

Supplementary Material

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Nanopore-sequencing characterization of the gut microbiota of *Melolontha melolontha* larvae: contribution to protection against entomopathogenic nematodes?

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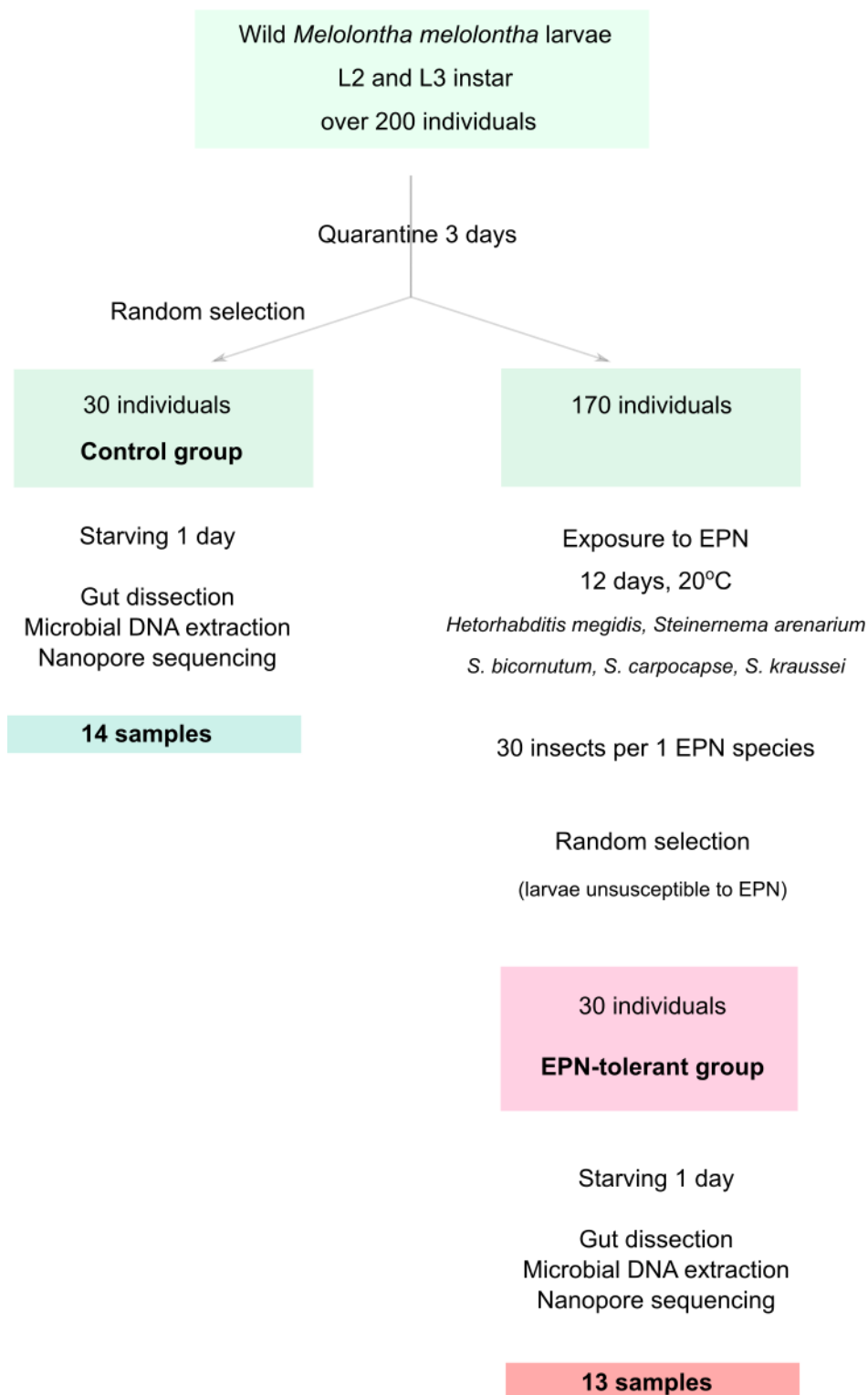


