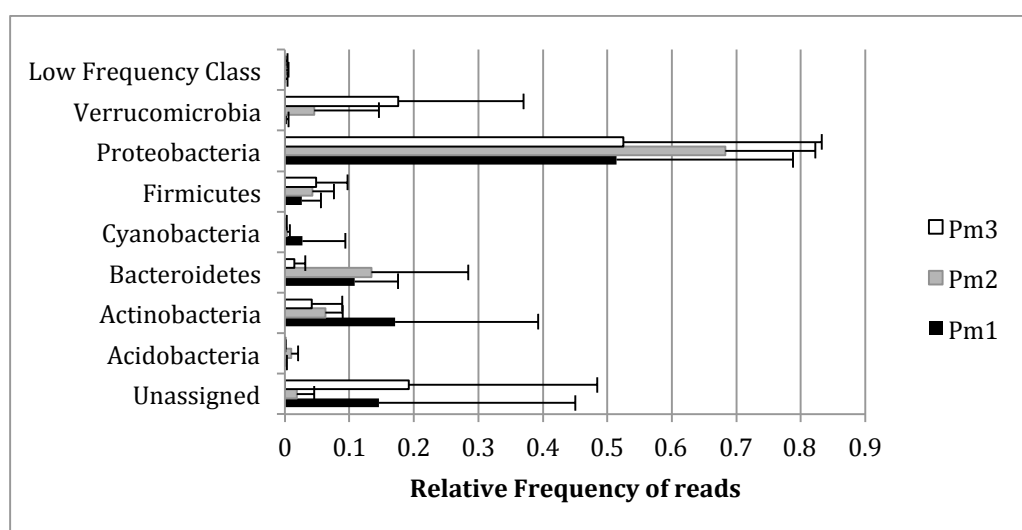


Figure 2: Rarefaction curves of the number of observed OTUs (A) and Shannon index (B) for each species based on Forward dataset. Error bars were calculated from the variance of the respective parameter drawn in 10 randomizations at each sample size.

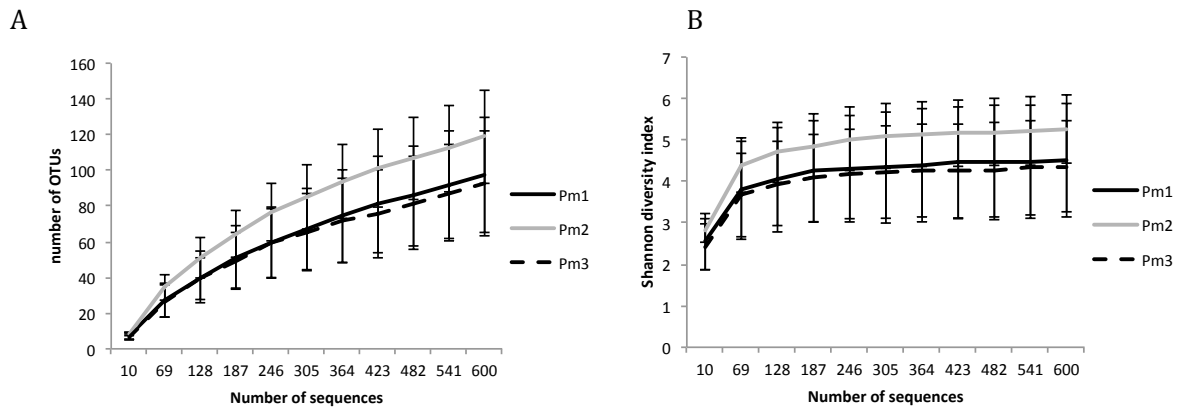
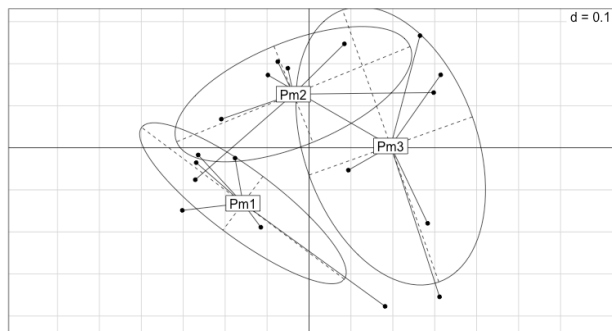
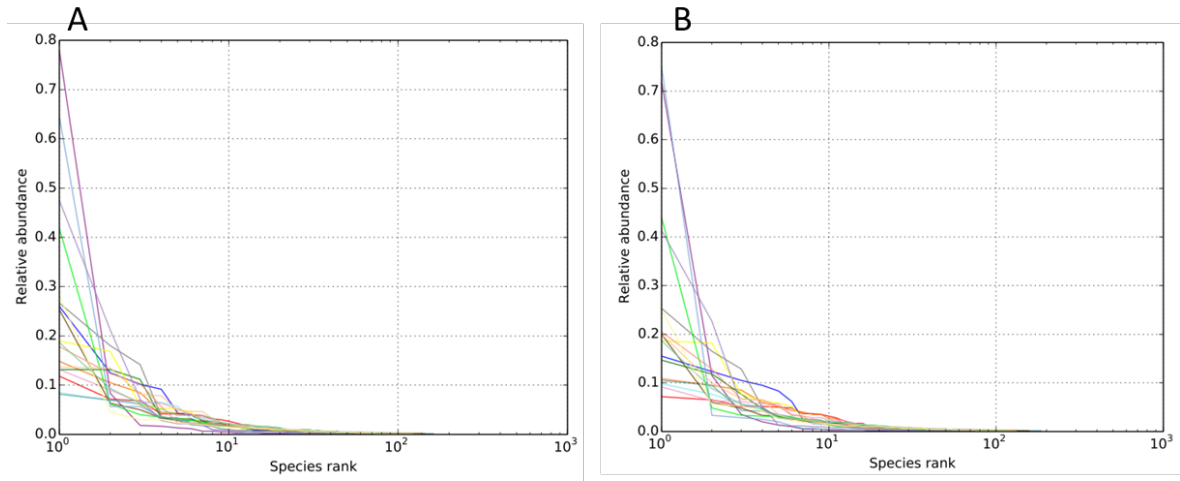


Figure 3: Principal coordinates analysis plot based on Generalized Unifrac distances between 18 nematode specimens after rarefaction of the forward dataset at 600 sequences per specimen. Intraspecific distances for Pm1, Pm2 and Pm3 are encircled.



Appendix S6: Rank abundance plots of OTUs from each of the 18 field specimens. Reads were generated with the 454 platform. Note the log scale of the X-axis. A/ Forward dataset; B/ Reverse dataset. Each line corresponds to one specimen.



Appendix S7: Taxonomic assignment of the core OTUs.

OTUs that are present in all six specimens of species Pm1, Pm2 and Pm3 are indicated by "x". Core OTUs that are present in at least one specimen of the other species are indicated by 'l'.

Forward 97%: OTUs generated using the Forward dataset with OTU clustering at 97%; Reverse 97%: OTUs generated based on the Reverse dataset with OTU

OTU ID	Pm1	Pm2	Pm3	Taxonomy
Forward 97%				
209124	x	x	l	Bacteroidetes, Flavobacteriia, Flavobacteriales, Weeksellaceae, Cloacibacterium
4330856	x	x	l	Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae,
741010	x	l	l	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Cellvibrio
New.ReferenceOTU34	x	l	l	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Cellvibrio
4334053	x	x	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae,
699789		l	x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
4449456	l	l	x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
284413	l	x	l	Proteobacteria, Gammaproteobacteria, Xanthomonadales, Sinobacteraceae,
154604	l	x	l	Proteobacteria, Gammaproteobacteria, Xanthomonadales, Sinobacteraceae,
Reverse 97%				
200979	l	x	l	Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae, Microbacterium
263590	l	x	l	Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae, Microbacterium
4437011	l	x	l	Bacteroidetes, Flavobacteriia, Flavobacteriales, Weeksellaceae, Chryseobacterium
310003		l	x	Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae
4330856	x	x	l	Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae,
4295954	l	l	x	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae, Acidovoraxcaeni
4455981	x	l	l	Proteobacteria, Deltaproteobacteria, Bdellovibrionales, Bacteriovoracaceae,
741010	x	l	l	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Cellvibrio
2967255	x	l	l	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Cellvibrio
4334053	x	x	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae
720489			x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
4449456		l	x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
4449458	l	x	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
Reverse 99%				
New.CleanUp.ReferenceOTU8140	l	x	l	Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae, Microbacterium
1102856	x	l	l	Actinobacteria, Actinobacteria, Actinomycetales, Propionibacteriaceae, Propionibacterium, acnes
1038849	x	x	l	Bacteroidetes, Flavobacteriia, Flavobacteriales, Weeksellaceae, Chryseobacterium
4330856	x	x	l	Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae
822041		l	x	Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae
1061429	x	x	l	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae
New.CleanUp.ReferenceOTU11073	x	l	l	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Cellvibrio
1025018	x	x	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae
1089344	x	x	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae
972803	l	l	x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
960682			x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
New.CleanUp.ReferenceOTU6513			x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
New.CleanUp.ReferenceOTU8300	l	l	x	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Acinetobacter
1105959	x	l	l	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Enhydrobacter

Appendix S2 Summary of the sequence data from the MiSeq run on the specimens of the food experiment. Summary of the sequence data from a separate MiSeq run containing three biological replicas of the *E. coli* suspension are also provided.

