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The microbiota associated with the invasive *Popillia japonica* (Coleoptera: Scarabaeidae) and evaluation of the entomopathogenic activity of the isolated nematodes

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Table of Contents

Abstract

Popillia japonica Newman (Coleoptera: Scarabaeidae) is a highly polyphagous invasive beetle originating from Japan. It attacks more than 300 plant species, causing severe damage, and can adapt rapidly to new environments. Recently, several studies have been carried out in order to study insect-associated microorganisms and their impact on insect physiology. These microbiota-based studies have also investigated the impact of symbionts on their host's potential to adapt to changing conditions, thus contributing to an insect's invasive potential. However, to date, no study has been reported regarding the microbial community associated with *P. japonica* and the factors that may influence the composition of these communities. Therefore, our group decided to investigate the bacterial community associated with *P. japonica* at different developmental stages (i.e., larvae, pupae and adults (male, female) using NGS approaches. The aim of this thesis is to investigate the microbiota associated with the three gut regions of different developmental stages of *P. japonica* (i.e., larvae, pupae and adults) in order to address the following biological questions: 1) Do the developmental stages (i.e., larvae vs. adults) influence the bacterial community associated with *P. japonica*? 2) Are the bacterial communities which are associated with the three gut regions different? 3) Does the soil have an impact on shaping the bacterial community associated with the different developmental stages of *P. japonica* (i.e., larvae, pupae and adults)? Further, we seek to: 4) investigate the isolation and molecular characterization of the nematode associated with the third larvae of *P. japonica* and the microbiota associated with the isolated nematode and 5) test the entomopathogenic activity of the nematode associated with the third larvae of *P. japonica*.

Regarding the *P. japonica* bacterial community, our results show that soil microbes represent an important source of gut bacteria for *P. japonica* larvae; but, as the insect develops, its gut microbiota richness and diversity decrease substantially. These changes are in parallel with changes in community composition. Regarding the nematode associated with the third larvae of *P. japonica*, the results of both the BLAST search and the reconstruction of a phylogenetic tree using the maximum-likelihood method from 18S rRNA sequences confirm the attribution of the isolated nematode to the genus *Oscheius*. The isolated nematode leads to the mortality of more than 50% of the host after five days. The microbiota study of the nematode shows that its bacterial community is dominated by bacteria belonging to the genus *Ochrobactrum*, which includes entomopathogenic species.

The results achieved during the Ph.D. program and reported in the present thesis were published as research articles:

1- Chouaia B, Goda N, Mazza G, Alali S, Florian F, Gionechetti F, Callegari M, Gonella E, Magoga G, Fusi M, Crotti E, Daffonchio D, Alma A, Paoli F, Roversi PF, Marianelli L, Montagna M. 2019. Developmental stages and gut microenvironments influence gut microbiota dynamics in the invasive beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae). Environ Microbiol. 21:4343-4359.

2- Goda N, Alali S, Mirzaei M, Brunetti M. Potentially entomopathogenic nematode isolated from *Popillia japonica* (Coleoptera: Scarabaeidae): bioassay, molecular characterization and the associated microbiota. submitted to the Turkish Journal of Agriculture and Forestry.

Introductory chapter

1. Introduction

The insect is the largest group among the arthropod phylum, and beetles are the largest insect order forming the order Coleoptera, with over 400,000 described species representing about 40% of all insect-described species so far (Bouchard *et al*., 2011). Beetles are found in almost every habitat occupied by insects. They feed on a wide variety of plants, fungi, decomposing dead animals, plant debris and other invertebrates. The invasive beetles have serious economic consequences for agriculture and forestry (Greathead *et al*.,1992). For instance, the Colorado potato beetle *Leptinotarsa decemlineata* is considered to be a serious agricultural pest which causes complete defoliation in the case of several Solanum plants; likewise, the invasive beetle *Agrilus mali* can cause significant economic losses of wild apple in the Tianshan (West China) forests.

The gut microbiota associated with insects impact their physiology and ecology, which play an important role in insects' evolution, nutrition, reproduction and protection during their developmental stages (Engel and Moran, 2013). For instance, the p-endosymbiont *Buchnera* provides essential amino acids to aphids that are not present on plant phloem (Shigenobu *et al*., 2000), as well as the gut bacteria *Enterococcus faecalis* associated with the omnivorous beetle (Lundgren and Lehman, 2010). The gut bacteria increase weight gain and affect the expression of genes governing insulin and vitellogenin levels in the young adult honeybees (Zheng *et al*., 2017). The gut symbiont is also involved in insecticide resistance in *Riptortus pedestris* (Hemiptera) and *Bactrocera dorsalis* (Diptera) (Xia *et al*., 2018). Meanwhile, the cellulolytic activity of the gut bacteria associated with the invasive subcortical beetle, *Agrilus planipenni*, has resulted in extensive mortality in urban and forest ash trees (Vasanthakumar *et al*., 2008). Studying and understanding symbiotic bacteria behavior have underlined their impact on economically important insects, such as invasive beetles, and how they facilitate their invasive success. Consequently, the importance of symbiotic bacteria associated with insects, as a promising tool in insect pest management, has increased, opening the way for new ideas and better solutions to improve methods for the management of invasive beetles by investigating their microbial community's composition and manipulating them in a way that guarantees better control and management of those pests (van den Bosch and Welte, 2017).

In this introductory chapter, we have provided examples of studies on microbiota associated with forest and agricultural invasive beetles. We have also discussed the biology, worldwide distribution and management of *P. japonica*, which is the main scope of this thesis.

2. Microbiota of invasive beetles

2.1. Microbiota of beetles consider forest pests

Agrilus mali (Coleoptera: Buprestidae) is an invasive wood borer beetle which attacks wild apple forests (*Malus sieversii*) in Western China, causing severe damage and tree death (Xia *et al.,* 2018) (Table.1). The different developmental stages of the intestinal microbial communities of *A. mali* (larvae, pupae and newly emerging adults) have been investigated, with the results showing that the core taxa are assigned to genera such as *Klebsiella, Stenotrophomonas, Serratia, Enhydrobacter, Achromobacter, Corynebacterium, Micrococcus* and *Acinetobacter.* The persistence of such taxa in the gut microbiota during different development stages indicates that they play an important role in the fitness of *A. mali*, for example, *Stenotrophomonas* is reported to have cellulose degradation capabilities *(*Zhang *et al.,* 2018*).* Another study found that the role of both bacteria *Pantoea* sp. and *Pseudomonas orientalis* is associated with *A. mali* in the breakdown of plant cell wall compounds: *Pantoea* sp. is able to synthesize the four

enzymes responsible for plant cell wall degradation and *P. orientalis* engages in lignin peroxidase activity (Bozorov *et al.,* 2019).

A previous study was carried out to characterize the gut microbial communities across different life stages of *Agrilus planipennis* Fairmaire, an invasive phloem-feeding and wood-boring beetle which causes extensive mortality in urban and forest ash trees. The genera *Pseudomonas, Bacillus, Staphylococcus, Rhodococcus* and *Streptomyces* were isolated from larvae, prepupae and adults. The persistence of such bacteria through the developmental stages indicates that they have a specific role in both the fitness and the adaptability of the invasive beetle *A. planipennis*, for example, *Streptomyces* spp. is capable of digesting carboxymethylcellulose (Vasanthakumar *et al.,* 2008).