Figure S1. Schematic diagram of the course of the experiment

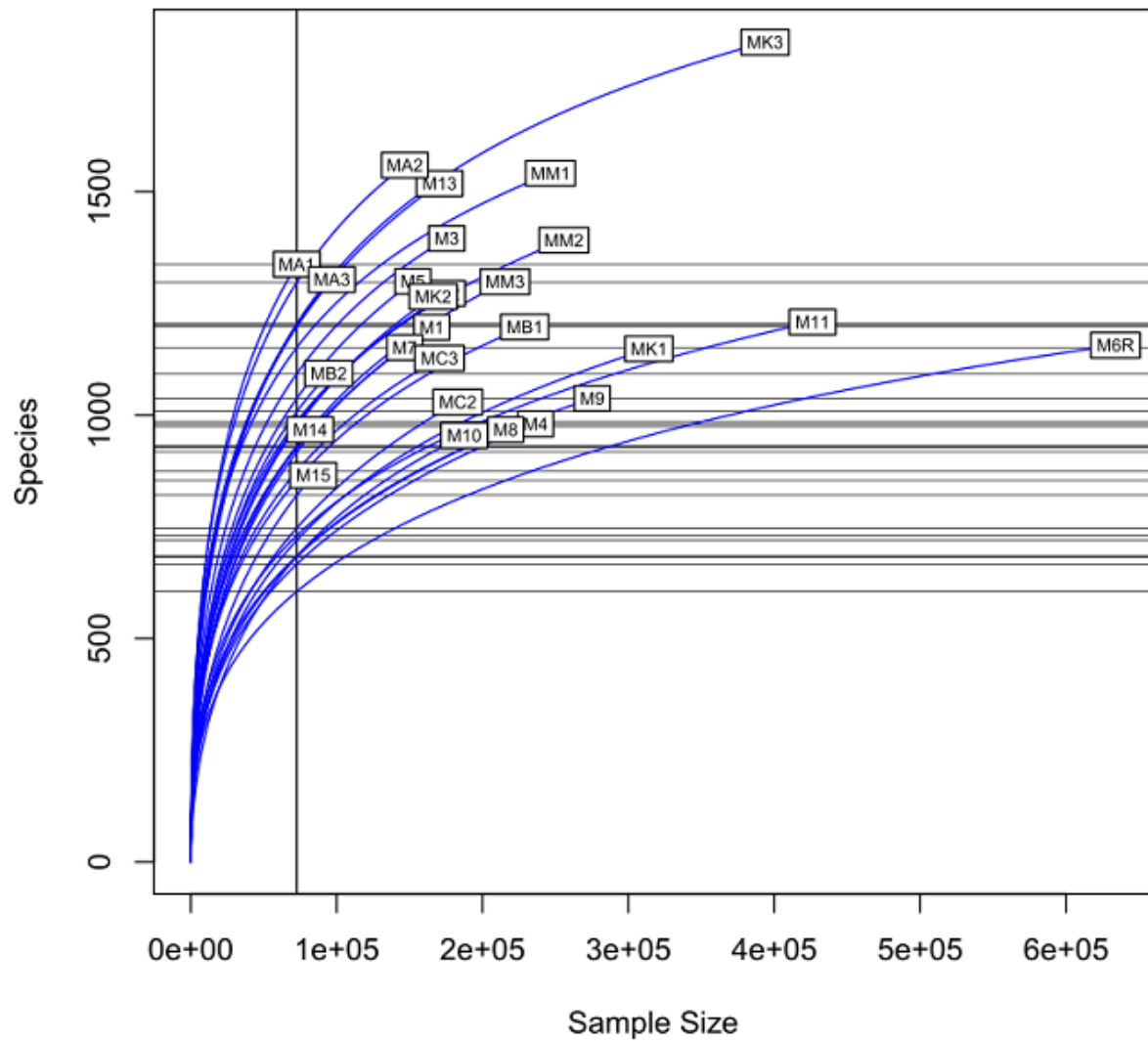


Figure S2. Rarefaction curves