

## [Short Communication]

## The composition of hindgut microbiota of *Periplaneta japonica* in the presence of thelastomatid parasitic nematodes

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Thelastomatid nematodes (Nematoda: Oxyurida: Thelastomatoidea) are obligatory parasites that occur naturally in the hindgut of arthropods. Their origin and impact in the host is still unknown. Previous studies showed that the presence of thelastomatid nematodes in the gut of cockroaches (*Periplaneta fuliginosa* and *P. americana*) could influence the composition of their hindgut microflora. Through a metagenomic approach (16S rRNA V3-V4 sequencing), we have characterized the hindgut microbiome of *P. japonica* in the presence of thelastomatid nematodes (L1986, natural parasitic nematode *Protrellus* sp. present as a natural infection condition; and L1987, non-native parasitic nematode *Leidynema appendiculatum* present as an artificial infection condition). The hindgut microbiome of *P. japonica* in both conditions were mainly composed of Bacteroidetes, Firmicutes and Proteobacteria. Moreover, the natural and artificial infection by thelastomatid nematodes lead to shifts in the relative abundance of these main resident flora as seen in *P. americana*. The OTUs percentage of Bacteroidetes and Proteobacteria were higher in *P. japonica* infected with *Protrellus* sp. (L1986) than in *P. japonica* infected by *L. appendiculatum* (L1987), while OTUs from Firmicutes phylum was higher in L1987 than in L1986. This study fosters a detailed investigation in the role played by these animal parasites in their host insect. *Nematol. Res.* 48(1), 19–26 (2018).

Key words: Cockroach, *Leidynema appendiculatum*, microbiome, *Protrellus*

*Periplaneta japonica*, the Japanese cockroach or Yamato cockroach, is a domiciliary pest originated from Japan, affecting other Asian countries (China and south-eastern Russia) and recently found in New York (USA) (Evangelista *et al.*, 2013). Adapted to northern climates, *P. japonica* is capable of surviving under freezing conditions through the strategic selection of

microhabitats and by displaying freeze tolerance (Tanaka *et al.*, 2012; Mullins, 2015). Recently, we discovered that *P. japonica* is naturally infected by thelastomatid parasitic nematodes from genus *Protrellus* and is capable of being artificially infected by the broad host range nematode, *Leidynema appendiculatum* (Ozawa and Hasegawa, 2018). These thelastomatid nematodes are not pathogenic parasites for the cockroaches, still their function in the biology of the insect is unknown (Ozawa *et al.*, 2014; Ozawa *et al.*, 2016; Sriwati *et al.*, 2016).

Cockroaches are interesting insect models to study multi-trophic interactions due to their long-term evolution and resilience (Mullins, 2015). As key-elements of insect's lifestyle, microbial communities (in particular gut residents) are involved in a wide range of functions (*i.e.* colonization and resistance to parasites and/or pathogens, diet breakdown, nutrient recycling and production of pheromones and/or kairomones) (Engel and Moran, 2013). The diversity of these communities is host-dependent, mainly determined by its habitat, diet, developmental stage and phylogeny (Yun *et al.*, 2014). Recently, the gut microbiome of *Periplaneta fuliginosa* and *P. americana* were investigated considering the presence of these nematodes (Vicente *et al.*, 2016). The authors suggested that the presence of thelastomatid nematodes could influence the composition of hindgut microflora, which was more biodiverse than the communities of non-infected hindgut and with major shifts in the relative abundance of the most representative taxa Bacteroidetes, Firmicutes and Proteobacteria (Vicente *et al.*, 2016).

Following our previous findings, here we briefly report, for the first time, the composition of the hindgut microbiome of the Japanese cockroach *P. japonica* in the presence of thelastomatid nematodes using a metagenomic approach.