Food experiment																							
Samples	sequences				Good's coverage	Samples	sequences				Good's coverage	Samples	sequences				Good's coverage						
	raw sequences	after denoising	After open reference picking	#OTU >1			raw sequences	after denoising	After open reference picking	#OTU >1			raw sequences	after denoising	After open reference picking	#OTU >1		raw sequences	after denoising	After open reference picking	#OTU >1		
Pm181a	54180	52894	51587	710	0.99	Pm1E1	397754	391753	198450	2418	0.99	Pm3B1	481421	478578	470440	2871	0.99	Pm3E1	570058	565998	557309	2603	0.99
Pm181b	57938	56179	54788	789	0.99	Pm1E2	79175	78438	77121	1150	0.99	Pm3B2	505853	484182	437742	6875	0.97	Pm3E2	369151	362186	362186	7864	0.98
Pm181c	71544	68788	65762	777	0.99	Pm1E3	440255	443384	433624	2154	0.99	Pm3B3	418275	393985	303985	3979	0.99	Pm3E3	252063	257821	252830	1982	0.99
Pm181	183662	178061	173137	1536	0.99	Pm1E4	246629	243122	238173	1596	0.99	Pm3B4	460394	416041	410721	2632	0.99	Pm3E4	439684	434603	426370	3410	0.99
Pm182	258860	257274	253159	2048	0.99	Pm1E5	997926	989814	972963	3541	0.99	Pm3B5	511810	456470	449560	1546	0.99	Pm3E5	352990	349212	343401	2238	0.99
Pm183	322913	303985	303985	6875	0.97	Pm1E6	308058	306201	302576	1568	0.99	Pm3B6	399667	389922	306457	2540	0.99	Pm3E6	277667	275888	271753	1990	0.99
Pm184	251187	245546	245673	1640	0.99	Pm1E7	574878	572322	565391	1463	1.00	Pm3B7	532983	336437	329652	3324	0.99	Pm3E7	377883	375053	369177	2049	0.99
Pm185	782833	775715	762658	4060	0.99	Pm1E8	203608	202362	200038	1118	0.99	Pm3B8	532823	528457	517876	1517	0.99	Pm3E8	496613	493721	487073	1857	0.99
Pm186	784471	776525	761842	3369	0.99	Pm1E9	265059	263801	260447	1760	0.99	Pm3B9	445151	231459	228153	2271	0.99	Pm3E9	336438	333999	328833	2310	0.99
Pm187	340165	335639	329048	2337	0.99	Pm1E10	389187	387422	383213	1817	0.99	Pm3B10	446659	442031	433946	2595	0.99	Pm3E10	372684	369814	364285	2277	0.99
Pm188	160028	158096	155969	1802	0.99													Pm3E10a	137776	136409	133859	1293	0.99
Pm189	364867	362186	362186	7864	0.87													Pm3E10b	108618	107838	106476	903	0.99
Pm1810	122397	121013	118399	1697	0.99													Pm3E10c	126290	125507	123950	849	0.99
3571383	3518640	3466056	21774			3911299	3878619	3631996	10584		4735036	4107562	3888532	15621			3855231	3818295	3763217	16963			
Stock and bacterial mix																							
Samples	sequences				Good's coverage	Samples	sequences				Good's coverage	Samples	sequences				Good's coverage						
	raw sequences	after denoising	After open reference picking	#OTU >1			raw sequences	after denoising	After open reference picking	#OTU >1			raw sequences	after denoising	After open reference picking	#OTU >1		raw sequences	after denoising	After open reference picking	#OTU >1		
Pm1C1	203843	202833	198450	1873	0.99	Pm3C1a	43253	42930	41982	597	0.99	bactmixa	278123	276469	270737	2197	0.98						
Pm1C2	272281	271027	266233	2120	0.99	Pm3C1b	69039	68610	59285	601	0.99	bactmixb	379113	375540	367033	2795	0.98						
						Pm3C1c	44938	44634	42521	397	1.00												
						Pm3C1	157230	156174	144188	1053	0.99												
						Pm3C2	248463	246977	242761	1550	0.99												
476124	473860	464693	2767		405693	403151	386949	1903		657236	652009	637770	5651										
Escherichia coli suspension (sequenced on a separate MiSeq run as part of a follow-up experiment)																							
Samples	sequences				Good's coverage																		
	raw sequences	after filtering	After open reference picking	#OTU >1																			
EcolIA	22509	22149	21306	573	0.99																		
EcolIB	27226	26493	25648	538	0.99																		
EcolIC	28914	28159	27300	546	0.99																		

Appendix S9: Taxonomic composition of the bacterial communities at the phylum, class and family level of the food experiment based on MiSeq sequencing of the 16S rRNA gene

The microbiomes of all samples were clearly dominated by Proteobacteria and Bacteroidetes (Fig. 1). Proteobacteria was the most dominant phylum in both food treatments of Pm1 (63% for B treatment and 72% for E treatment) and Pm3 (55% for B treatment and 37% for E treatment). Bacteroidetes was the second most dominant phylum for both species (>7.5% in all four treatments). The Actinobacteria were also present in all specimens, but reached much lower abundances than the Proteobacteria and Bacteroidetes. The Planctomycetes, Firmicutes and Cyanobacteria had low frequencies and were found in 95%, 95% and 93 % of all the samples, respectively. The samples in which these phyla were absent belonged to the *E.coli* treatment. A high frequency of unassigned OTUs was prominent in all specimens of Pm3 (4.8% and 6.3% for Pm1B and Pm1E, respectively and 55.1% and 37.3% for Pm3B and Pm3E, respectively).

Within the Proteobacteria, the Alpha and Gammaproteobacteria reached comparable ratios in the microbiomes of the four food treatments. For Pm1B, almost all specimens were dominated by Alphaproteobacteria, while half of the specimens of the *E.coli* treatment were dominated by Alphaproteobacteria and the other half by Gammaproteobacteria (Fig 2a). For Pm3, the Gammaproteobacteria dominated in almost all specimens, regardless of the food that had been offered (Fig2b). Within the Alphaproteobacteria 10 orders and 35 families were found. Both food treatments of Pm1 and Pm3 were dominated by the Rhizobiales (with high abundances of the Rhizobiaceae Fig 3). The family of the Rhodobacteraceae was abundant in all Pm1 specimens, but for Pm3 this family was more abundant in the specimens that received *E. coli* as food source. The Rhodobacteraceae was also the most abundant family in the stock cultures of both species (Fig. 3). Within the Gammaproteobacteria 18 orders and 54 families were found. Vibrionales was the most abundant order for both stock cultures, the *E.coli* treatment of Pm1 and Pm3 and the bacterial mixture treatment of Pm1 and consisted almost exclusively of Pseudoalteromonadaceae and Vibrionaceae (Fig. 4). Pm1 and Pm3 worms that had been fed the bacterial mixture further contained a higher amount of Alteromonadaceae than those worms that had been fed *E. coli*. Importantly, the composition at the family level appears to be different between worms that had been fed the bacterial mixture and those that had been fed *E. coli*, and the composition of the bacterial mix was similar to the composition of the worms that had been fed this mixture (Fig. 4). The Beta, Delta and Epsilon Proteobacteria contained only 9, 11 and 1 order, respectively.

Within the Bacteroidetes 7 orders and 26 families were observed. Flavobacteriales was the most abundant order (more than 60%) for both food treatments and the stock culture of Pm1 and Pm3. Flavobacteriaceae was the most abundant family within this order for all these treatments, and was more abundant in the Pm1 specimens than in the Pm3 specimens. In contrast, the order Saprospirales (with almost exclusively the family Saprospiraceae) was prominent in almost all samples, but reached higher abundances in all Pm3 specimens than in the Pm1 specimens. It was also the dominant family in the pure bacterial mixture (Fig. 5). For the bacterial mixture treatment of Pm1, the Cryomorphaceae was also present in a high

abundance in several specimens (Fig. 5), while this family was almost absent in the other treatment and species.