from nanopore sequencing of the 16S rRNA gene

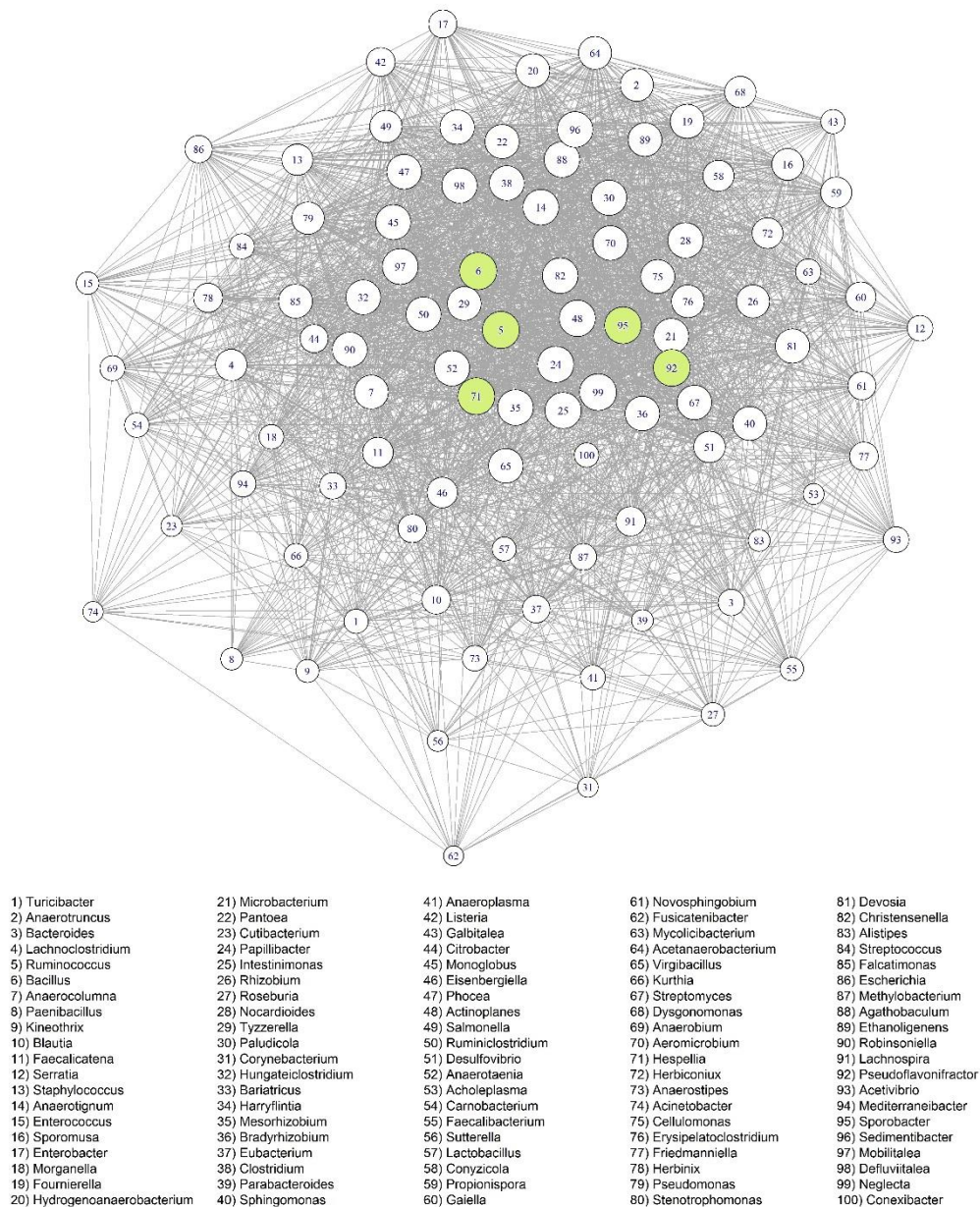
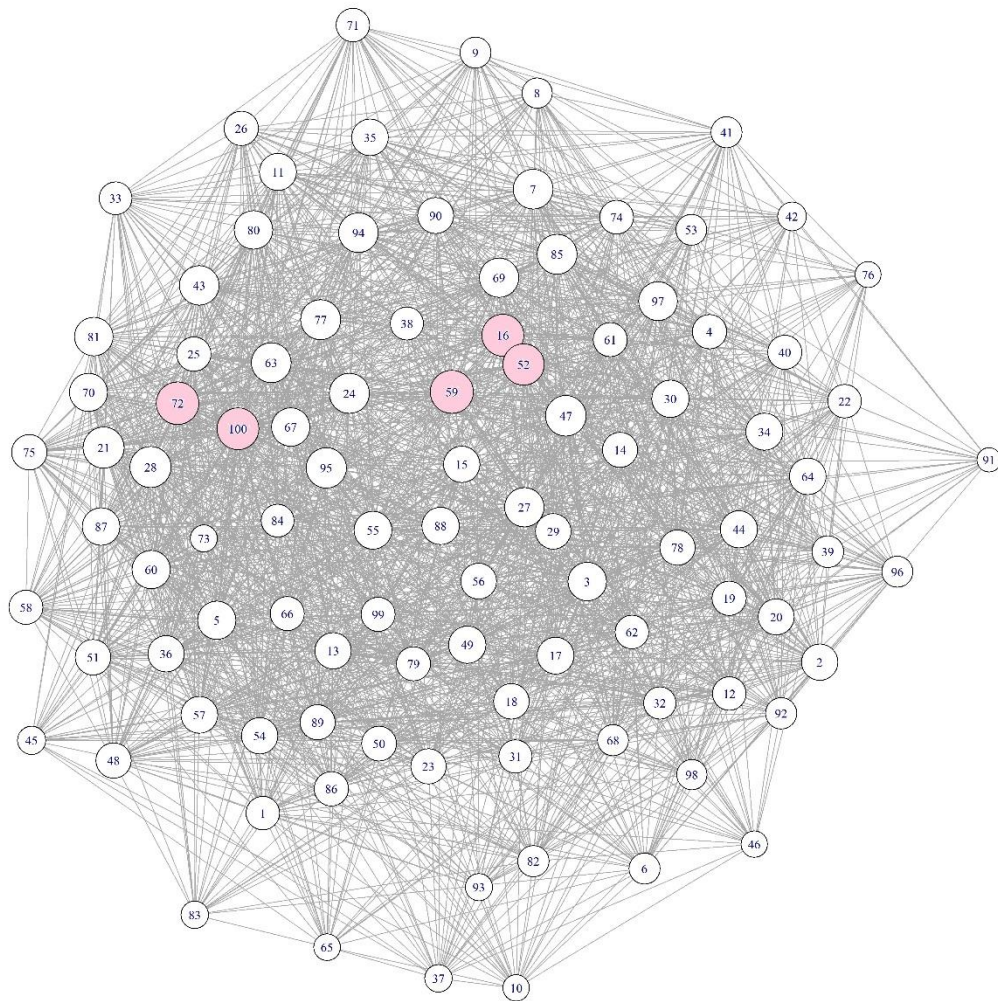