### MATERIALS AND METHODS

*Periplaneta japonica* Miyoshi (artificially infected with *L. appendiculatum*) and *P. japonica* Akita (naturally infected with *Protrellus* sp.) strains have been maintained in the Hasegawa Laboratory (Chubu University, Japan) since, respectively, 2013 and 2015, under the conditions described in Vicente *et al.* (2016). Three adult males from each cockroach strain ( $n = 6$ ; average size:  $22.79 \pm 5.52$  mm) were dissected to obtain the whole gut system. Hindgut sections were separated, carefully washed with sterile 0.01M PBS (phosphate-buffered saline) pH7.4, and stored at  $-80^{\circ}\text{C}$  until further usage. The procedures for total DNA extraction from hindgut, PCR confirmation of

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nematode's presence (Table S1), and V3–V4 16S rRNA library preparation (Table S1) were performed as described in Vicente *et al.* (2016). Individual-to-individual variation of core gut microbiome of cockroaches is considered very low (Tinker and Ottesen, 2016). DNA samples for each condition were pooled in equimolar concentrations, fulfilling the standard concentration of 16S rRNA gene library protocol from Illumina MiSeq System. 16S rRNA libraries ( $n = 2$ ) were sequenced at Hokkaido System Science using Illumina MiSeq 300-bp Pair-End (301 cycles x2), and deposited in NCBI under the Bioproject PRJNA343690 and accessions SRS2483108 (L1986, *P. japonica* infected with *Protrellus* sp.) and SRS1705542 (L1987, *P. japonica* artificially infected with *L. appendiculatum*). Post-sequencing processing analysis was performed in QIIME version 1.9.1 (Caporaso *et al.*, 2010) and following the strategy presented in Edgar (2010) and Vicente *et al.* (2016). The sampling depth was adjusted to 30,000 sequences per library after rarefaction curves review (Fig. S1). For microbial community characterization (alpha-diversity), the following estimates were determined at 97% genetic distance: Chao 1 estimator (Chao, 1984), operational taxonomic units observed (OTUs), Simpson and Shannon diversity indexes. To compare community structures (beta-diversity), two additional libraries were also considered: *P. americana* with natural parasites *Hammerschmidtella diesingi* and *Thelastoma bulhoesi* (L1983; NCBI accession: SRR3225595) and *P. americana* artificially infected with *L. appendiculatum* (L1984; NCBI accession: SRR3225596). For this analysis, sampling depth was also adjusted for 30,000 sequences per library (Fig. S2) and the phylogenetic unweighted UniFrac metrics (qualitative measure that accounts for the phylogenetic distance and the presence and absence of OTUs) was used for PCoA (principal coordinates analysis) (Mardia *et al.*, 1979). Statistical significance ( $P$ -value with Bonferroni correction less than 0.01) between communities was computed using unweighted UniFrac with 999 Monte Carlo permutations.

## RESULTS AND DISCUSSION

Prior to sequencing, the presence of parasitic nematodes (*Protrellus* sp. and *L. appendiculatum*) in all hindgut samples ( $n = 6$ ) were confirmed through PCR amplification of D2/D3 fragment of the 28S rRNA gene (Table S1, Fig. S2) and subsequent sequencing (data not shown). The number of sequences per library was, respectively, 67,482 in L1986 (*P. japonica* naturally

infected with *Protrellus* sp.) and 68,147 in L1987 (*P. japonica* artificially infected with *L. appendiculatum*), which were then adjusted to 30,000 sequences/library after rarefaction curves inspection (Fig. S1). The characterization of both *P. japonica* libraries is presented in Table 1 and Fig. 1. In terms of alpha-diversity, both hindgut communities (natural versus artificial) show very similar results in terms of OTUs abundance (Chao1 and OTUs observed) as well as in diversity and community evenness (Simpson diversity index: 0.99; Shannon index: 7.37–7.84) (Table 1). A total of 665 OTUs observed were observed in L1986, and 526 OTUs in L1987. These OTUs (at 97% level) were assigned to 17 phyla in L1986 (Table 1), whereas L1987 only accounted for 13 of these phyla. The most representative phyla taxa in both communities were Bacteroidetes (41% in L1986; 34% in L1987), Firmicutes (38% in L1986; 42% in L1987) and Proteobacteria (12% in L1986; 18% in L1987). For both libraries, the major families belonging to Bacteroidetes phylum were Porphyromonadaceae (23.1% in L1986; 18.7% in L1987) with genus *Dysgonomas* (12.8% in L1986; 6.3% in L1987) and *Parabacteroides* (5.4% in L1986; 5.8% in L1987), and Rikenellaceae (9.8% in L1986; 5.7% in L1987). In term of Firmicutes,

Table 1. Alpha-diversity indexes and phylum relative abundance of hindgut microbial communities of *Periplaneta japonica* naturally infected with *Protrellus* sp. (L1986) or artificially infected with *Leidyneema appendiculatum* (L1987). OTU is defined as operational taxonomical units.