The Actinobacteria consisted of 7 orders and 47 families with the order Actinomycetales comprising more than 95%. Within this order 33 families occurred with Microbacteriaceae being the most dominant in all the treatments (at least 70%) (Fig. 6).

Figure 1: Taxonomic assignments of reads at the phylum level with a relative frequency ≥ 0.025 for the four treatments of the food experiment, and for the two stock cultures. Taxa with frequencies below 0.025 are pooled in a 'Low frequency' class. Bars represent average values with standard deviations of ten specimens for each food treatment (Pm1B: Pm1 nematodes fed the bacterial mix; Pm 3B: Pm3 nematodes fed the bacterial mix; Pm1E: Pm1 nematodes fed *E.coli*; Pm3E: Pm3 nematodes fed *E. coli*) and two replicates from the agar of stock cultures of Pm1 (Pm1C) and Pm3 (Pm3C).

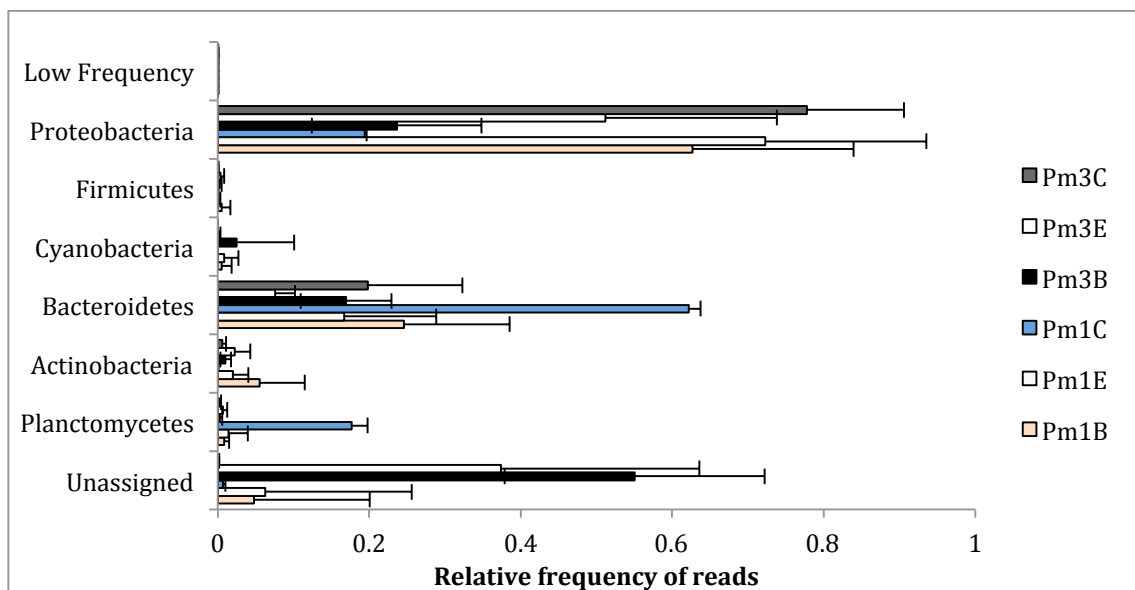


Figure 2: Taxonomic assignment of reads at the class level within Proteobacteria from the food experiment. A/ all 22 samples of Pm1. Pm1B1-10: 10 biological replicas of the bacterial mixture treatment; Pm1E1-10: 10 biological replicas of the *E. coli* treatment; Pm1C1-2: two biological replicas of the agar from Pm1 stock cultures; bactmixa-b: two biological replicas of the bacterial mix. B/ all 22 samples of Pm3. Sample codes as for Pm1.

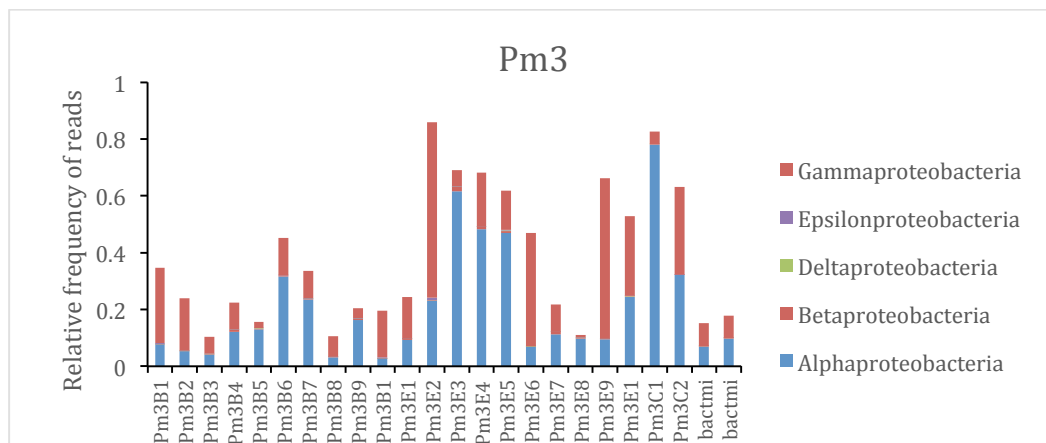
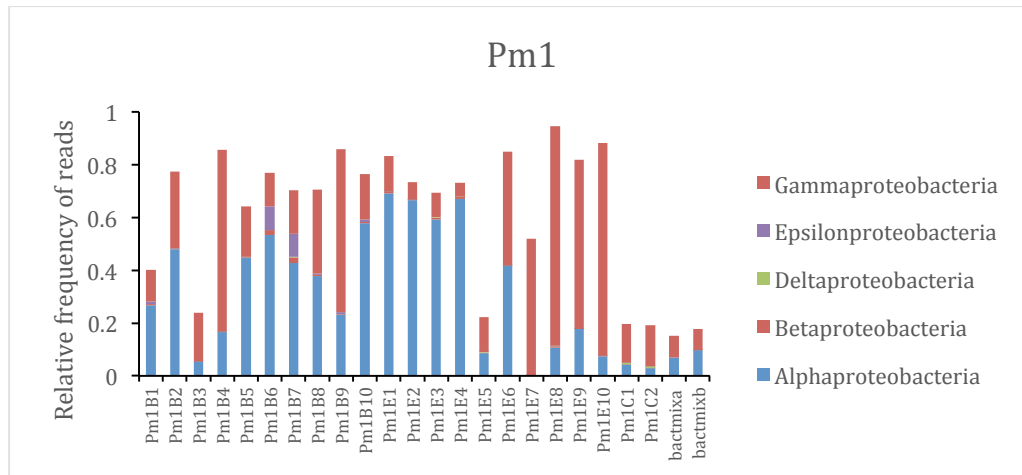


Figure 3: Taxonomic assignment of reads at the family level within Alphaproteobacteria for the food experiment. The six most abundant families are shown, the 29 remaining families are pooled in a “Low Frequency Class”. A/ all 22 samples of Pm1. Pm1B1-10: 10 biological replicas of the bacterial mixture treatment; Pm1E1-10: 10 biological replicas of the *E. coli* mixture treatment; Pm1C1-2: two biological replicas of the agar from Pm1 stock cultures; bactmixa-b: two biological replicas of the bacterial mix. B/ all 22 samples of Pm3. Sample codes as for Pm1.