Figure S3. Microbiome network of the midgut bacterial genera found in the control group of *M. melolontha* larvae



- | | | | | |
|------------------------------|-------------------------|-----------------------|----------------------------|--------------------------|
| 1) Turicibacter | 21) Microbacterium | 41) Anaeroplasm | 61) Novosphingobium | 81) Devosia |
| 2) Anaerotruncus | 22) Pantoea | 42) Listeria | 62) Fusicatenibacter | 82) Christensenella |
| 3) Bacteroides | 23) Cutibacterium | 43) Galbitalea | 63) Mycolicibacterium | 83) Alistipes |
| 4) Lachnospirillum | 24) Papillibacter | 44) Citrobacter | 64) Acetanaerobacterium | 84) Streptococcus |
| 5) Ruminococcus | 25) Intestinimonas | 45) Monoglobus | 65) Virgibacillus | 85) Falcitimonas |
| 6) Bacillus | 26) Rhizobium | 46) Eisenbergiella | 66) Kurthia | 86) Escherichia |
| 7) Anaerocolumna | 27) Roseburia | 47) Phocaea | 67) Streptomyces | 87) Methylobacterium |
| 8) Paenibacillus | 28) Nocardioide | 48) Actinoplanes | 68) Dysgonomonas | 88) Agathobaculum |
| 9) Kineothrix | 29) Tyzzerella | 49) Salmonella | 69) Anaerobium | 89) Ethanologenes |
| 10) Blautia | 30) Paludicola | 50) Ruminiclostridium | 70) Aeromicrobium | 90) Robinsoniella |
| 11) Faecalicatena | 31) Corynebacterium | 51) Desulfovibrio | 71) Hespelia | 91) Lachnospira |
| 12) Serratia | 32) Hungateiclostridium | 52) Anaerotaenia | 72) Herbiconiux | 92) Pseudoflavonifractor |
| 13) Staphylococcus | 33) Bariatrus | 53) Acholeplasma | 73) Anaerostipes | 93) Acetivibrio |
| 14) Anaerotruncus | 34) Harryflintia | 54) Carnobacterium | 74) Acinetobacter | 94) Mediterraneibacter |
| 15) Enterococcus | 35) Mesorhizobium | 55) Faecalibacterium | 75) Cellulomonas | 95) Sporobacter |
| 16) Sporomusa | 36) Bradyrhizobium | 56) Sutterella | 76) Erysipelatoclostridium | 96) Sedimentibacter |
| 17) Enterobacter | 37) Eubacterium | 57) Lactobacillus | 77) Friedmanniella | 97) Mobilitalea |
| 18) Morganella | 38) Clostridium | 58) Conyzicola | 78) Herbinix | 98) Defluviitalea |
| 19) Fournierella | 39) Parabacteroides | 59) Propionispora | 79) Pseudomonas | 99) Neglecta |
| 20) Hydrogenoanaerobacterium | 40) Sphingomonas | 60) Gaiella | 80) Stenotrophomonas | 100) Conexibacter |