Bark beetles (Coleoptera: Scolytidae; alt. Curculionidae: Scolytinae) form part of an economically and ecologically important group of invasive beetles which attack subcortical trees. In particular, the genus *Dendroctonus*, which is distributed in Central and North America, attack trees from the Pinaceae family (Vasanthakumar *et al*., 2008). The gut bacterial communities associated with *Dendroctonus rhizophagus* have been investigated using both culture-dependent and culture-independent methods, with the results

showing that *Stenotrophomonas* and *Rahnella* genera are the most frequently found bacteria throughout the *D. rhizophagus* life cycle. In vitro, *Stenotrophomonas maltophilia, Ponticoccus gilvus* and *Kocuria marina* have revealed the presence of cellulolytic activity, while the capability of *Stenotrophomonas maltophilia, Rahnella aquatilis, Raoultella terrigena, Ponticoccus gilvus* and *Kocuria marina* in nitrogen fixation and cellulose breakdown underline the important role of these bacteria in helping *D. rhizophagus* development. Another study has been carried out to investigate the bacterial communities associated with 13 *Dendroctonus* species from infested pine trees in Mexico and the US (Morales-Jiménez *et al., 2012*) (Table.1). According to the findings, Proteobacteria were the most abundant, followed by Firmicutes, Fusobacteria, Actinobacteria, and Deinococcus-Thermus, while, at the genus level, the core bacteriome was composed of the genera *Enterobacter, Pantoea, Pseudomonas, Rahnella, Raoultella* and *Serratia.* A further study investigated the composition of the bacterial community in the gut of the pine engraver, *Ips pini* (Say) (Coleoptera), known for colonizing red pine, which is distributed across North America, using culture-dependent and culture-independent methods (Delalibera *et al., 2007*). The results revealed that two bacterial genera, *Pantoea* and S*tenotrophomonas*, were found in all life stages of *I. pini*, indicating a simple gut flora compared with wood colonizing insects, such as wood borers and termites, and highlighting their role in helping *I. pini* development (Delalibera *et al., 2007*).

The bamboo snout beetle *Cyrtotrachelus buqueti* (Coleoptera: Curculionidae), regarded as a serious pest, is found in China and other Southeast Asian countries and attacks the shoots of bamboo tree species such as *Bambusa and Dendrocalamopsis, Phyllostachys pubescens* and *Neosinocalamus affinis* (Yang *et al.*, 2017). Luo and colleagues investigated the role of the gut symbiotic microbiota of *C. buqueti* on bamboo lignocellulose degradation in vitro. Using 16sRNA sequencing, the gut symbiotic microbiota of adult and larvae *C. buqueti* were identified. The results revealed that *Lactococcus, Serratia, Dysgonomonas* and *Enterococcus* represent approximately 84% to 94% of the total gut symbiotic microbiota of adult and larvae *C. buqueti*. Interestingly, after analyses of the resident CAZyme genes using the genomes of the aforementioned microbes, it was possible to understand the role of the gut symbiotic microbiota of *C. buqueti* in lignocellulose degradation (Luo *et al.*, 2019).

2.2. Microbiota associated with beetles considered pest of crops

Octodonta nipae (Coleoptera: Chrysomelidae) is an invasive beetle, which was introduced into China in 2001. The beetle feeds on palm trees' young leaves causing them to shrink and curl and their young stems to die (Peng *et al.*, 2018). The bacterial communities of different developmental life stages (eggs, larvae, pupae and adults) and the reproductive organs of *O. nipae* have been investigated. It was

subsequently reported that the majority of the taxa belong to the phyla Proteobacteria, Actinobacteria and Firmicutes and the families Dermabacteraceae, Anaplasmataceae and Enterobacteriaceae, while the genera *Serratia* and *Lactococcus* were dominant in eggs, and *Pantoea* and *Brachybacterium* were dominant in both larvae and pupae microbiota. Members of the Anaplasmataceae and Enterobacteriaceae families play important roles such as nitrogen fixation, host protection against the pathogen, degradation of uric acid and increasing host fitness (Ali *et al.,* 2019).

Table 1: Summary of the beetle pests.

Insect species	**Family**	**Feeding Behavior**	**Type of Pest**
1- Agrilus mali	Buprestidae	polyphagous	Forest pest
2- Agrilus planipennis	Buprestidae	polyphagous	Forest pest
3- Dendroctonus rhizophagus	Curculionidae		Forest pest
4- Dendroctonus frontalis Zimmermann	Curculionidae: Scolytinae	polyphagous	Forest pest
5- Dendroctonus valens	Curculionidae	polyphagous	Forest pest
6- Epilachna vigintioctopunctata	Coccinellidae	polyphagous	Crop pest
7- Holotrichia parallela	Scarabaeidae	monophagous	Crop pest
8- Hylobius abietis	Curculionidae	polyphagous	Forest pest
9- Hypothenemus hampei	Curculionidae	monophagous	Crop pest
10- Ips pini	Curculionidae	polyphagous	Forest pest
11- Leptinotarsa decemlineata	Chrysomelidae	polyphagous	Crop pest
12- Dendroctonus ponderosae	Curculionidae	polypahgous	Forest pest
13- Cyrtotrachelus buqueti	Curculionidae		
14- Octodonta nipae	Chrysomelidae	polyphagous	Crop pest

The large black chafer *Holotrichia parallela* (Coleoptera: Scarabaeidae) is an economically significant peanut pest in China whose larvae attack peanuts. The bacterial community associated with the hindgut *H. parallela* larvae from different geographic locations and instars were investigated using the 16S rRNA clone library and denaturing gradient gel electrophoresis. The results showed that the bulk of the taxa

belong to the Firmicutes and Proteobacteria phyla and the Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae, Desulfovibrionaceae and Rhodocyclaceae families, while the genera *Bacteroidetes* and *Firmicutes* represented the dominant group in the first and (second, third etc.) instars, respectively (Huang and Zhang, 2013).

The spotted leaf beetle *Epilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae) is a serious pest, with both adult and larvae forms attacking solanaceous and cucurbitaceous plants, causing serious damage and the loss of crop production. The gut-associated bacteria in *E. vigintioctopunctata* has been characterized in order to explore the relationship between the bacteria associated with the beetle and their roles in *E. vigintioctopunctata* development. The results of 16S rRNA partial gene sequencing revealed the presence of *Bacillus subtilis* (EVI16), *B. vietnamensis* (EVI09) and *B. anthracis* (EVI07). *B. subtilis* is known as a microbial insecticide for the effective management of insect pests, while *B. anthracis* is known to cause anthrax in mammals (Ramachandiran *et al.*,2018).

The pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae: Molytinae), can cause damage, reaching 80% mortality, when it feeds on the stem of bark conifer seedlings. In one study, the gut bacterial communities of different populations of *H. abietis* across Europe have been characterized and compared with those of other beetles that occupy similar ecological niches. The results indicate that some OTUs within the Enterobacteriaceae from *H. abietis* are closely related to those of bark beetles (*Dendroctonus ponderosae*, *D. frontalis*, *D. valens*, *D. rhizophagous* and *Ips pini*), and that the microbiota of *H. abietis* are different from those of closely related beetles feeding on a different diet. The study also reported that members of the Enterobacteriaceae family are involved in terpenoid degradation and the detoxification of plant secondary metabolites, which supports *H. abietis* development. This confirms that ecological niches (Berasategui *et al.*, 2016).

Coffea species are small trees that are economically important in countries that produce coffee beans. The coffee berry borer *Hypothenemus hampei* (Coleoptera; Curculionidae: Scolytinae) is one of the most destructive threats to coffee beans. After characterizing the bacterial composition of the *H. hampei* microbial communities in specimens from major coffee-producing regions (Hawaii, India, Indonesia, Kenya, Mexico and Puerto Rico), it was suggested that a core of organisms detected across all locations belongs to the orders Pseudomonadales, Enterobacteriales, Turicibacteriales, Rhizobiales, Alteromonadales and Actinomycetales, with the most abundant being Pseudomonadales (*Pseudomonas* spp.). This particular study also highlighted the role of *Pseudomonas fulva* in the degradation of caffeine

in vivo, i.e., it enables *H. hampei* to subsist on caffeine as a source of carbon and nitrogen (Ceja-Navarro *et al.*, 2015).