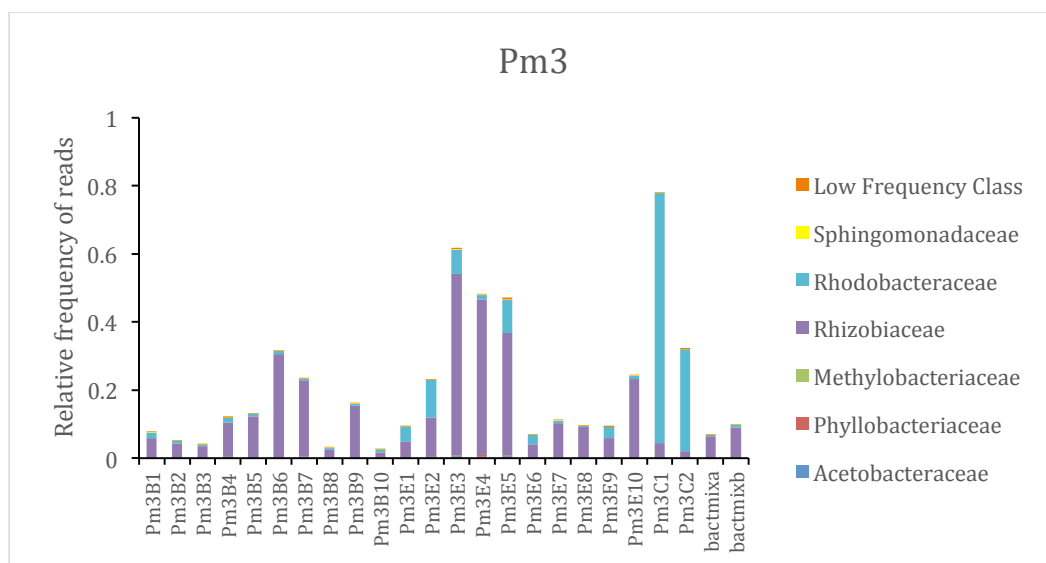
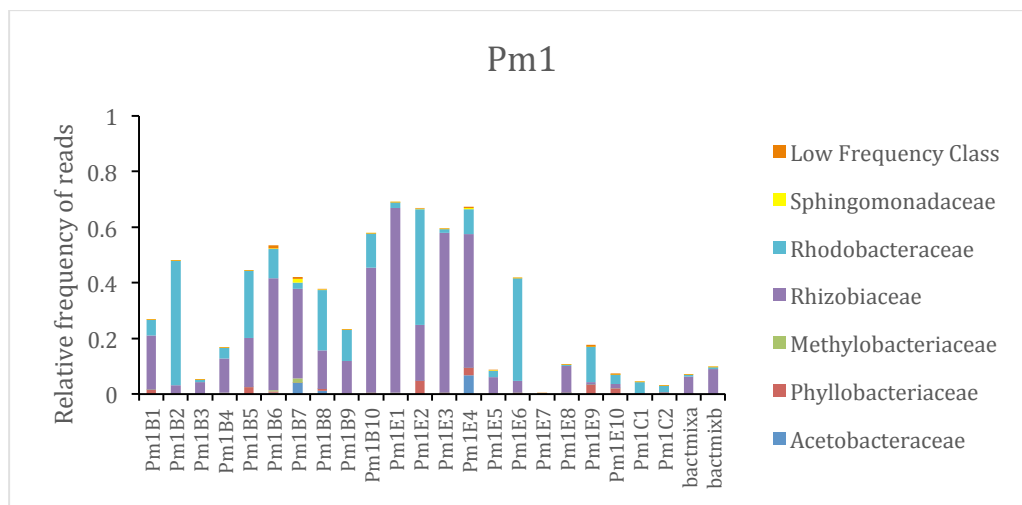


Figure 5: Taxonomic assignment of reads at the family level within Bacteroidetes for the food experiment. The five most abundant families are shown, the 21 remaining families are pooled in a “Low Frequency Class “. A/ all 22 samples of Pm1. Pm1B1-10: 10 biological replicas of the bacterial mixture treatment; Pm1E1-10: 10 biological replicas of the *E. coli* treatment; Pm1C1-2: two biological replicas of the agar from Pm1 cultures; bactmixa-b: two biological replicas of the bacterial mix. B/ all 22 samples of Pm3. Sample codes as for Pm1.

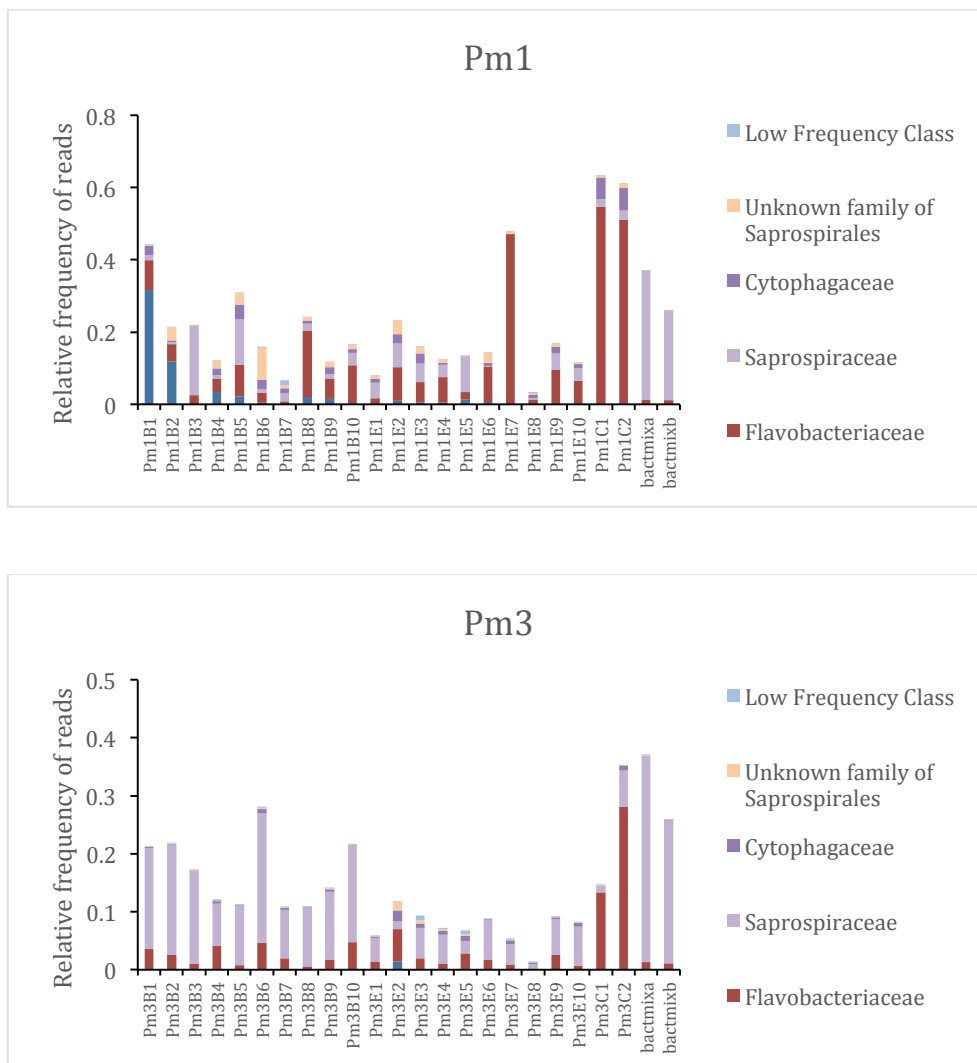
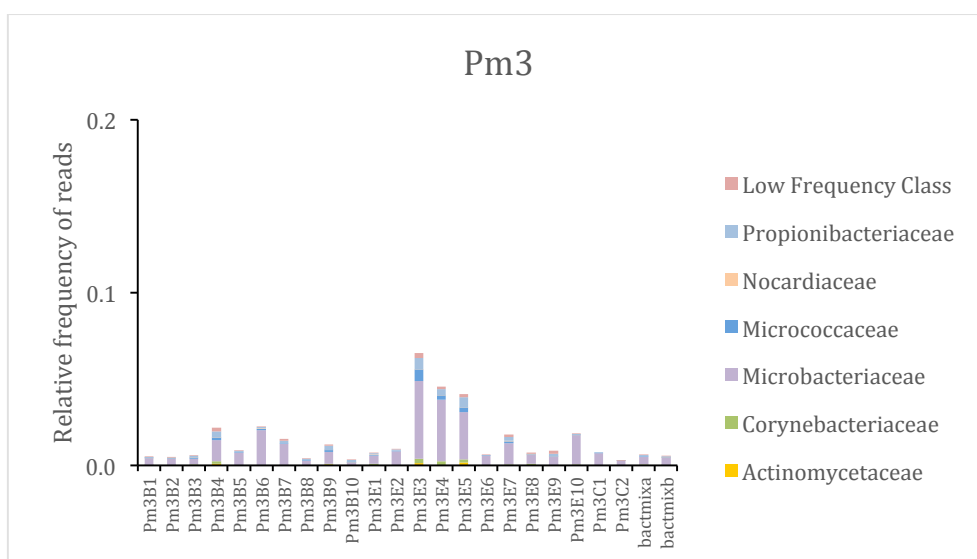
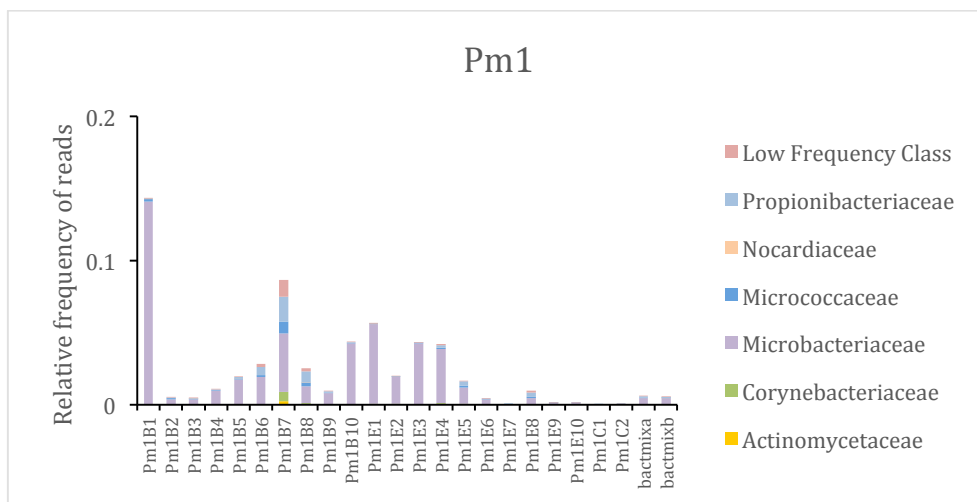