Alpha-diversity	Library ID	
	L1986	L1987
OTUs observed	665	526
Chao1	717	603
Simpson index	0.99	0.99
Shannon index	7.84	7.37
Phylum relative abundance (%)		
Unassigned; Other	1.38	2.71
Euryarchaeota	0	0.11
Actinobacteria	0.82	0.39
Bacteroidetes	33.55	41.3
Chloroflexi	0	0.01
Deferribacteres	0.16	0.32
Elusimicrobia	0	0.26
Firmicutes	42.42	37.61
Fusobacteria	1.56	2.43
GN02	0.03	0.1
Planctomycetes	0.54	0.39
Proteobacteria	18.16	12.4
SR1	0	0.25
Spirochaetes	0.06	0.15
Synergistetes	0.09	0.07
TM7	0.13	0.07
Tenericutes	0.63	0.97
Verrucomicrobia	0.48	0.47

Enterococcaceae (less than 5% in L1986; 5.9% in L1987), Lachnospiraceae (11.1% in L1986; 9.4% in L1987) and Ruminococcaceae (8.2% in L1986; 9.2% in L1987) were the most representative families. Desulfovibrionaceae (8.8% in L1986; 14.1% in L1987) was the most marked family in phylum Proteobacteria. The percentage of unassigned taxa (others) was nearly 3% in L1986 and 1% in L1987. For community structure analysis, two new libraries were considered: *P. americana* with natural parasites (*H. diesingi* and *T. bulhoesi*, L1983), and *P. americana* with artificial parasite (*L. appendiculatum*, L1984) (Vicente *et al.*, 2016). All libraries were considered statistically different (*P*-value Bonferroni-corrected < 0.01) using the unweighted UniFrac metrics (Table S3). PCoA analysis (Fig. 2) corroborates these results. Interestingly, this analysis also shows that L1983 and L1986, both hindgut microbial communities with

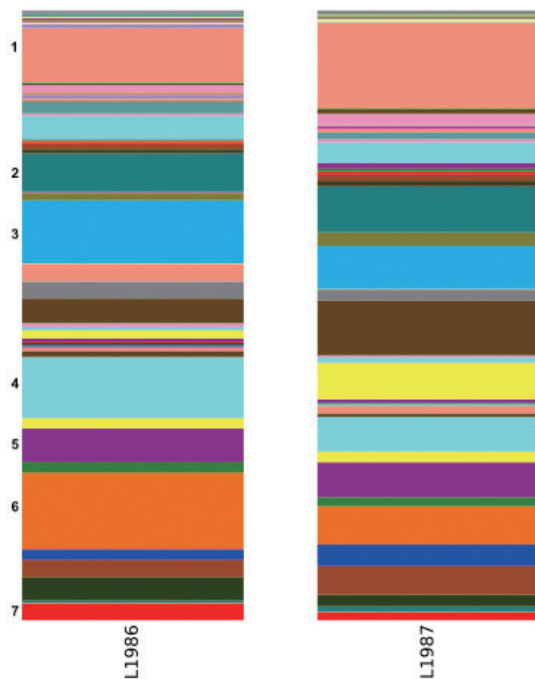


Fig. 1. Bar-chart of hindgut microbiota of *Periplaneta japonica* naturally infected with *Protrellus* sp. (L1986) or artificially infected with *Leidynea appendiculatum* (L1987). Numbers indicate taxonomical identification (Phylum, Class, Order, Family, Genus) of each color-group: (1) Proteobacteria, Deltaproteobacteria, Desulfovibrionales, Desulfovibrionaceae; (2) Firmicutes, Clostridia, Clostridiales, Ruminococcaceae; (3) Firmicutes, Clostridia, Clostridiales Lachnospiraceae; (4) Bacteroidetes, Bacteroidia, Bacteroidales, Rikenellaceae; (5) Bacteroidetes, Bacteroidia, Bacteroidales, Porphyromonadaceae, Parabacteroides; (6) Bacteroidetes, Bacteroidia, Bacteroidales, Porphyromonadaceae, Dysgonomonas; and (7) Unassigned. Detailed description of genus-relative abundance is presented in supplemental Table S2 and Fig. S3.

natural parasites cluster closer than L1984 and L1987 (Fig. 2), possibly suggesting that the artificial infection by non-natural thelastomatid parasites may cause a shift in the hindgut microbiota of host cockroaches, as also seen in Vicente *et al.* (2016).