The Colorado potato beetle *Leptinotarsa decemlineata* is a major pest in the case of solanaceous crops, such as potato (*Solanum tuberosum*), tomato (*S. lycopersicum*), and eggplant (*S. melongena*). The microbiota associated with this beetle have been investigated, in which 16S rDNA sequences identified *Leclercia adecarboxylata*, *Acinetobacter* and *Pseudomonas putida* as the core microbiota associated with *L. decemlineata* (Muratoglu *et al.*, 2011).

3.The Japanese beetle *Popillia japonica* (invasion and distribution, biology, and control)

3.1. History of invasion and distribution worldwide

The Japanese beetle, *Popillia japonica* (Newman, 1841) (Coleoptera: Scarabaeidae), is a polyphagous threat which is native to Japan and the far east of Russia. It was first discovered in North America in 1916, near Riverton, New Jersey, before spreading across the US. In Japan, it is common and not considered to be a pest. On the contrary, in the US, *P. japonica* is a restricted and quarantined pest in all states (Potter and Held, 2002).

In the EPPO region, *P. japonica* has recently established itself in the Azores and the Canton of Ticino in Switzerland. In Canada, the beetle has established itself in Southern Quebec, New Brunswick and Nova Scotia (EPPO, 2018). *P. japonica* was recorded for the first time in Italy when an outbreak was reported within the Ticino Valley Natural Park in Northern Italy in 2014 (Pavesi, 2014). In that year, the infested area and the number of adults per lure traps increased to 80 km^2 and about 28,000 adults using 64 double-lure traps, respectively. In 2016, the infested area increased to be about 500 km^2 while there were 14.7 million adults by 2,100 traps. In 2017, about 800 km^2 were reported to be infested and more than 48 million adults were caught by 2,100 traps(Marianelli *et al.*, 2019). The history of the invasion of *P. japonica* indicates the severe threat posed by the beetle and how rapidly the population increases, especially the Italian population, which falls within the scope of this thesis.

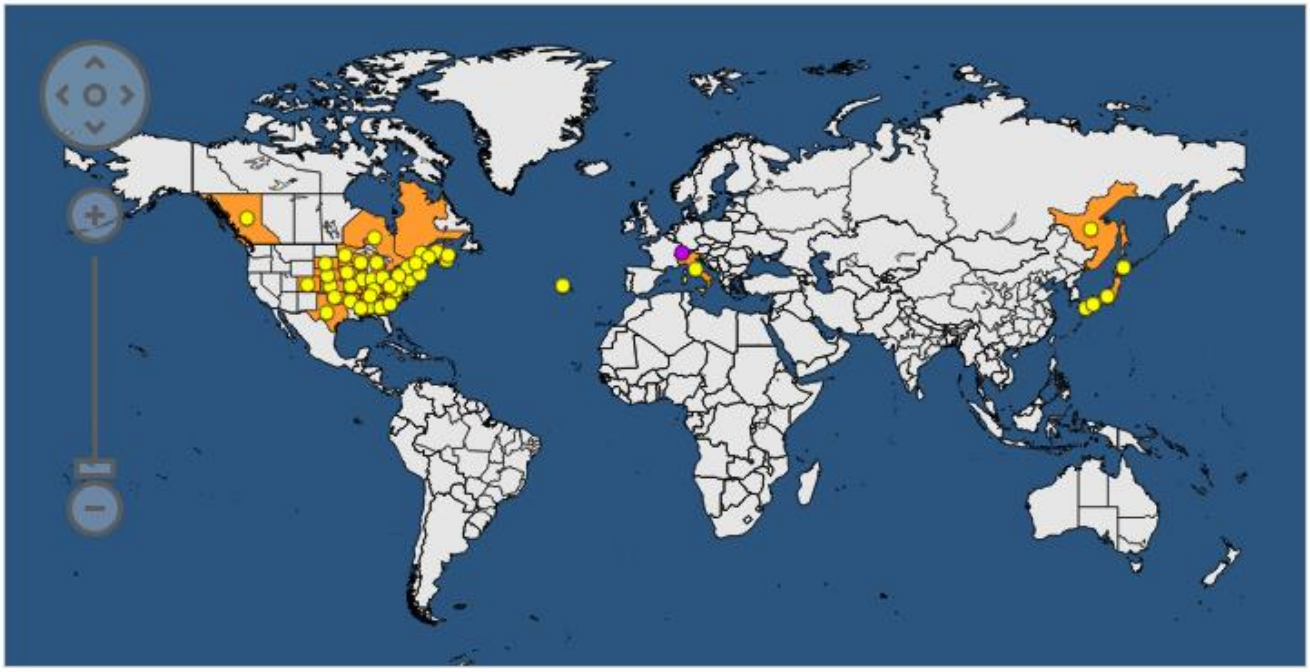

Figure 1: worldwide distribution of *Popillia japonica* from (https://gd.eppo.int/taxon/POPIJA/distribution)

3.2. Biology

The life cycle of *P. japonica* is generally is one year or two years in particularly cold climates. In the native area of Japan, it takes one year, but, in the cooler areas in the north of Honshu, individuals take two years to complete development (Clausen, 1927). In the US, in states such as Pennsylvania and New Jersey, it has been reported that *P. japonica* individuals complete their life cycle within 12 months, compared with two years in Massachusetts. Meanwhile, in Canada, the life cycle can take one or two years depending on the temperature and, in Italy, the life cycle is completed in one year (Bragard *et al.*, 2018).

After the emergence of adults, mating, oviposition and larval development differ from year to year, depending on the temperature (Fleming, 1927). Generally, adults emerge in the summer (June-July) and feed on the foliage and fruit of hosts, living for 30-45 days. In Italy, while adults peak in July, some adults can be active until September and rarely into October. In the Azores, adults can be found in the period between May and November (Bragard *et al.*, 2018).

After adults mate, the females lay eggs, between 40 and 60 in total, in the soil to oviposit a single egg or a group of eggs, once or several times. Depending on the temperature, eggs usually hatch after about two weeks. After oviposition, the hatched larvae feed on decaying matter before attacking the roots of

different plants (PLH, 2018). Larvae are involved in three larval instars: the first instar lasts two to three weeks; the second starts after three to four weeks; in the third instar, larvae live at deeper levels of the soil, preparing to overwinter. At depths of 10-20 cm, they can presumably avoid cooler or freezing temperatures (Bragard *et al*., 2018).

In the warm spring, the larvae form a chamber in which they pupate before emerging in mid-summer, after which the life cycle is repeated. *P. japonica* is a polyphagous plant pest which, in the adult form, attacks over 300 plants species such as *Acer* and *Betula*, shrubs such as *Rosa*, soft fruit crops such as *Fragaria* and field crops such as *Asparagus officinalis*, *Glycine max* and *Zea mays*. Larvae are known to feed on the roots of grasses (e.g., Festuca, Poa, Lolium) and pasture plants, such as Trifolium. In particular, they are pests in the case of lawns, golf courses and pastures, as well as feed on the roots of vegetables and nursery stock (Bragard *et al*., 2018).

3.3. Control

Due to the economic impact of *P. japonica*, several control strategies and tools are used to limit the impact of this threat in the agricultural sector, such as phytosanitary tools which seek to prevent the entry of pests into areas free of infestation or entire countries by controlling larvae in nursery stock in line with specific procedures. There are also treatments aimed at prohibiting the pest's entry into plants via any soil in nurseries (Oliver *et al.*, 2007).