Figure 6: Taxonomic assignment of reads at the family level within Actinobacteria for the food experiment. The six most abundant families are shown, the 41 remaining families are pooled in a “Low Frequency Class “. A/ all 22 samples of Pm1. Pm1B1-10: 10 biological replicas of the bacterial mixture treatment; Pm1E1-10: 10 biological replicas of the *E. coli* treatment; Pm1C1-2: two biological replicas of the agar from Pm1 stock cultures; bactmixa-b: two biological replicas of the bacterial mix. B/ all 22 samples of Pm3. Sample codes as for Pm1.



Appendix S10: Graphs of alpha diversity (rarefaction curves of number of OTUs and Shannon Index, rank abundance plots) of the specimens of the food experiment.

Figure 1: Rarefaction curves of the number of observed OTUs for each treatment of the food experiment (Pm1B, Pm1E, Pm3B, Pm3E), the agar from the stock cultures (Pm1C and Pm3C) and the pure bacterial mixture. Error bars were calculated from the variance of 10 randomizations at each sample size.

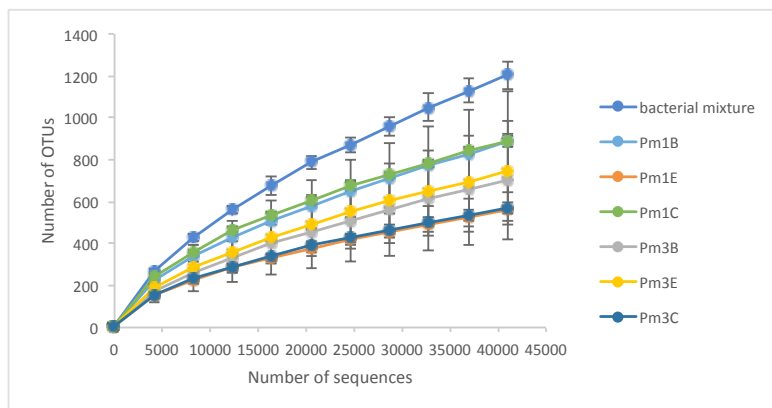


Figure 2: Rarefaction curves of the number of the Shannon Index for each treatment of the food experiment (Pm1B, Pm1E, Pm3B, Pm3E), the agar from the stock cultures (Pm1C and Pm3C) and the pure bacterial mixture. Error bars were calculated from the variance of 10 randomizations at each sample size.

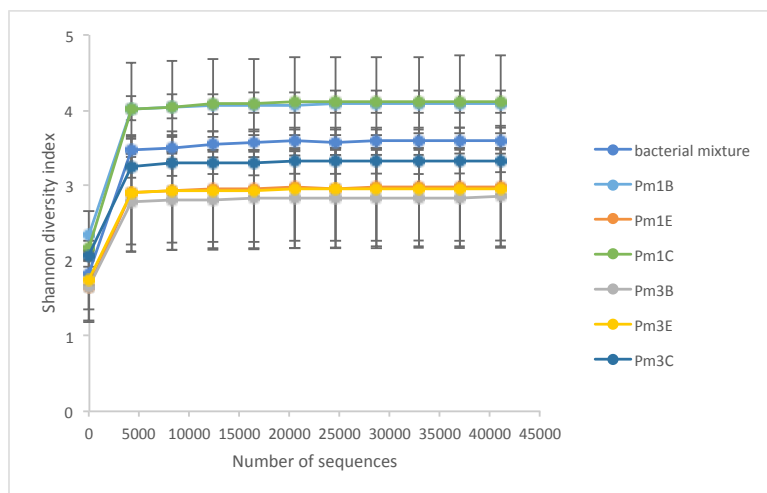


Figure 3: Rank abundance plots of OTUs from each of the 20 Pm1 specimens. Note the log scale of the X-axis. A/ *E. coli* fed worms; B/ Bacterial mix fed worms. Each line corresponds to one specimen.

A

B

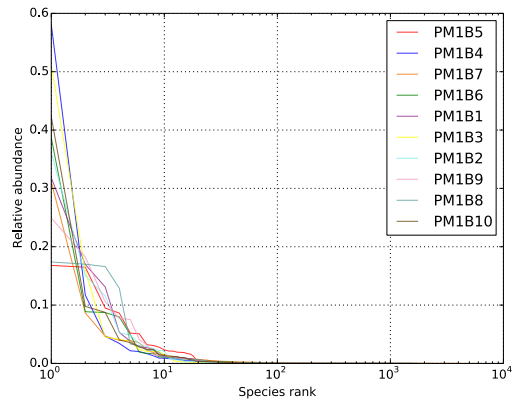
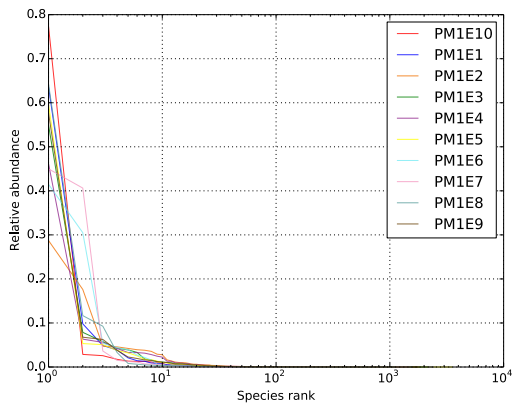
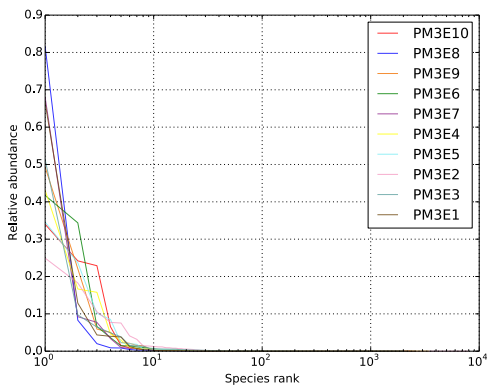
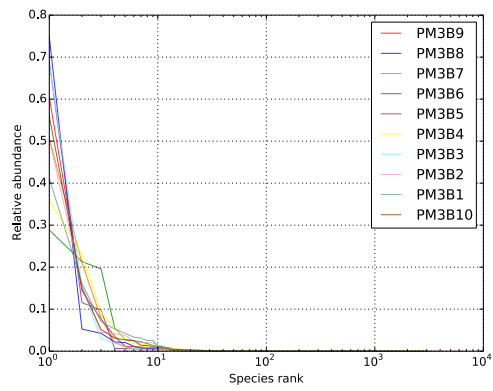


Figure 4: Rank abundance plots of OTUs from each of the 20 Pm3 specimens. Note the log scale of the X-axis. A/ *E. coli* fed worms; B/ Bacterial mix fed worms. Each line corresponds to one specimen.

A



B



Appendix S11: List of biomarker taxa identified by LeFSe for Pm1 and Pm3 from the food experiment. OTU ID and taxonomic assignment using Greengenes are included.