The hindgut microbial community of *P. japonica*, regardless of natural or artificial infection of thelastomatid nematodes, is characterized mostly for the presence of three major phyla, Bacteroidetes, Firmicutes and Proteobacteria, and, respectively, families Porphyromonadaceae and Rikenellaceae, Lachnospiraceae and Ruminococcaceae, and Desulfovibrionaceae. The same observations were seen in other cockroach species, such as *Blattella germanica* (Pérez-Cobas *et al.*, 2015), *Panesthia angustipennis* (Bauer *et al.*, 2015), *Salganea esakii* (Bauer *et al.*, 2015), *Shelfordella lateralis* (Schauer *et al.*, 2014) and, *P. americana* and *P. fuliginosa* (Vicente *et al.*, 2016). These phyla are even shared between wild-captured or lab-maintained cockroaches with different diet regimes and sampling time-points (Bauer *et al.*, 2015), therefore indicating a quite stable microbial community at family level (Mikaelyan *et al.*, 2015; Tinker and Ottesen, 2016). Regarding the presence of parasitic nematodes from Thelastomatoidea family, Vicente *et al.* (2016) firstly indicated that hindgut community of *P. fuliginosa* and *P.*

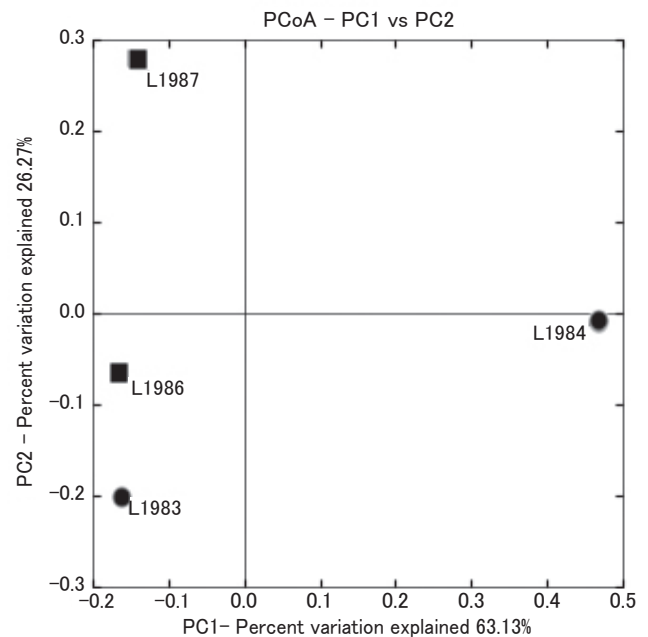


Fig. 2. Beta-diversity of hindgut communities of *Periplaneta japonica* (square) and *P. americana* (circle) with naturally (respectively, L1983 and L1986) or artificially (L1984 and L1987) infected thelastomatid nematodes. Principal coordinates analyses (PCoA) using unweighted UniFrac distance matrix. PCoA axes accounts for 89.4% of the variation.

*americana* with natural parasites were more biodiverse than the hindgut without parasites, and that for the case of *P. americana*, the natural co-infection with *H. diesingi* and *T. bulhoesi* in comparison with artificial infection with *L. appendiculatum* also indicated biodiversity differences. In the case of *P. japonica*, the same trend is perceived (Vicente *et al.*, 2016). Although at higher levels, the natural- and artificial-infection may not seem different, at genus-level we could denote differences of hindgut community structures (as seen in Fig. 2) whereas the natural community seems more diverse than the artificial community. We also observed that changes in the naturally versus artificially infected hindgut community may be cockroach species-related when comparing *P. japonica* with *P. americana*. In addition to previous observations, the present report emphasizes the need to continue investigation on the role of thelastomatid parasitic nematodes and gut microflora of cockroaches (Vicente *et al.*, 2016). This work completes the composition characterization of gut microflora of these major pest insects in Japan (*P. fuliginosa*, *P. americana*, and *P. japonica*) (Vicente *et al.*, 2016).