Other strategies such as the use of resistant or less susceptible cultivars have been applied, but they have not resulted in complete control being achieved (Ladd Jr *et al.*, 1986). Mass trapping is another important tool for the identification and delimitation of new *P. japonica* infestations, which is used in the US; however, it has not been effective at reducing established *P. japonica* infestations. Among the most common strategies used involves chemical control compounds such as carbamates, organophosphates and pyrethroid insecticides. Recently, neonicotinoid (imidacloprid) and molt accelerators (halofenozide) have been used for preventive larval control in turf (Potter and Held, 2002).

Due to the negative impact of chemical pesticides on the environment, searching for environmentally friendly tools has become crucial and using biological control agents has become inevitable and urgent. Parasitoids such as the *Tiphia vernalis* wasp parasitize overwintered grubs in the spring. *Istocheta aldrichi*, a tachinid fly which parasitizes adults, has also been successfully used and, while it provides some suppression effects, the level of control remains inadequate.

Microorganisms such as *Bacillus thuringiensis* have been effective in suppressing *P. japonica* grubs in turf plots, while entomopathogenic nematodes such as *Steinernema glaseri* and *Heterorhabditis bacteriophora* have been effectively applied to tackle larvae. However, the sensitivity of nematodes to heat, soil moisture and exposure to sunlight as well as biotic factors can compromise their field efficacy. In Italy, in order to control *P. japonica* and limit its spread beyond infested areas, several strategies have been applied. For example, the use of entomopathogenic nematodes (*Heterorhabditis bacteriophora*) in a field experiment resulted in a 45% reduction in *P. japonica* populations and fungi (*Metarhizium anisopliae*) (Marianelli *et al.,* 2019).

All previous control strategies that have been implemented has not been able to completely eradicate *P. japonica*. In addition, the lack of information about the microbiota associated with *P. japonica* prompts several biological questions about the composition and the role of P. *japonica* microbiota in helping the invasive beetle in its development and the colonization of novel habitats.

4. Conclusion

The microbiota associated with forest and agricultural invasive beetles, especially their bacterial symbionts, are helping their hosts in the processes of protection, colonization and adaptation in novel habitats. As with many other insects, invasive beetles' bacterial symbionts are mainly dominated by bacteria from the phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. Proteobacteria frequently harbor microbiota in various invasive beetles including both those considered to be crop pests and those considered to be forest pests. Moreover, the Enterobacteriaceae family has been reported to be present in both forest and agricultural invasive beetles, as well as the genus *Pseudomonas*. The associated symbiotic bacteria are playing an important role by enabling their host in the protection, adaptation and colonization of new habitats. For example, the genera *Pantoea* and *Pseudomonas orientalis* are contributing to the suppression of plant responses against *A. mail,* in which *Pantoea* sp. can synthesize enzymes responsible for plant cell wall degradation and *P. orientalis* engages in lignin peroxidase activity. Meanwhile, the genus *Streptomyces*, associated with *Agrilus planipennis,* is capable of digesting carboxymethylcellulose. Further, the genera *Lactococcus, Serratia, Dysgonomonas* and *Enterococcus,* associated with *C. buqueti*, play an important role in lignocellulose degradation. In terms of protection, the genus *Pseudomonas*, which is present in most invasive beetles, such as *D. rhizophagus, I. pini, A. planipennis, A. mali, Cryptocephalus* spp. and *H. hampei*, is engaged in many critically important activities such as degradation and protecting its host from the pathogens of plants. The beetle *P. japonica* is a serious threat worldwide. The lack of information about the microbiota associated with *P. japonica*

undermines the importance of studying this topic and investigating the microbiota associated with *P. japonica* – this is the main goal of this thesis.

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First study: Developmental stages and gut microenvironments influence gut microbiota dynamics in the invasive beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae)

Summary

Popillia japonica Newman (Coleoptera: Scarabaeidae) is a highly polyphagous invasive beetle originating from Japan. This insect is highly resilient and able to rapidly adapt to new vegetation. Insect-associated microorganisms can play important roles in insect physiology, helping their hosts to adapt to changing conditions and potentially contributing to an insect's invasive potential. Such symbiotic bacteria can be part of a core microbiota that is stably transmitted throughout the host's life cycle or selectively recruited from the environment at each developmental stage. The aim of this study was to investigate the origin, stability, and turnover of the bacterial communities associated with an invasive population of *P. japonica* from Italy. Our results demonstrate that soil microbes represent an important source of gut bacteria for *P. japonica* larvae, but as the insect develops, it's gut microbiota richness and diversity decreased substantially, paralleled by changes in community composition. Notably, only 16.75% of the soil bacteria present in larvae are maintained until the adult stage. We further identified the micro-environments of different gut sections as an important factor shaping microbiota composition in this species, likely due to differences in pH, oxygen availability and redox potential. In addition, *P. japonica* also harbored a stable bacterial community across all developmental stages, consisting of taxa well-known for the degradation of plant material, namely the families Ruminococcaceae, Christensenellaceae, and Lachnospiraceae. Interestingly, the family Christensenallaceae had so far been observed exclusively in humans. However, the Christensenellaceae OTUs found in *P. japonica* belong to different taxonomic clades within this family.

1. Introduction

Insects are the most diverse and abundant animal clades (Foottit and Adler, 2009). The diversification and evolutionary success of insects have been partially attributed to their ability to establish associations with different beneficial microorganisms (e.g., Douglas, 2014; Corbin et al., 2017; Sudakaran et al., 2017; Heddi and Zaidman-Rémy, 2018). These microorganisms can play key roles for different physiological functions such as the supply of essential nutrients missing from unbalanced diets; contributing to the digestion of recalcitrant food components; protection from predators, parasites and

pathogens; and controlling mating and reproductive systems (e.g., Leftwich et al., 2017; Muhammad et al., 2017).

As for essentially all animals, microbial communities are particularly prominent in the digestive tract (e.g., Douglas, 2015, 2018; Clayton et al., 2018; Münger et al., 2018). The insect gut is generally structured into foregut, midgut, and hindgut, presenting a multitude of micro-environments suitable for microbial colonization. Differences in morphology and Physico-chemical properties between different gut sections can greatly influence the microbial colonization patterns and community structure depending on the host species. Gut bacteria have the potential to provide many beneficial services to their hosts and insects display a wide range in degree of dependence on gut bacteria for basic functions. Paramount to the evolution of intimate associations with gut microorganisms in the development of secure transmission routes between host individuals and generations. The lack of such mechanism in most insect species may hinder the establishment of such long-term associations. With the exception of social insects, such as termites and ants, where social interactions provide opportunities for the transfer of gut bacteria (Zhukova et al., 2017), insects had to develop original ways in order to transmit the important components of their gut microbiota (Fukatsu and Hosokawa, 2002; Gonella et al., 2012; Hosokawa et al., 2013; Mason et al., 2019). These "heritable" gut bacteria have been shown to play crucial roles in the nutrition, protection against different pathogens and xenobiotics, modulation of immune responses, and even extending life span (Roh et al., 2008; Kim et al., 2016; Daisley et al., 2018; Obata et al., 2018). Several factors can influence the gut microbiota structure and composition. Among these factors, the most important ones are diet and environment, but other factors (e.g., age) can also be at play (Wong et al., 2011; Montagna, Chouaia, et al., 2015; Montagna, Gómez-Zurita, et al., 2015; Montagna et al., 2016; Sanders et al., 2017; Tiede et al., 2017; Vacchini et al., 2017; Anderson et al., 2018). Although various factors can influence the insect gut microbiota, the existence of a shared core microbial community in some species could indicate that there are mechanisms (e.g. vertical transmission) favoring the presence of certain members of the gut microbiota. Several studies have investigated this possibility by tracking the changes in gut microbiota composition along the developmental stages of different insect species. These studies showed that the transmission of the gut microbiota throughout the different developmental stages may depend on the usefulness of certain bacteria (Zhukova et al., 2017; Malacrinò et al., 2018). For instance, the bacterial communities of fruit flies (Tephritideae) change throughout the insect's developmental stages to respond to the physiological needs of the host (Aharon et al., 2013; Malacrinò et al., 2018). In holometabolous insects, the pupal stage generally represents a bottleneck where most of