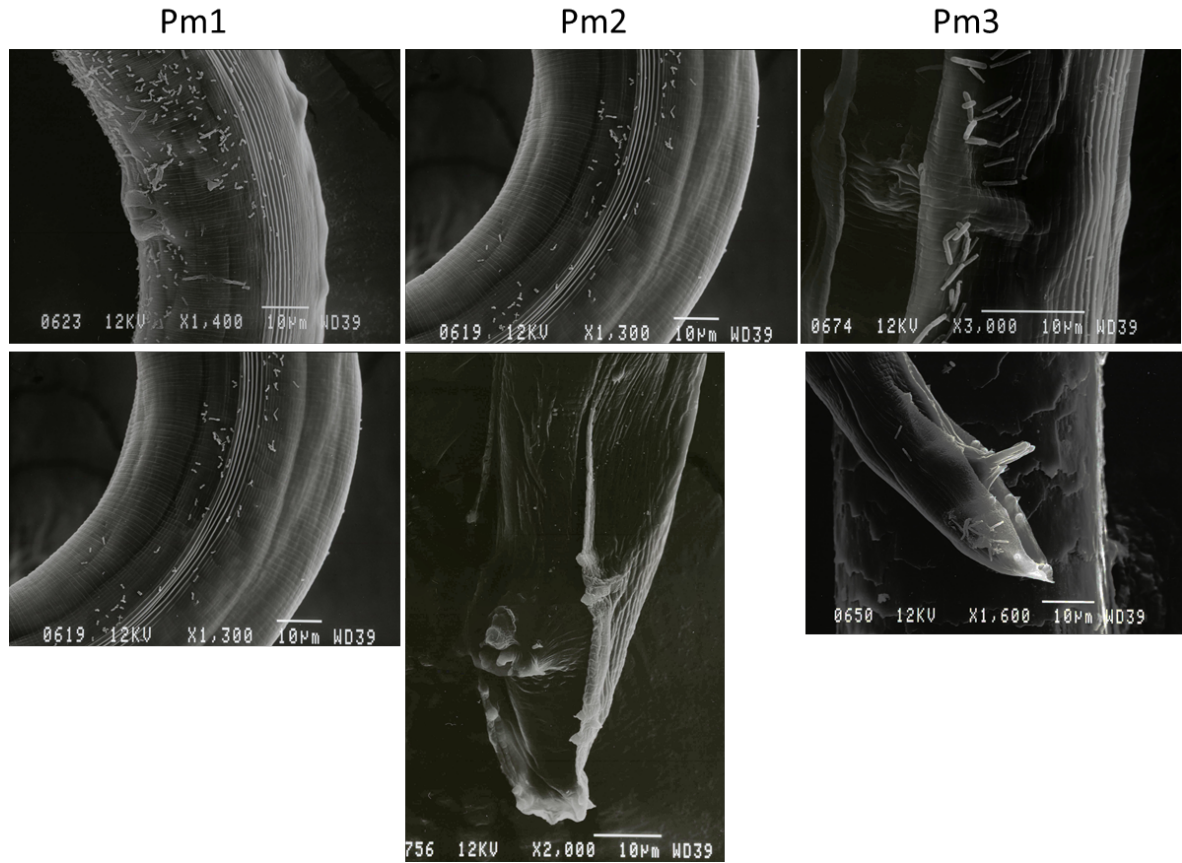
Biomarker taxa for Pm1		Biomarkers for Pm3	
OTU ID	Taxonomy	OTU ID	Taxonomy
24346	Bacteroidetes	278303	Bacteroidetes
NewReferenceOTU2348	Bacteroidetes	NewCleanUpReferenceOTU29602	Bacteroidetes
NewReferenceOTU2103	Bacteroidetes	147086	Bacteroidetes
394758	Bacteroidetes	NewReferenceOTU4131	Bacteroidetes
783545	Bacteroidetes	NewReferenceOTU2357	Bacteroidetes
NewReferenceOTU590	Bacteroidetes	NewReferenceOTU2438	Bacteroidetes
NewReferenceOTU1082	Bacteroidetes	NewReferenceOTU2455	Bacteroidetes
114234	Bacteroidetes	NewCleanUpReferenceOTU155365	Bacteroidetes
643716	Bacteroidetes	NewReferenceOTU2305	Bacteroidetes
210280	Bacteroidetes	4313779	Bacteroidetes
249383	Bacteroidetes	NewReferenceOTU631	Bacteroidetes
1032085	Bacteroidetes	147086	Bacteroidetes.Flavobacteria
NewReferenceOTU4008	Bacteroidetes	NewCleanUpReferenceOTU29602	Bacteroidetes.Flavobacteria
NewReferenceOTU1030	Bacteroidetes	278303	Bacteroidetes.Flavobacteria
4321726	Bacteroidetes	NewReferenceOTU2455	Bacteroidetes.Flavobacteria
NewReferenceOTU4188	Bacteroidetes	NewReferenceOTU2305	Bacteroidetes.Flavobacteria
549386	Bacteroidetes	NewReferenceOTU2438	Bacteroidetes.Flavobacteria
NewReferenceOTU4168	Bacteroidetes	NewReferenceOTU4131	Bacteroidetes.Flavobacteria
149448	Bacteroidetes	NewCleanUpReferenceOTU155365	Bacteroidetes.Flavobacteria
NewReferenceOTU4195	Bacteroidetes	NewCleanUpReferenceOTU155365	Bacteroidetes.Flavobacteria.Flavobacteriales
NewReferenceOTU4168	Bacteroidetes.Flavobacteria	NewCleanUpReferenceOTU29602	Bacteroidetes.Flavobacteria.Flavobacteriales
NewReferenceOTU4195	Bacteroidetes.Flavobacteria	147086	Bacteroidetes.Flavobacteria.Flavobacteriales
1032085	Bacteroidetes.Flavobacteria	NewReferenceOTU2438	Bacteroidetes.Flavobacteria.Flavobacteriales
249383	Bacteroidetes.Flavobacteria	278303	Bacteroidetes.Flavobacteria.Flavobacteriales
4321726	Bacteroidetes.Flavobacteria	NewReferenceOTU4131	Bacteroidetes.Flavobacteria.Flavobacteriales
84346	Bacteroidetes.Flavobacteria	NewReferenceOTU2305	Bacteroidetes.Flavobacteria.Flavobacteriales
149448	Bacteroidetes.Flavobacteria	NewReferenceOTU2455	Bacteroidetes.Flavobacteria.Flavobacteriales
NewReferenceOTU1082	Bacteroidetes.Flavobacteria	NewCleanUpReferenceOTU155365	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
643716	Bacteroidetes.Flavobacteria	NewReferenceOTU4131	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
NewReferenceOTU590	Bacteroidetes.Flavobacteria	NewCleanUpReferenceOTU29602	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
783545	Bacteroidetes.Flavobacteria	NewReferenceOTU2438	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
NewReferenceOTU2348	Bacteroidetes.Flavobacteria	NewReferenceOTU2305	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
NewReferenceOTU2103	Bacteroidetes.Flavobacteria	147086	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
NewReferenceOTU4008	Bacteroidetes.Flavobacteria	NewReferenceOTU2455	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
394758	Bacteroidetes.Flavobacteria	NewReferenceOTU2455	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae
NewReferenceOTU4188	Bacteroidetes.Flavobacteria	NewCleanUpReferenceOTU29602	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
114234	Bacteroidetes.Flavobacteria	NewReferenceOTU2305	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
NewReferenceOTU4168	Bacteroidetes.Flavobacteria.Flavobacteriales	147086	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
394758	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU4131	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
NewReferenceOTU4008	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU2455	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
NewReferenceOTU2103	Bacteroidetes.Flavobacteria.Flavobacteriales	278303	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
4321726	Bacteroidetes.Flavobacteria.Flavobacteriales	NewCleanUpReferenceOTU155365	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
114234	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU2438	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga
1032085	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU2438	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga.slytica
643716	Bacteroidetes.Flavobacteria.Flavobacteriales	NewCleanUpReferenceOTU29602	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga.slytica
149448	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU4131	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga.slytica
NewReferenceOTU590	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU2305	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga.slytica
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NewReferenceOTU1082	Bacteroidetes.Flavobacteria.Flavobacteriales	278303	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gCellulophaga.slytica
NewReferenceOTU2348	Bacteroidetes.Flavobacteria.Flavobacteriales	4313779	Bacteroidetes.Saprospirae
783545	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU631	Bacteroidetes.Saprospirae
NewReferenceOTU4188	Bacteroidetes.Flavobacteria.Flavobacteriales	NewReferenceOTU2357	Bacteroidetes.Saprospirae
NewReferenceOTU2103	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae	NewReferenceOTU631	Bacteroidetes.Saprospirae.Saprospirales
NewReferenceOTU4008	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae	NewReferenceOTU2357	Bacteroidetes.Saprospirae.Saprospirales
NewReferenceOTU4168	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae	4313779	Bacteroidetes.Saprospirae.Saprospirales
NewReferenceOTU4188	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae	NewReferenceOTU2357	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae
NewReferenceOTU4195	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g	4313779	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae
NewReferenceOTU2103	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g	NewReferenceOTU631	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g
NewReferenceOTU4188	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g	4313779	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g
NewReferenceOTU4195	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g	NewReferenceOTU2357	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g
NewReferenceOTU4168	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g.s	NewReferenceOTU2357	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g.s
NewReferenceOTU2103	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g.s	NewReferenceOTU631	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g.s
NewReferenceOTU4188	Bacteroidetes.Flavobacteria.Flavobacteriales.Cryomorphaceae.g.s	4313779	Bacteroidetes.Saprospirae.Saprospirales.Saprospiraceae.g.s
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NewReferenceOTU1082	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	160928	Proteobacteria
4321726	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	530962	Proteobacteria
1032085	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	NewReferenceOTU2428	Proteobacteria
NewReferenceOTU2348	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	NewCleanUpReferenceOTU73063	Proteobacteria
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		NewReferenceOTU1394	Proteobacteria