Figure S4. Microbiome network of the midgut bacterial genera in the EPN-resistant group of *M. melolontha* larvae

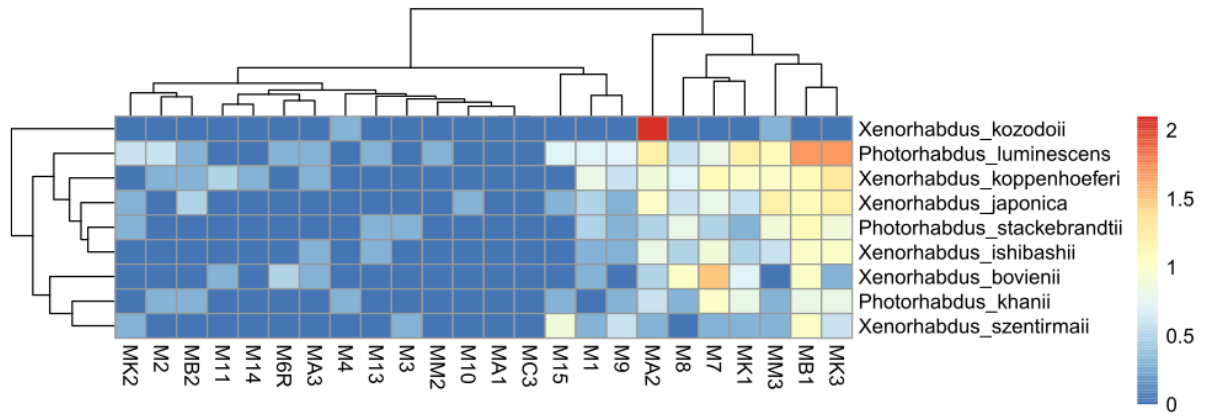


Figure S5. Heatmap showing the relative abundance of *Xenorhabdus* and *Photorhabdus* spp. detected in the midguts of all tested individuals

Table S1a. NGS sequencing statistics and bacterial diversity indicators for the midgut microbiota of the control group of *M. melolontha* larvae

Sample name	Nematode exposure	Sampling site	Developmental stage of larva	Total number of bacterial reads	Classification rate genus level (%)	Classification rate species level (%)	Sobs	Shannon	Simpson	Evenness
M1	No	ZF	L2	176,411	93.7	69.2	865	3.4	0.91	0.14
M2	No	ZF	L2	185,004	95.0	86.8	928	4.2	0.94	0.14
M3	No	PF	L2	182,284	96.3	86.9	1,045	3.8	0.86	0.12
M4	No	PF	L2	255,949	92.3	88.3	640	3.7	0.92	0.14
M5	No	PF	L2	160,319	95.0	86.6	934	4.4	0.97	0.14
M6R	No	KG	L2	675,822	93.8	90.8	595	3.7	0.93	0.15
M7	No	KG	L2	165,194	88.8	88.7	855	4.3	0.97	0.14
M8	No	KG	L3	227,905	94.7	88.7	643	3.6	0.92	0.14
M9	No	KG	L3	303,510	90.7	89.5	662	4.1	0.97	0.15
M10	No	KF	L3	191,361	97.9	93.5	635	3.9	0.91	0.14
M11	No	KF	L3	456,497	93.4	91.6	724	4.3	0.97	0.15
M13	No	KF	L3	181,730	93.9	84.7	1,113	4.5	0.97	0.14
M14	No	KF	L2	87,463	93.7	89.6	801	4.3	0.97	0.14
M15	No	KF	L2	86,641	96.9	89.8	720	3.6	0.90	0.14

Table S1b. NGS sequencing statistics and bacterial diversity indicators for the midgut microbiota of the EPN-resistant group of *M. melolontha* larvae