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### Supplemental data

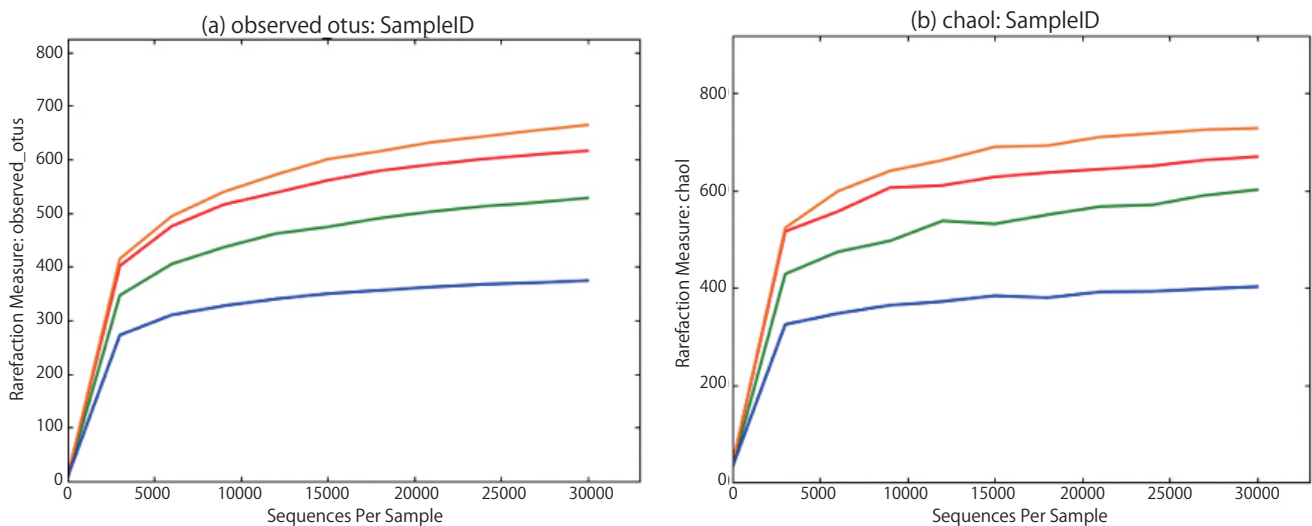


Fig. S1. Rarefaction curves based on the observed OTUs per sequences/sample. Orange, L1986; Red, L1983; Green, L1987; Blue, L1984.

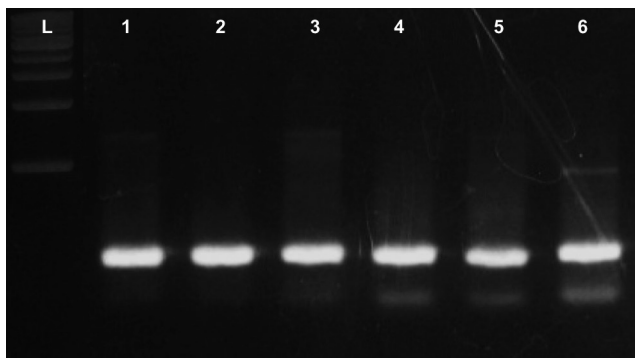


Fig. S2. PCR products from six hindgut samples, amplified by nematode species-specific primers. L1-3, *Leidynema appendiculatum*. L4-6, *Protrellus* sp.