the larval gut microbiota is lost and adult insects may have to resort to indirect ways (e.g. via environmental transmission) to insure the transfer of beneficial bacteria from larvae to adults (Zhukova et al., 2017). For instance, in certain bee species, certain bacterial taxa are not trans-stadially transmitted but re-acquired from the environment (McFrederick et al., 2014). While the gut microbiota is not constant across the developmental stages in most insects, in some cases the microbial community can be relatively stable throughout the developmental stages. This has been observed in some Tephritid flies as well as in the Black Soldier Fly *Hermetia illucens* and in the moth *Plodia interpunctella* (Mereghetti et al., 2017; Yong et al., 2017; De Smet et al., 2018).

In the present study, we focused on the highly polyphagous invasive Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae, Fig. S1a). This invasive insect is listed in the EPPO Annex 2 due to the damages caused to different crops and turfs (EPPO, 2000). Native to Japan and the far east of Russia (Fleming, 1972), this beetle became an established pest in North America in the early 1900's (Switzer et al., 2009), in the Azores in the early 1970's (Vieira, 2008) and more recently in continental Europe, where it was recorded for the first time in Italy in 2014 (EPPO, 2014; Pavesi, 2014) and in Switzerland in 2017 (EPPO, 2017). Several laboratory and field trials have been carried out to limit the spread of this pest in mainland Europe and to evaluate the environmental resilience of the infested areas (Mazza et al., 2017; Paoli, Marianelli, Binazzi, et al., 2017; Paoli, Marianelli, Torrini, et al., 2017; Marianelli, Paoli, Sabbatini Peverieri, et al., 2018; Marianelli, Paoli, Torrini, et al., 2018). The damages to plants are caused by the different developmental stages of the beetle: the larvae, being underground dwellers, feed on the plant roots and soil organic matter while adults, living in an above-ground environment, feed on leaves and floral parts of different plant species (Fleming, 1972; Vieira, 2008).

Insect-associated bacteria can potentially contribute to an insect's invasive potential by helping their hosts to adapt to changing environmental conditions. Such symbiotic bacteria can be part of a core microbiota that is stably transmitted throughout the host's life cycle or selectively recruited from the environment at each developmental stage. The aim of this study was to investigate microbiota dynamics in an invasive population of *P. japonica* from Italy. Specifically, we addressed the following questions: i) Does *P. japonica* harbor a stable core microbiota or are the bacteria mainly acquired from the surrounding environment (i.e. rhizospheric soil exploited by larvae and pupae vs aerial environment exploited by adults)? ii) Is the gut microbiota maintained across the post-embryonic developmental stages (i.e. larvae, pupae, and adults) or is there a major turnover due to insect development? iii) Do different gut micro-environments impact microbial community structure?

2. Materials and methods

2.1. Collection and processing of insect and soil samples

Four campaigns were organized from June to September 2017 to collect insect samples at different developmental stages of the insect. The different stages and instars (in the case of larvae: larval instar 1 – L1; larval instar 2 – L2; larval instar 3 – L3) of the insects were collected in Oleggio (Novara, Italy; 45°36′ N, 08°38′ E, altitude ca. 230 m a.s.l.). Simultaneously, at each sampling expedition, 10 soil samples were taken from the sampled area and combined into a single sample representative of the area, leading to the collection of three soil samples. Insects were preserved in absolute ethanol while soil samples in 50 ml vials, kept refrigerated on the field and then stored at -20°C before processing. All insects were surface sterilized before dissection using the protocol described in Montagna and colleagues (Montagna, Chouaia, et al., 2015). 90 individuals (i.e. 15 individuals of each larval instar, 15 pupae, 15 males, 15 females) were dissected under sterile conditions, and the gut (Fig. S1b) was removed in sterile Ringer solution. The insect alimentary canal was then aseptically separated into its three compartments (i.e. foregut, midgut, and hindgut). For each developmental stage and larval instar, five homologous gut compartments were pooled together in a single sample, resulting in three biological replicates for each sample category. These samples were used for DNA extraction (see table S1 for detail on the samples).

Additionally, male adults (N=9) and L3 larvae (N=6) were collected and immediately processed in order to measure physicochemical properties (pH level, redox potential, oxygen concentration) of different gut regions. Specimens were anesthetized at 4°C for 3’ before their dissection.

2.2. DNA extraction, amplicon library preparation, sequencing and bioinformatics

The DNA was extracted from each sample (consisting of five homologous gut compartments for a defined insect instar and developmental stage) using the phenol-chloroform methods (Doyle and Doyle, 1990) with the modifications described in Mereghetti and colleagues (Mereghetti et al., 2017). The DNA was then eluted in 50 µl of sterile water (Sigma-Aldrich, Saint Louis, Missouri, USA). A DNA extraction blank was performed as control to monitor for contamination of environmental bacterial DNA. DNA from soils was extracted using PowerSoil DNA Isolation Kit MO BIO Laboratories Inc., Carlsbad, CA) following manufacturer's instructions. Three independent DNA extractions were performed for each of the three representative soil samples.