Sample name	Nematode exposure	Sampling site	Developmental stage of larva	Total number of bacterial reads	Classification rate genus level (%)	Classification rate species level (%)	Sobs	Shannon	Simpson	Evenness
MA1	Yes	KF	L2	77,759	93.5	86.2	1,165	4.9	0.98	0.14
MA2	Yes	KF	L2	158,972	92.3	82.7	1,217	4.8	0.97	0.14
MA3	Yes	KF	L2	104,015	93.0	84.5	1,083	5.1	0.99	0.14
MB1	Yes	KF	L2	249,252	91.8	86.8	813	3.9	0.94	0.14
MB2	Yes	KF	L2	99,846	94.8	87.7	896	4.4	0.96	0.14
MC2	Yes	KF	L2	190,503	96.1	94.1	701	2.4	0.65	0.10
MC3	Yes	KF	L3	177,462	96.1	92.7	829	4.7	0.98	0.15
MK1	Yes	KF	L2	326,700	96.1	88.9	691	3.6	0.93	0.14
MK2	Yes	KF	L2	173,240	95.9	89.6	924	4.1	0.96	0.14
MK3	Yes	KF	L3	422,249	93.2	87.1	1,201	4.7	0.98	0.14
MM1	Yes	KF	L2	259,941	94.9	80.1	1,112	4.8	0.98	0.14
MM2	Yes	KF	L2	275,099	93.0	86.6	965	4.2	0.96	0.14
MM3	Yes	KF	L3	222,727	96.7	91.9	874	3.9	0.91	0.13

Table S2. Proportions of the top twenty most abundant shared bacterial genera detected in the midgut of *M. melolontha* larvae

	M10	M11	M13	M14	M15	M1	M2	M3	M4	M5	M6R	M7	M8	M9	MA1	MA2	MA3	MB1	MB2	MC2	MC3	MK1	MK2	MK3	MM1	MM2	MM3
<i>Turicibacter</i>	76.14	9.73	10.88	0.33	0.46	0.06	0.01	0.17	0.06	2.26	0.27	0.23	0.06	3.40	0.08	0.50	0.58	1.85	0.02	5.14	47.18	11.63	1.00	2.73	0.50	0.28	38.88
<i>Bacteroides</i>	0.01	5.08	0.09	1.36	10.59	5.95	2.10	4.08	0.84	10.79	21.58	3.42	4.02	2.81	11.24	4.19	0.34	1.33	15.75	0.44	0.49	19.62	5.79	9.78	0.17	0.84	0.36
<i>Anaerotruncus</i>	0.16	8.06	6.37	8.29	2.36	0.11	0.82	2.64	5.25	2.62	7.14	5.80	20.73	1.78	0.05	0.61	2.06	0.88	0.86	55.27	3.59	0.07	0.32	3.65	6.57	4.19	0.13
<i>Lachnoclostridium</i>	0.02	2.17	1.83	11.36	0.73	0.52	22.34	3.29	23.08	1.89	3.83	8.80	1.01	1.57	0.55	0.32	1.24	6.14	1.26	0.96	1.59	0.61	0.14	0.70	2.05	1.41	0.06
<i>Ruminococcus</i>	0.02	3.47	1.27	2.57	0.58	0.44	3.11	0.86	4.51	1.61	2.07	4.18	2.55	5.53	0.51	0.32	0.96	2.28	1.78	5.24	1.06	7.05	5.82	4.13	0.64	3.91	0.23
<i>Anaerocolumna</i>	0.01	0.50	0.55	0.31	0.01	0.13	2.52	0.80	8.84	0.53	4.31	4.03	0.59	1.17	0.01	0.02	2.22	10.03	0.68	1.96	4.38	0.21	0.02	1.42	0.15	1.70	0.07