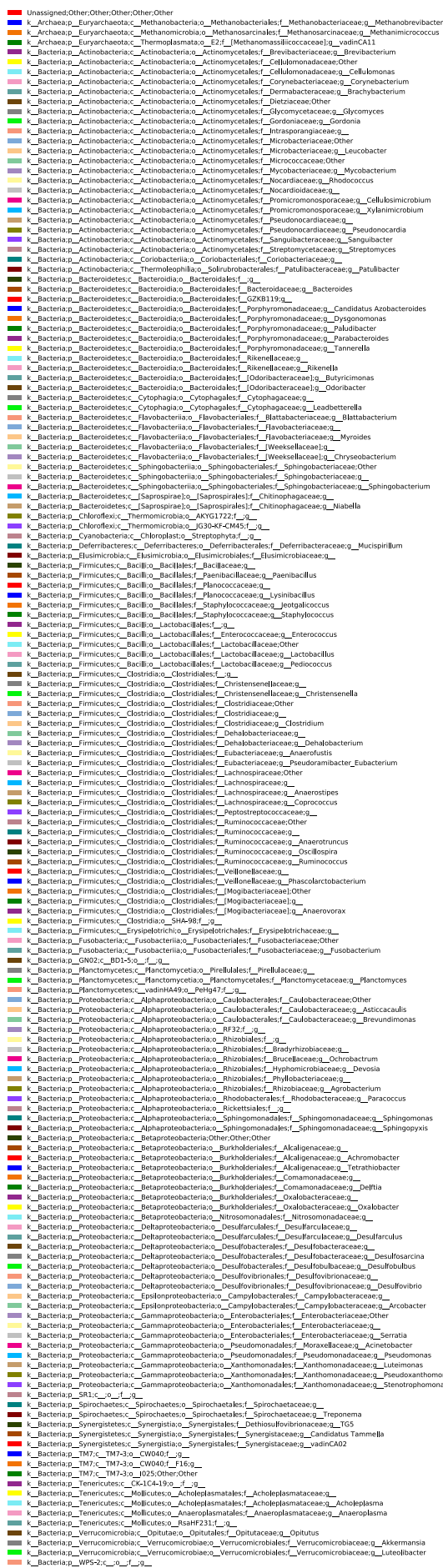


Fig. S3. Detailed description of genus-relative abundance of hindgut microbiota of *Periplaneta japonica* naturally infected with *Protrellus* sp. (L1986) or artificially infected with *Leidyneia appendiculatum* (L1987).

Table S1. List of primers used in this study. Bold region highlighted in 16S V3-V4 region primers indicate the Illumina overhang adapter sequences.

Primers (5'-3')	
<i>Leidyneema appendiculatum</i> specific primer based in D2D3 region	
Forward	GCTGGTTGCCAGGCTCTACTAC
Reverse	CCGCACCACCGCAGGCCAGCAT
<i>Protellus</i> sp. specific primer based in D2D3 region	
Forward	TTGTTGTTTCGTCGGCCTCGATG
Reverse	CGATTTCGAGATTCAACCAACGTC
16S V3-V4 region	
Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

Table S2. Genus abundance (%) for each library. The rarefaction level was 30000 sequences/library.

Kingdom	Phylum	Class	Order	Family	Genus	Library ID	
						L1986	L1987
Unassigned						2.7%	1.4%
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanimicrococcus	0.1%	0.0%
Archaea	Euryarchaeota	Thermoplasmata	E2	Methanomassiliococcaceae	vadinCA11	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	other	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Dermabacteraceae	Brachybacterium	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	other	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Glycomycetaceae	Glycomyces	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	Gordonia	0.0%	0.1%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Intraporangiaceae	other	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococccaceae	other	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiodaceae	other	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0.0%	0.0%
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	0.0%	0.0%
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	other	0.3%	0.7%
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	Patulibacter	0.0%	0.0%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	2.7%	4.9%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	GZKB119	other	0.1%	0.0%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Odoribacteraceae	Butyricimonas	0.1%	0.0%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Odoribacteraceae	Odoribacter	1.0%	0.6%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Candidatus Azobacteroides	1.6%	3.4%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Dysgonomonas	12.8%	6.3%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter	1.6%	1.4%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	5.4%	5.8%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Tannerella	1.7%	1.8%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenella	0.1%	0.0%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	other	9.8%	5.7%
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	other	other	3.7%	2.0%
Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	other	0.0%	0.0%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Blattabacteriaceae	Blattabacterium	0.4%	1.2%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	other	0.0%	0.0%
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	other	0.0%	0.3%
Bacteria	Bacteroidetes	Saprosiriae	Saprosiriales	Chitinophagaceae	other	0.0%	0.0%
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	0.2%	0.0%
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	other	0.0%	0.0%
Bacteria	Chloroflexi	Thermomicrobia	JG30-KF-CM45	other	other	0.0%	0.0%
Bacteria	Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Mucispirillum	0.3%	0.2%
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	other	0.3%	0.0%
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	other	0.0%	0.0%
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	0.0%	0.0%
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	other	0.2%	0.0%
Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	0.0%	0.0%
Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	1.5%	5.9%
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.7%	0.3%
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	other	0.5%	0.9%
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	0.0%	0.0%
Bacteria	Firmicutes	Bacilli	Lactobacillales	other	other	0.6%	0.6%
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenella	0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	other	3.0%	1.7%
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	0.2%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	other	2.8%	0.1%
Bacteria	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	Dehalobacterium	0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	other	0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	1.0%	2.4%

Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	other	0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		10.1%	7.1%
Bacteria	Firmicutes	Clostridia	Clostridiales	Mogibacteriaceae	Anaerovorax	0.1%	1.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Mogibacteriaceae	other	0.1%	0.1%
Bacteria	Firmicutes	Clostridia	Clostridiales	Mogibacteriaceae		0.2%	0.4%
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae		0.0%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerotruncus	0.1%	0.2%
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	0.7%	0.7%
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	other	0.4%	0.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	0.8%	1.0%
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		6.2%	7.3%
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		0.3%	0.4%
Bacteria	Firmicutes	Clostridia	Clostridiales			4.1%	9.0%
Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae		3.8%	3.3%
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	2.0%	1.0%
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	other	0.5%	0.5%
Bacteria	GN02	BD1-5				0.1%	0.0%
Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae		0.0%	0.0%
Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	0.0%	0.0%
Bacteria	Planctomycetes	vadinHA49	PeHg47			0.4%	0.5%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	other	0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	RF32			0.5%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	0.1%	0.3%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0.0%	0.1%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae		0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales			0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0.0%	0.1%
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales			0.0%	0.0%
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.0%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Tetrathiobacter	0.0%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae		0.0%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae		0.2%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Oxalobacter	0.0%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae		0.0%	0.0%
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae		0.0%	0.0%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	Desulfarculus	0.0%	0.0%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae		1.2%	1.9%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfosarcina	0.1%	0.0%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae		0.3%	0.8%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus	0.1%	0.1%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.1%	0.1%
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae		8.7%	14.1%
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacteriales	Campylobacteraceae		0.0%	0.1%
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	other	0.6%	0.0%
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	0.0%	0.0%
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae		0.3%	0.4%
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.0%	0.0%
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.0%	0.0%
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	0.0%	0.0%
Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	0.1%	0.1%
Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae		0.1%	0.0%
Bacteria	SR1					0.3%	0.0%
Bacteria	Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	TG5	0.1%	0.1%
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Candidatus Tammella	0.0%	0.0%
Bacteria	Tenericutes	CK-1C4-19				0.2%	0.2%
Bacteria	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	0.0%	0.0%
Bacteria	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae		0.2%	0.2%
Bacteria	Tenericutes	Mollicutes	RsaHF231			0.6%	0.3%
Bacteria	TM7	TM7-3	CW040			0.0%	0.0%
Bacteria	TM7	TM7-3	CW040			0.1%	0.0%
Bacteria	TM7	TM7-3	I025	Other	Other	0.0%	0.1%
Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	0.0%	0.1%
Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	0.5%	0.4%

Table S3. Statistical differences between all pairwise libraries comparisons in hindgut communities of *P. japonica* and *P. americana* with naturally or artificially infected parasitic nematodes. Unweighted UniFrac distance matrix was as significant as test with 999 Monte Carlo permutation.

Sample 1	Sample 2	<i>P</i> value	<i>P</i> value (Bonferroni corrected)
L1983	L1984	0	≤ 0.01
L1983	L1986	0	≤ 0.01
L1983	L1987	0	≤ 0.01
L1984	L1986	0	≤ 0.01
L1984	L1987	0	≤ 0.01
L1986	L1987	0	≤ 0.01