394758	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	NewCleanUpReferenceOTU249675	Proteobacteria
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249383	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	198609	Proteobacteria
114234	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	346035	Proteobacteria
84346	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	NewReferenceOTU2279	Proteobacteria
643716	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae	4304357	Proteobacteria
NewReferenceOTU2348	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g	785565	Proteobacteria
1032085	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g	NewReferenceOTU315	Proteobacteria
249383	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g	NewCleanUpReferenceOTU249675	Proteobacteria.Alphaproteobacteria
1032085	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g.s	NewReferenceOTU315	Proteobacteria.Alphaproteobacteria
249383	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g.s	NewCleanUpReferenceOTU73063	Proteobacteria.Alphaproteobacteria
NewReferenceOTU2348	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.g.s	NewReferenceOTU1394	Proteobacteria.Alphaproteobacteria
84346	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gMaribacter	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria
84346	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gMaribacter	4304357	Proteobacteria.Alphaproteobacteria
394758	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewCleanUpReferenceOTU249675	Proteobacteria.Alphaproteobacteria.Rhizobiales
1032085	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae
114234	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewCleanUpReferenceOTU249675	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae.gAgrobacterium
643716	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewCleanUpReferenceOTU249675	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae.gAgrobacterium.s
783545	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewReferenceOTU315	Proteobacteria.Alphaproteobacteria.Rhodobacterales
4321726	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewCleanUpReferenceOTU73063	Proteobacteria.Alphaproteobacteria.Rhodobacterales
NewReferenceOTU590	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria.Rhodobacterales
149448	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella	NewReferenceOTU1394	Proteobacteria.Alphaproteobacteria.Rhodobacterales
4321726	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	4304357	Proteobacteria.Alphaproteobacteria.Rhodobacterales
149448	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	NewReferenceOTU315	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae
NewReferenceOTU1082	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae
NewReferenceOTU590	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	4304357	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae
643716	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	NewReferenceOTU1394	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae
783545	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	NewCleanUpReferenceOTU73063	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g
394758	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	4304357	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g
114234	Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.gWinogradskyella.sthalssocola	NewReferenceOTU315	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g
549386	Bacteroidetes.Saprosiriae	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g
NewReferenceOTU1030	Bacteroidetes.Saprosiriae	NewReferenceOTU73063	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g
210280	Bacteroidetes.Saprosiriae	NewReferenceOTU2110	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g.s
210280	Bacteroidetes.Saprosiriae.Saprosirales	4304357	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g.s
NewReferenceOTU1030	Bacteroidetes.Saprosiriae.Saprosirales	NewCleanUpReferenceOTU73063	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g.s
549386	Bacteroidetes.Saprosiriae.Saprosirales	NewReferenceOTU315	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g.s
549386	Bacteroidetes.Saprosiriae.Saprosirales	NewReferenceOTU1394	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.g.s
210280	Bacteroidetes.Saprosiriae.Saprosirales.g	NewReferenceOTU2428	Proteobacteria.Gammaproteobacteria
549386	Bacteroidetes.Saprosiriae.Saprosirales.g	346035	Proteobacteria.Gammaproteobacteria
210280	Bacteroidetes.Saprosiriae.Saprosirales.g.s	NewReferenceOTU2279	Proteobacteria.Gammaproteobacteria
549386	Bacteroidetes.Saprosiriae.Saprosirales.g.s	820978	Proteobacteria.Gammaproteobacteria
NewReferenceOTU1030	Bacteroidetes.Saprosiriae.Saprosirales.g.s	160928	Proteobacteria.Gammaproteobacteria
NewReferenceOTU100	Proteobacteria	198609	Proteobacteria.Gammaproteobacteria
591923	Proteobacteria	NewReferenceOTU2190	Proteobacteria.Gammaproteobacteria
NewReferenceOTU2343	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria
NewReferenceOTU921	Proteobacteria	785565	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU169	Proteobacteria	160928	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU2218	Proteobacteria	198609	Proteobacteria.Gammaproteobacteria.Vibrionales
593700	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria.Vibrionales
561165	Proteobacteria	NewReferenceOTU2279	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU1033	Proteobacteria	NewReferenceOTU2190	Proteobacteria.Gammaproteobacteria.Vibrionales
NewCleanUpReferenceOTU150954	Proteobacteria	785565	Proteobacteria.Gammaproteobacteria.Vibrionales
1108208	Proteobacteria	820978	Proteobacteria.Gammaproteobacteria.Vibrionales
176420	Proteobacteria	NewReferenceOTU2428	Proteobacteria.Gammaproteobacteria.Vibrionales
750031	Proteobacteria	346035	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
NewReferenceOTU1334	Proteobacteria	NewReferenceOTU2428	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
251914	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
252007	Proteobacteria	785565	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
542278	Proteobacteria	198609	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g
836362	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g
309489	Proteobacteria	785565	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g
NewReferenceOTU1521	Proteobacteria	820978	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g
NewCleanUpReferenceOTU130132	Proteobacteria	NewReferenceOTU2428	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g
NewReferenceOTU3035	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
NewReferenceOTU471	Proteobacteria	198609	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
NewReferenceOTU2011	Proteobacteria	530962	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
158140	Proteobacteria	785565	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
326373	Proteobacteria	820978	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
NewReferenceOTU408	Proteobacteria	NewReferenceOTU2428	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae.g.s
NewReferenceOTU3401	Proteobacteria	346035	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae
140787	Proteobacteria	160928	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae
808031	Proteobacteria	NewReferenceOTU2279	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae
36441	Proteobacteria	NewReferenceOTU2190	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio
		NewReferenceOTU2279	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio

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79817	Proteobacteria	160928	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio
159398	Proteobacteria	NewReferenceOTU2190	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio
NewCleanUpReferenceOTU220298	Proteobacteria	346035	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio.s
NewCleanUpReferenceOTU117670	Proteobacteria	160928	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio.s
NewCleanUpReferenceOTU92419	Proteobacteria	NewReferenceOTU2279	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio.s
586746	Proteobacteria	NewReferenceOTU2190	Proteobacteria.Gammaproteobacteria.Vibrionales.Vibrionaceae.gVibrio.s
84384	Proteobacteria	NewReferenceOTU13759	Unassigned
153173	Proteobacteria	NewReferenceOTU1875	Unassigned
NewReferenceOTU1989	Proteobacteria	NewReferenceOTU1504	Unassigned
140920	Proteobacteria	NewReferenceOTU1550	Unassigned
NewReferenceOTU419	Proteobacteria	NewReferenceOTU1045	Unassigned
NewReferenceOTU2435	Proteobacteria	NewReferenceOTU2401	Unassigned
590614	Proteobacteria	NewReferenceOTU2750	Unassigned
4353625	Proteobacteria	NewReferenceOTU54	Unassigned
NewReferenceOTU2796	Proteobacteria	NewReferenceOTU3705	Unassigned
830290	Proteobacteria	NewReferenceOTU3574	Unassigned
NewReferenceOTU3191	Proteobacteria	NewReferenceOTU2014	Unassigned
830696	Proteobacteria	NewCleanUpReferenceOTU101342	Unassigned
518661	Proteobacteria	NewCleanUpReferenceOTU216626	Unassigned
NewReferenceOTU1187	Proteobacteria	NewCleanUpReferenceOTU83057	Unassigned
140829	Proteobacteria	NewReferenceOTU1877	Unassigned
140860	Proteobacteria	NewReferenceOTU1507	Unassigned
562023	Proteobacteria	NewReferenceOTU1172	Unassigned
518661	Proteobacteria.Alphaproteobacteria	NewReferenceOTU2213	Unassigned
NewReferenceOTU2011	Proteobacteria.Alphaproteobacteria	NewReferenceOTU2420	Unassigned
593700	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1043	Unassigned
NewReferenceOTU1334	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1848	Unassigned
NewReferenceOTU1187	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3347	Unassigned
NewReferenceOTU1989	Proteobacteria.Alphaproteobacteria	NewReferenceOTU949	Unassigned
NewReferenceOTU471	Proteobacteria.Alphaproteobacteria	NewCleanUpReferenceOTU236908	Unassigned
590614	Proteobacteria.Alphaproteobacteria	NewReferenceOTU2197	Unassigned
NewReferenceOTU1033	Proteobacteria.Alphaproteobacteria	NewCleanUpReferenceOTU201181	Unassigned
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NewCleanUpReferenceOTU220298	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3153	Unassigned
NewReferenceOTU921	Proteobacteria.Alphaproteobacteria	NewReferenceOTU2168	Unassigned
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750021	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1376	Unassigned
NewCleanUpReferenceOTU130132	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3457	Unassigned
NewReferenceOTU419	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1442	Unassigned
NewReferenceOTU169	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3095	Unassigned
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542278	Proteobacteria.Alphaproteobacteria	NewReferenceOTU2684	Unassigned
NewReferenceOTU2343	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1502	Unassigned
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NewReferenceOTU2796	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3078	Unassigned
1108208	Proteobacteria.Alphaproteobacteria	NewReferenceOTU3922	Unassigned
326373	Proteobacteria.Alphaproteobacteria	NewCleanUpReferenceOTU147402	Unassigned
NewCleanUpReferenceOTU117670	Proteobacteria.Alphaproteobacteria	NewReferenceOTU1787	Unassigned
NewReferenceOTU2435	Proteobacteria.Alphaproteobacteria.Rhizobiales	NewReferenceOTU1236	Unassigned
NewCleanUpReferenceOTU220298	Proteobacteria.Alphaproteobacteria.Rhizobiales	NewReferenceOTU1493	Unassigned
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NewReferenceOTU2796	Proteobacteria.Alphaproteobacteria.Rhizobiales	NewReferenceOTU1422	Unassigned
518661	Proteobacteria.Alphaproteobacteria.Rhizobiales	NewReferenceOTU1042	Unassigned
NewReferenceOTU1989	Proteobacteria.Alphaproteobacteria.Rhizobiales	NewReferenceOTU1160	Unassigned
NewCleanUpReferenceOTU220298	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae	NewReferenceOTU1756	Unassigned
518661	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae	NewReferenceOTU1872	Unassigned
NewReferenceOTU3035	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae		
NewReferenceOTU2796	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae		
NewReferenceOTU1989	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g		
NewReferenceOTU2796	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g		
NewCleanUpReferenceOTU220298	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g		
NewReferenceOTU1989	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g		
NewReferenceOTU3035	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g		
NewReferenceOTU1989	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g.s		
NewReferenceOTU2796	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g.s		
NewReferenceOTU3035	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g.s		
NewCleanUpReferenceOTU220298	Proteobacteria.Alphaproteobacteria.Rhizobiales.Phylobacteriaceae.g.s		
NewReferenceOTU2435	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae		
NewReferenceOTU2435	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae.gAgrobacterium		
NewReferenceOTU2435	Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhizobiaceae.gAgrobacterium.s		
NewReferenceOTU2343	Proteobacteria.Alphaproteobacteria.Rhodobacterales		
NewReferenceOTU100	Proteobacteria.Alphaproteobacteria.Rhodobacterales		
NewReferenceOTU1334	Proteobacteria.Alphaproteobacteria.Rhodobacterales		

590614	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.gOctadecabacter
NewReferenceOTU169	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.gOctadecabacter.s
590614	Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.gOctadecabacter.s
837366	Proteobacteria.Gammaproteobacteria
836362	Proteobacteria.Gammaproteobacteria
562023	Proteobacteria.Gammaproteobacteria
591923	Proteobacteria.Gammaproteobacteria
158140	Proteobacteria.Gammaproteobacteria
561165	Proteobacteria.Gammaproteobacteria
79817	Proteobacteria.Gammaproteobacteria
830290	Proteobacteria.Gammaproteobacteria
586746	Proteobacteria.Gammaproteobacteria
140787	Proteobacteria.Gammaproteobacteria
153173	Proteobacteria.Gammaproteobacteria
808031	Proteobacteria.Gammaproteobacteria
NewCleanUpReferenceOTU92419	Proteobacteria.Gammaproteobacteria
309489	Proteobacteria.Gammaproteobacteria
36441	Proteobacteria.Gammaproteobacteria
4353625	Proteobacteria.Gammaproteobacteria
84384	Proteobacteria.Gammaproteobacteria
830696	Proteobacteria.Gammaproteobacteria
176420	Proteobacteria.Gammaproteobacteria
140920	Proteobacteria.Gammaproteobacteria
NewReferenceOTU3191	Proteobacteria.Gammaproteobacteria
251914	Proteobacteria.Gammaproteobacteria
NewReferenceOTU1521	Proteobacteria.Gammaproteobacteria
140829	Proteobacteria.Gammaproteobacteria
140860	Proteobacteria.Gammaproteobacteria
252007	Proteobacteria.Gammaproteobacteria
19398	Proteobacteria.Gammaproteobacteria
NewReferenceOTU3401	Proteobacteria.Gammaproteobacteria
79817	Proteobacteria.Gammaproteobacteria.Alteromonadales
79817	Proteobacteria.Gammaproteobacteria.Alteromonadales.Alteromonadaceae
79817	Proteobacteria.Gammaproteobacteria.Alteromonadales.Alteromonadaceae.g
140860	Proteobacteria.Gammaproteobacteria.Vibrionales
586746	Proteobacteria.Gammaproteobacteria.Vibrionales
176420	Proteobacteria.Gammaproteobacteria.Vibrionales
830696	Proteobacteria.Gammaproteobacteria.Vibrionales
140787	Proteobacteria.Gammaproteobacteria.Vibrionales
19398	Proteobacteria.Gammaproteobacteria.Vibrionales
561165	Proteobacteria.Gammaproteobacteria.Vibrionales
NewCleanUpReferenceOTU92419	Proteobacteria.Gammaproteobacteria.Vibrionales
837366	Proteobacteria.Gammaproteobacteria.Vibrionales
309489	Proteobacteria.Gammaproteobacteria.Vibrionales
36441	Proteobacteria.Gammaproteobacteria.Vibrionales
836362	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU3191	Proteobacteria.Gammaproteobacteria.Vibrionales
140920	Proteobacteria.Gammaproteobacteria.Vibrionales
591923	Proteobacteria.Gammaproteobacteria.Vibrionales
808031	Proteobacteria.Gammaproteobacteria.Vibrionales
140829	Proteobacteria.Gammaproteobacteria.Vibrionales
158140	Proteobacteria.Gammaproteobacteria.Vibrionales
252007	Proteobacteria.Gammaproteobacteria.Vibrionales
251914	Proteobacteria.Gammaproteobacteria.Vibrionales
4353625	Proteobacteria.Gammaproteobacteria.Vibrionales
830290	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU3401	Proteobacteria.Gammaproteobacteria.Vibrionales
NewReferenceOTU1521	Proteobacteria.Gammaproteobacteria.Vibrionales
153173	Proteobacteria.Gammaproteobacteria.Vibrionales
84384	Proteobacteria.Gammaproteobacteria.Vibrionales
562023	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
562023	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
808031	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
836362	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
561165	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
19398	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
140829	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
309489	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
84384	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
251914	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
140920	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
140860	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
4353625	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
140787	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae
153173	Proteobacteria.Gammaproteobacteria.Vibrionales.Pseudoalteromonadaceae

Appendix S12: SEM pictures of nematode specimens from Pm1, Pm2 and Pm3 with bacteria attached to the cuticula. Top row: vulva region of the females (laterofrontal view for Pm1, lateral view for Pm2 and ventral view for Pm3); bottom row: males. For Pm1, this is a lateral view of the mid body region, for Pm2 a ventral view of the tail posterior region with bursa and spicula and for Pm3 a lateral view of the posterior body region with ejected spicula.



Appendix S13: Number of shared OTUs between each nematode specimen and each replica of the bacterial mix.