<i>Bacillus</i>	2.24	0.74	9.64	2.51	6.23	17.72	1.18	1.04	0.16	0.69	0.15	0.65	0.37	1.43	0.97	0.31	2.74	1.48	1.23	0.27	2.41	1.89	0.45	2.26	1.72	0.19	2.64
<i>Kineothrix</i>	0.01	0.17	0.64	2.99	0.59	0.96	3.88	0.27	3.71	0.69	0.15	1.27	0.23	6.38	0.48	0.09	0.90	5.81	2.29	0.14	0.69	1.46	0.17	0.49	0.54	10.93	0.22
<i>Faecalicatena</i>	0.00	0.31	0.13	0.53	0.09	0.18	3.78	0.15	2.57	0.26	0.48	2.11	0.17	7.65	0.03	0.05	0.47	3.00	2.46	0.25	1.09	1.76	0.24	0.33	0.05	12.25	0.24
<i>Blautia</i>	0.01	0.60	0.33	1.24	1.03	1.64	2.89	0.73	3.66	1.14	1.20	1.20	0.61	2.63	1.63	1.24	1.54	8.88	2.95	0.43	0.99	0.32	4.39	0.74	0.26	1.56	0.03
<i>Paludicola</i>	0.01	6.97	0.37	0.62	0.05	0.05	0.33	0.23	0.61	1.26	6.66	0.70	1.20	0.21	0.00	0.05	0.31	0.44	0.06	0.17	1.33	0.03	0.26	0.18	0.15	0.24	0.00
<i>Paenibacillus</i>	1.00	1.09	0.58	0.79	0.76	0.10	0.05	35.98	0.30	0.81	0.28	0.50	0.19	0.23	0.74	0.33	0.52	0.11	0.06	0.20	0.21	0.17	0.08	0.30	0.32	0.45	0.26
<i>Serratia</i>	0.01	0.06	0.01	0.01	0.05	0.05	0.03	0.10	0.00	0.93	0.32	9.06	8.23	0.02	0.36	0.19	2.06	14.48	0.21	0.00	0.00	0.16	0.02	0.20	0.03	0.00	1.81
<i>Staphylococcus</i>	0.51	0.18	0.03	0.11	0.37	14.86	1.77	0.04	0.01	1.57	0.01	0.06	0.13	1.26	0.19	0.02	0.46	1.53	1.75	0.12	0.32	4.32	2.60	2.26	0.06	0.00	0.10
<i>Hydrogenoanaerobacterium</i>	0.03	2.08	2.15	1.56	0.26	0.01	0.22	0.42	1.35	0.73	2.83	1.38	2.84	0.72	0.02	0.12	0.49	0.34	0.27	4.59	1.71	0.01	0.04	0.85	0.43	0.96	0.00
<i>Enterobacter</i>	0.01	0.00	0.02	0.00	0.05	0.35	0.68	0.03	0.00	0.02	0.01	0.05	0.02	0.95	0.09	2.95	0.00	0.31	0.03	0.00	0.06	1.22	2.59	2.74	0.00	0.01	17.03
<i>Sporomusa</i>	0.10	1.84	0.22	2.91	2.74	0.04	0.20	0.27	1.08	0.65	1.50	1.35	3.59	1.20	0.14	0.16	1.11	2.99	0.29	2.26	1.29	0.41	0.14	0.98	0.52	1.39	0.04
<i>Fournierella</i>	0.05	3.37	1.16	1.67	0.53	0.08	0.33	0.70	1.76	1.10	0.75	1.74	4.47	0.59	0.11	0.30	0.65	0.42	0.60	1.67	1.10	0.10	1.05	0.86	0.86	0.88	0.03
<i>Papillibacter</i>	0.01	1.53	0.11	1.87	0.50	0.08	0.17	0.55	1.54	0.50	0.91	3.04	1.44	7.19	0.01	0.03	1.25	0.26	0.17	0.14	0.24	2.04	0.13	0.86	0.42	0.39	0.02
<i>Enterococcus</i>	0.17	0.06	0.06	0.07	0.00	7.38	0.01	0.21	0.04	0.05	0.02	0.04	13.74	0.03	0.35	0.01	0.04	4.77	0.84	0.02	0.79	0.81	0.02	0.10	0.01	0.12	1.10