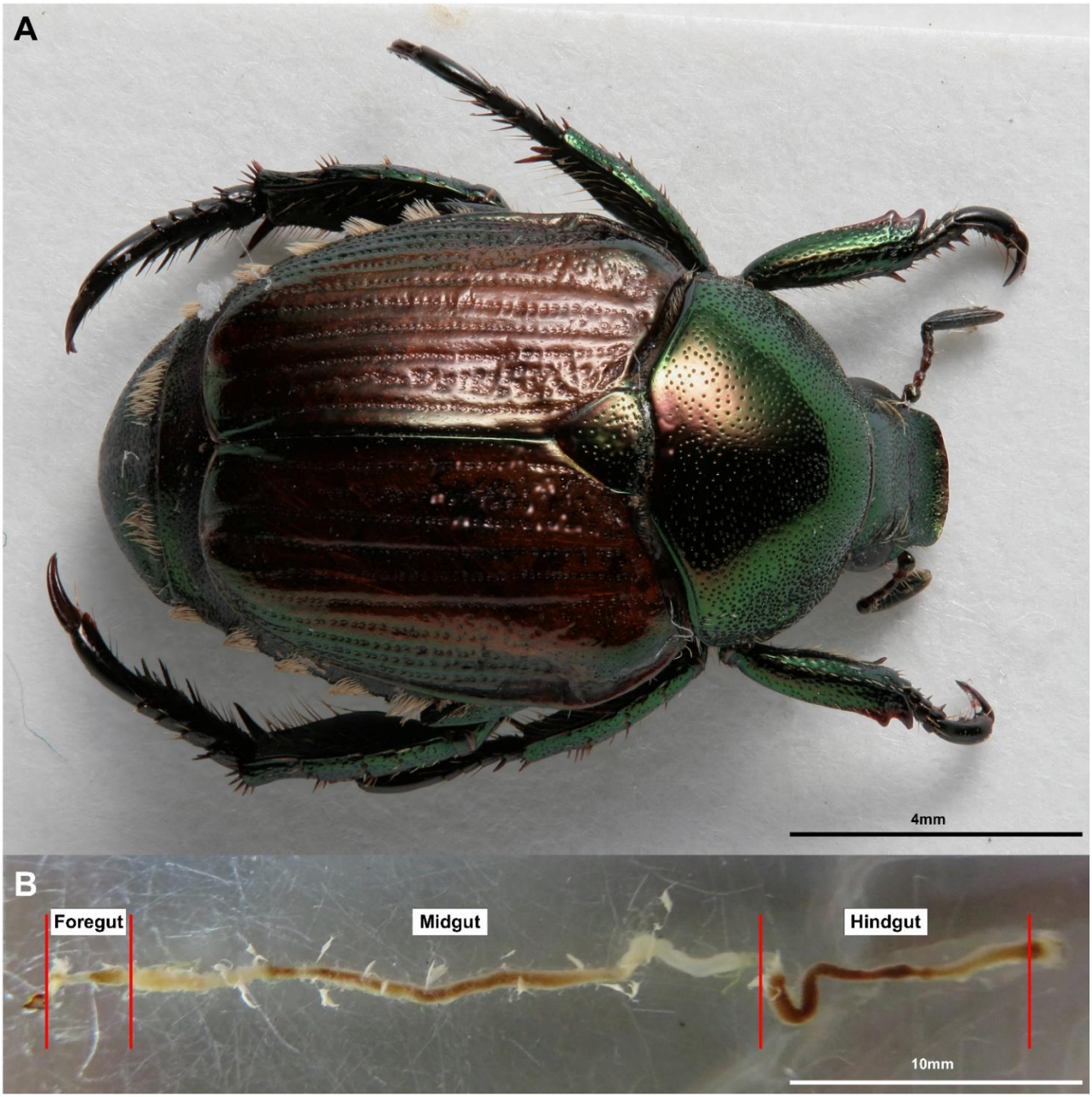

Figure S1: 1a. Male adult specimen of *Popillia japonica*. 1b. Gut of an adult P. japonica with the different sections delimited.

The extracted DNA was used as template for the amplification of the V4 hypervariable region of the 16S rRNA gene using the PCR primers 515F (Caporaso et al., 2011) and a blend of reverse primers 802R (Claesson et al., 2009) and 806R (Caporaso et al., 2011) in order to reduce amplification bias. Forward and reverse primers were tailed with two different GC rich sequences, enabling barcoding with a second amplification. Each sample was first amplified in 20 µl reaction volume containing 8 µl HotMasterMix

5 Prime 2.5X (Quanta Bio), 0.4 µl BSA (20 µg/µl) (Sigma-Aldrich), 1 µl EvaGreen™ 20X (Biotium), 0.8µl 515 F (10 µM) (- 5' modified with unitail 1 5'-CAGGACCAGGGTACGGTG-3'), 0.4 µl 802 R (10 µM) (- 5' modified with unitail 2 5'-CGCAGAGAGGCTCCGTG-3'), 0.4 µl 806 R (10 µM) (- 5' modified with unitail 2 5'-CGCAGAGAGGCTCCGTG-3'), and 1 µl (50 ng) of DNA template. The PCR amplifications were performed in a CFX 96™ PCR System (Bio-Rad) with 34 cycles of 94°C for 20 s, 52°C for 20 s, 65°C for 40 s and a final extension of 65°C for 2 min. The second PCR amplification was performed in 25 µl reaction volume containing the same reagents as the first PCR but with 1.5 µl barcoded/TrP1 primers (10 µM) and with 1 µl of the first PCR amplification in the following conditions: 8 cycles of 94°C for 10 s, 60°C for 10 s, 65°C for 40 s and a final extension of 72°C for 3 min. After labeling each sample with a specific Ion Torrent (Ion Express) DNA barcode, each single library was quality checked with agarose gel electrophoresis, quantified with Qubit Fluorometer (Thermo Fisher Scientific) then pooled with the other libraries in equimolar amounts. The final product was then sequenced using the Ion Torrent PGM System. Libraries preparation and sequencing were performed at the Life Sciences Department of Trieste University, Italy. Four samples (see table S1a for details) were excluded from the following analyses since they did not have enough reads (less than 200). The reads of the remaining samples were analyzed using QIIME version 1.9.1 (Caporaso et al., 2010). In detail, adapters were removed, and low-quality reads filtered (Phred < 20, read length < 250pb). Uclust (Edgar, 2010) was used to cluster the 16S rRNA sequences into Operational Taxonomic Units (OTUs) with a similarity cut-off of 97%. Chimeras were removed using Chimeraslayer. A representative sequence for each identified OTUs was aligned to Green-genes (http://greengenes.lbl.gov/) using Pynast (Caporaso et al., 2010). Taxonomic assignment was performed comparing the representative OTUs to Green-genes (release 13.8). Rare OTUs (i.e., singletons and OTUs < 10) and OTUs identified as chloroplast were discarded. The resulting OTU table was then used for the subsequent analyses.

2.3. Diversity analyses

Bacterial OTU richness, diversity and evenness were calculated using the package Vegan (Dixon, 2003; Oksanen et al., 2018), implemented under the R software (R Project 3.0.2; http://cran.r-project.org/) adopting the species richness estimator Chao 1 (Chao, 1984), the Shannon H' index (Shannon, 1948) and the Pielou's evenness (Pielou, 1975), after sub-sampling the OTU table to obtain a total of 25,000 sequences per sample. Alpha diversity indices were compared between different groups (i.e. tissues, developmental stages) using two-sample t-tests with 999 Monte Carlo permutations.

In order to evaluate if the structures of the bacterial communities associated with soil and the different developmental stages of *P. japonica* were driven by species competition or by environmental factors, thus resulting in a community dominated by closely related species (Webb et al., 2002; Mouquet et al., 2012; O'Dwyer et al., 2012), the mean pairwise distance between all taxa in the bacterial communities (MPD; Webb et al., 2002) was used as metric for phylogenetic structure. To allow the comparison between the bacterial communities of the different types, null models maintaining species occurrence frequency constant were estimated. Standard effect size and relative position of each bacterial community with respect to the null MDP distribution, generated by 999 randomizations of the null model, were calculated using the ses.mpd function implemented in the Rpackage picante (Kembel et al., 2010). This standardized metric quantifies the relative excess or deficit in the phylogenetic diversity for each community with respect to the entire species pool. Negative values reflect a relative phylogenetic clustering of the species, while positive values indicate a relative phylogenetic evenness (or overdispersion). SESMDP values were visualized as box-plots based on sample type (i.e., soil, larvae, pupae, adults) and statistical differences among sample types were assessed using Welch's one-way ANOVA (Welch, 1951), since SESMDP values were normally distributed based on Shapiro-Wilk test (Royston 1982) (p-value > 0.05), but the variance between groups was not homogeneous based on Levene test (Levene, 1960) (p-value < 0.001). Hence, we used the Tamhane post-hoc test for multiple comparisons without homoscedasticity.

The spatial (across the three gut regions) and temporal shifts (across developmental stages) of the *P. japonica* bacterial community (presence/absence) were estimated using the Sørensen-based multiple-site dissimilarity (βSOR; Baselga, 2010) implemented in the R package betapart (Baselga and Orme, 2012). The turnover and nestedness components of this β-diversity were calculated using Simpson-based multiple-site dissimilarity (βSIM; Baselga, 2010) and nestedness-resultant multiple-site dissimilarity (βNES; Baselga, 2010), respectively. In addition, for each β-diversity component, the pairwise dissimilarity values among the microbiotas of all analysed groups (i.e. soil, larvae, pupae and adults) were calculated using the betapair function of the R package betapart (Baselga and Orme, 2012) and visualized through heatmaps using heatmap.2 from the R package gplots.

In order to assess the difference in the microbiota structure among soil and insect samples, the sub-sampled OTU table was subjected to a nonparametric one-way analysis of similarity ANOSIM (Clarke, 1993), implemented in the vegan library and based on the Bray-Curtis dissimilarity (999 permutations

permuting within gut samples of the same individuals in order to account for the non-independence of the observations (Bray and Curtis, 1957).

The sub-sampled OTU table, after the removal of soil community samples, was used as input for a Nonmetric Multi-Dimensional Scaling (NMDS; Kruskal, 1964) biplot based on the Bray–Curtis dissimilarity (Bray and Curtis, 1957), in order to graphically ordinate samples and assess the differences among: i) the developmental stages (i.e. larvae, pupae and adults); ii) the three gut regions, and iii) to evaluate the impact of the gut physicochemical properties on the microbiotas associated with third instar larvae and adults. NMDS analyses were performed using the metaMDS function implemented in the R package Vegan (Dixon, 2003; Oksanen et al., 2018). The correlation between the microbiota composition and the tested factors (i.e. developmental stages, gut sections, gut physicochemical properties) was investigated by fitting the NMDS ordination scores with the envfit Vegan function (Dixon, 2003; Oksanen et al., 2018). The permutation of the community composition-based dissimilarity matrix (taking into account the non-independence of the different gut samples of the same individuals) allowed assessment of the significance of the fitted factors and vectors, and a squared correlation coefficient (R2) was calculated.

To determine the level of specificity of the microbiota composition associated with each developmental stage or gut region, model predictions were generated using Random Forest regressors based on the relative abundance OTU table (Knights et al., 2011). In order to classify the microbiota samples based on the host developmental stage or gut region, the supervised_learning.py script from the QIIME pipeline was used. cv10 was used as error correction method with 999 replicate trees.

2.4. Changes in microbiota composition

In order to identify OTUs shared between the different insect developmental stages and the soil, we only focused on OTUs that were typical for a given sample type (i.e. larvae, pupae, adults, soil). To this end, an OTU was considered "present" in a given sample type only when it occurred in at least 66% of the biological replicates of that sample type (in most cases, 2 out of 3 biological replicates). These OTUs are hereafter referred to as "core OTUs". The "core OTUs" specific to or shared among the different developmental stages and the soil were visualized through a Venn diagram. In addition, a bipartite network analysis (Dormann et al., 2008) of the bacterial community associated with the *P. japonica* (larvae, pupae, and adults) and the bulk soil was performed using the pairwise dissimilarity matrix generated from the OTU table adopting the Bray-Curtis dissimilarity index (Bray and Curtis, 1957).

Cytoscape (Shannon et al., 2003) was used to visualize the network. Differentially abundant taxa were determined after data normalization of the OTU table using the EdgeR package (version 3.16.5) with R (version 3.4.4). Differentially abundant OTUs were then ranked by their log2 fold change from the most differentially abundant to the least differentially abundant. Ranked OTUs were used to determine enriched families between different groups using the tmod package (version 0.36) with the CERNO test (Yamaguchi et al., 2008) and the Benjamini-Hochberg correction. The position of the OTUs belonging to enriched families along the continuum of ranked OTUs was also assessed visually using ROC curves (Receiver Operating Characteristic curves). The enriched families were then tested for their presence in all samples (supplementary table S3).

The OTU sequences of enriched taxa of interest (i.e. Christensenellaceae) were retrieved from the OTU file then aligned to complete or near-complete 16S rRNA sequences downloaded from the NCBI website (www.ncbi.nlm.nih.gov) using Clustal W. After gap removal, the evolution model was estimated using jModeltest according to the Akaike Information Criterion (AIC) parameter (Akaike, 1976). The phylogenetic tree was reconstructed using maximum likelihood with the Kimura 2 parameters model and 500 bootstraps. The phylogenetic tree was reconstructed and visualized using Mega X (Kumar et al., 2018).

In order to detect OTUs that are specific for a given gut section within the same developmental stage, the indicator value (Dufrêne and Legendre, 1997) was calculated using the R package indicspecies (De Cáceres and Legendre, 2009). Briefly, the indicator value of an OTU varies from 0 to 1 and attains its maximum value when all reads of an OTU occur in all samples of only one specific gut section. We tested the significance of the indicator value for each OTU with a Monte Carlo randomization procedure with 999 permutations.

2.5. Measurement of the gut physicochemical properties

Physico-chemical parameters of oxygen partial pressure (pO2), pH and redox potential were measured in the different sections of *P. japonica* gut (foregut, midgut, and hindgut) with microsensors and microelectrodes (Unisense, Aarhus, Denmark). Freshly dissected guts from both L3 larvae and males were placed on a layer of 2% (Low Melting Point) agarose prepared with Ringer's solution (7.2 g/L NaCl; 0.37 g/L KCl; 0.17 g/L CaCl2, pH 7.3-7.4) and immediately covered with a second layer of 0.5% agarose prepared with Ringer's solution (Šustr et al., 2014). Oxygen microsensors (OX-50), with a tip diameter of 50 μm, were calibrated after an overnight polarization in water saturated with air and in 0.1

M sodium dithionite anoxic solution by using the CAL 300 calibration chamber (Unisense, Aarhus, Denmark), following an overnight polarization. pH microelectrodes (PH-50), with a tip diameter of 50 μm, were calibrated with standard solutions at pH 4.0, 7.0 and 10.0. Redox potential microelectrodes (RD-50) had a tip diameter of 50 μm and were calibrated using saturated quinhydrone solutions at pH 4.0 and 7.0. Electrode potentials for microelectrodes were measured against Ag-AgCl reference electrodes by using a high-impedance voltmeter (Ri > 1014 Ω). Unisense microsensor multimeter allowed to measure the current and data were recorded by using SensorTracePRO software (Unisense, Aarhus, Denmark). Microsensors were positioned using a motorized micromanipulator (Unisense, Aarhus, Denmark). Measurements were carried out at room temperature.

3. Results

3.1. Alpha, beta and phylogenetic diversity of the gut microbiota

In this study, we analyzed the microbiota associated with three gut sections (foregut, midgut, hindgut) of the different developmental stages (L1, L2, L3, pupae, adult males and females) of *P. japonica*. For each sample type, 16S rRNA gene amplicons were obtained from three biological replicates, each containing the tissues of five individuals. In addition, we analyzed the microbiota of nine soil samples taken from the same habitat from which the insects were sampled. A total of 5175086 high-quality reads longer than 250 bp were kept after quality filtering and chimera removal. These reads clustered into 1612 OTUs. On average, 67299 high-quality reads grouped into 336 OTUs were obtained from larvae, 80249 reads/204 OTUs from pupae, 88397 reads/99 OTUs from adults and 148324 reads/1093 OTUs from soil samples (see Table S1a, Supporting Information, for details). Rarefaction curves of the observed OTU richness in 25,000 sub-sampled sequences showed that our sequencing effort was sufficient to capture the major part of the bacterial diversity associated with both insect and soil samples (Fig. S2). OTU richness and diversity (Fig. S2), as determined by the species richness estimator Chao1 and the Shannon Index of diversity, were higher in soil samples than in insect samples (Chao1: all t-tests $P < 0.01$; Shannon: all t-tests $P < 0.01$; see supplementary Table S1b for more details on the statistics for the different comparisons). Regarding the different developmental stages of *P. japonica*, OTU richness and diversity were the highest in the larvae (Chao 1: all t-tests $P < 0.01$; Shannon: all t-tests $P < 0.01$, see supplementary Table1 and Table S1b for all ecological indices). On the other hand, these indices were the lowest for adults (Chao 1: all t-tests $P < 0.01$; Shannon: all t-tests $P < 0.01$; Table1 and Table S1b). The different larval instars had similar richness and diversity with the Chao 1 and Shannon indices of 360.26 ± 52.2 and 4.99 ± 0.77, respectively, for L1 larvae, 313.92 ± 48.44 and 5.47 ± 0.28 for L2 larvae and 342.96 ±

43.02 and 5.74 ± 0.27 for L3 larvae (Chao 1: all t-tests p-value > 0.5; Shannon: all t-tests P > 0.5, Table S1b). It is noteworthy that the values of Pielou's evenness also followed a similar pattern, with the soil having the highest value (Pielou'J = 0.84; Table1), then larvae (Pielou'J = 0.67; Table1) and with pupae and adults having similar values (Pielou'J = 0.47 and 0.49 respectively; Table 1).