Table S3. Relative abundance of bacterial species exhibiting antagonistic activity against *Xenorhabdus* and *Photorhabdus* entomopathogens detected in the midgut of the EPN-resistant and control groups of insects

Species name	Abundance (mean in %) and 95% confidence interval	
	Control larvae	EPN-resistant larvae
<i>Serratia liquefaciens</i>	0.141 [0-0.325]	0.080 [0-0.179]
<i>Acinetobacter calcoaceticus</i>	0.046 [0-0.114]	0.073 [0.001-0.146]
<i>Citrobacter murlinae</i>	0.029 [0-0.061]	0.036 [0-0.077]
<i>Pseudomonas chlororaphis</i>	0.003 [0-0.01]	<0.001
<i>Chryseobacterium lathyr</i>	<0.001	<0.001
Antagonistic species in total	0.22 [0-0.47]*	0.19 [0.05-0.33]*

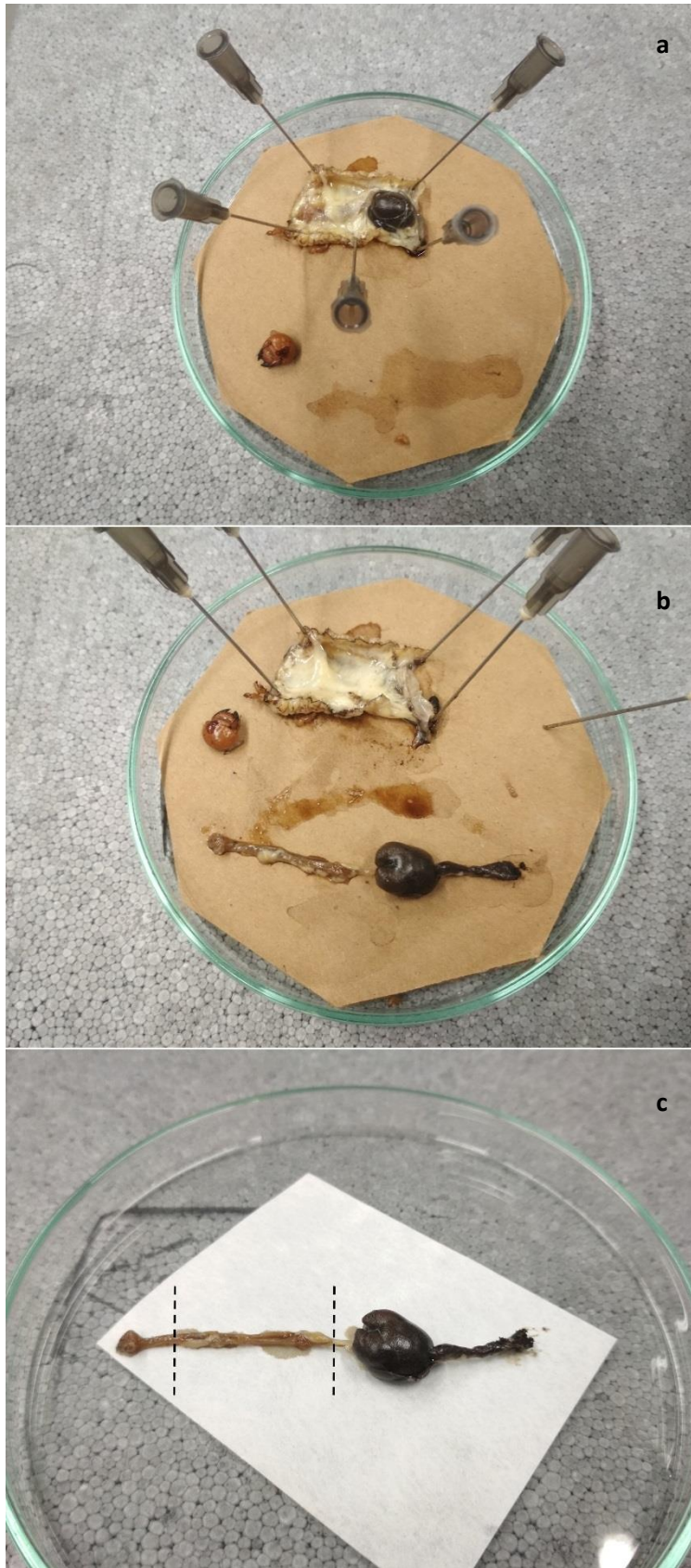
*no statistically significant differences between the groups

Table S4. Relative abundance of *Xenorhabdus* and *Photorhabdus* spp. detected in the midgut of the EPN-resistant and control groups of insects

Taxaname	Abundance (mean in %) and 95% confidence interval		
	Control insects	EPN-resistant insects	All insects
<i>Photorhabdus</i>	0.003 [0.001-0.005]*	0.008 [0.002-0.014]*	0.005 [0.002-0.009]
<i>P._luminescens</i>	n.d.	n.d.	0.003 [0.001-0.004]
<i>P._stackebrandtii</i>	n.d.	n.d.	0.001 [0-0.001]
<i>P._khanii</i>	n.d.	n.d.	0.001 [0-0.001]
<i>Xenorhabdus</i>	0.008 [0-0.015]*	0.016 [0-0.034]*	0.012 [0.003-0.021]
<i>X._kozodoii</i>	n.d.	n.d.	0.003 [0-0.01]
<i>X._koppenhoeferi</i>	n.d.	n.d.	0.001 [0.001-0.002]
<i>X._bovienii</i>	n.d.	n.d.	0.001 [0-0.003]
<i>X._japonica</i>	n.d.	n.d.	0.001 [0-0.002]
<i>X. ishibashii</i>	n.d.	n.d.	0.001 [0-0.001]
<i>X._szentirmaii</i>	n.d.	n.d.	0.001 [0-0.001]

Main abundant species (>0.001%) are indicated

*no statistically significant differences between the groups



Phot. S1. Stages of *M. melolontha* larva section (a, b) and an overview of the whole larval gut (c). The midgut is visible between the dashed lines.