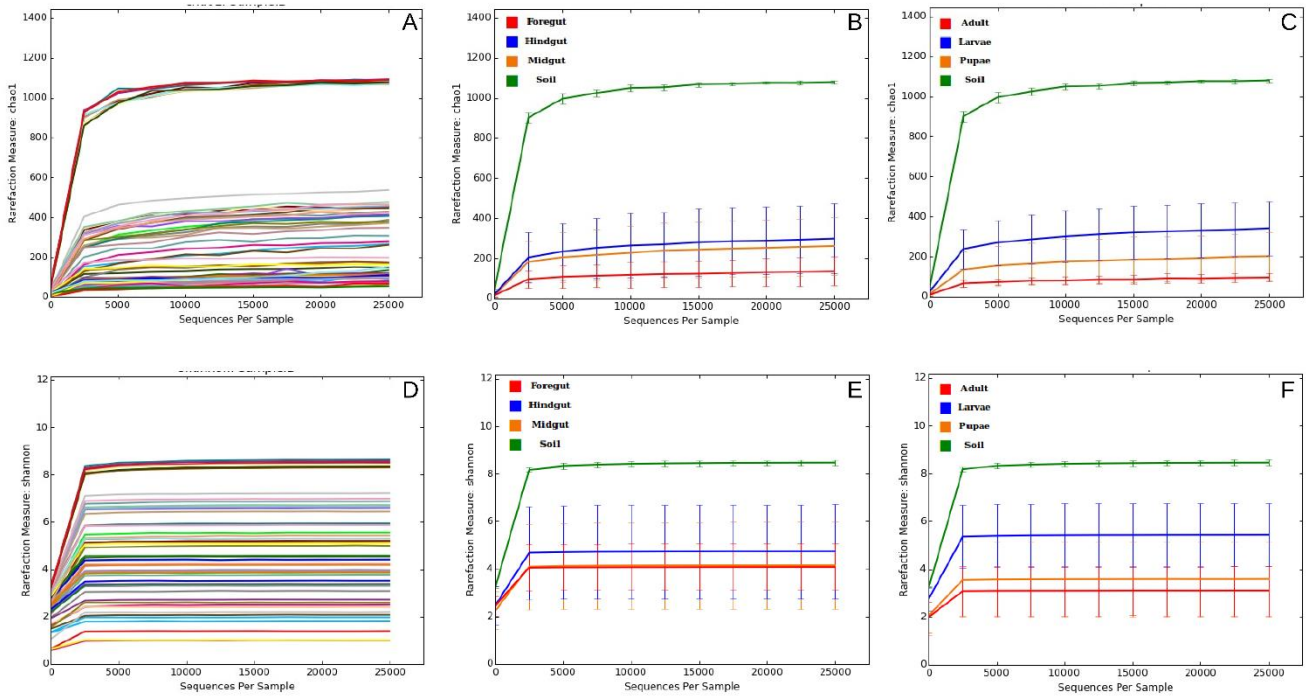

Figure S2: Alpha diversity parameters by sample or sample type. A: Chao1 index for all the samples. B: Chao1 index reported by gut section. C: Chao1 index reported by developmental stage. D: Shannon index for all the samples. E: Shannon index reported by gut section. F: Shannon index reported by developmental stage.

Table 1: Ecological indices by developmental stage (mean ± SE)

	Richness (Chao1)	**Diversity (Shannon)**	**Evenness (Pielou)**
Soil	1099 ± 1.35	5.88 ± 0.03	0.84 ± 0.00
Larvae	369.93 ± 28.95	3.77 ± 0.19	0.67 ± 0.03
Pupae	241.12 ± 43.51	2.49 ± 0.39	0.47 ± 0.06
Adults	129.65 ± 7.33	2.22 ± 0.18	0.49 ± 0.04

The standardized effect size of mean pairwise distance values (SES_MPD) of the bacterial communities associated with the samples ranged from positive values for soil bacterial communities (median value of SES_MPDSOIL = 0.78 associated with high quantiles, Table S1c) to negative values for bacterial communities associated with the larval and pupal stages (median values SES_MPDLARVAE = -3.38 and SES_MPDPUPAE= -3.9, low quantile values, Table S1c) (Fig. 1C). SES_MDP values were significantly different between sample types (one-way ANOVA, $F = 36.75$, $df1 = 3$, $df2=21.4$, $p < 0.001$), namely between larvae and soil (Tamhane post-hoc test, $p < 0.001$) and between larvae and adults (Tamhane post-hoc test, $p = 0.001$). The positive SES_MPD values for the soil communities indicate a phylogenetic overdispersion, as expected for communities characterized by high species richness and evenness such as those of soil. In contrast, the negative SES_MPD values for the bacterial communities associated with larvae and pupae indicate a phylogenetic clustering of these communities, possibly due to the selection towards certain closely related bacterial lineages by the insect gut environment or to the adaptation of these bacteria to the gut environment. Interestingly, the bacterial communities associated with adults were characterized by slightly negative SES_MPD values (median value of SES_MPDADULTS= -0.53; see Supplementary Table S1c), indicating a phylogenetic evenness of these communities (Fig. 1C). This increasing trend of SES_MPD values from larvae and pupae (negative values) towards adults (slightly negative values) contrasted with the trend of decreasing community species richness from larvae to adults (Fig. S3).

3.2. Factors affecting gut microbiota composition

Soil was different from the insect samples in terms of bacterial composition (adonis: $P < 0.001$, $R2 = 0.33$; anosim: $P < 0.001$, $R = 0.54$) with few OTUs shared between soil and the different insect developmental stages (Fig. 1A). Specifically, 891 OTUs out of the 1102 “core OTUs” of the soil were not found in the insect samples (Fig. 1B). On the other hand, only 35 “core OTUs” present in soil were also present in all the insect developmental stages (Fig. 1B).

Moreover, the nestedness component of the α-diversity between soil and the different insect developmental stage was very low (0.16 on average) and the turnover was high (0.84 on average) (Fig. S4), indicating that very few “core OTUs” were shared between soil and insect microbiotas while the variable fraction was high. Although more bacterial OTUs were shared between the insect samples (i.e. developmental stages and gut sections combined) than between insects and soil, these samples still formed distinct clusters as shown by NMDS analysis (Fig 2A). Specifically, insect developmental stages segregated along the first axis with the larvae microbiotas being clearly distinct from adult microbiotas,

while pupal microbiotas were intermediate. The second axis further separated the samples based on gut sections. For larvae and adults, the microbiotas of the different gut sections formed distinct clusters with the midgut microbiota being more different than the foregut and hindgut microbiotas. In contrast, the pupal microbiotas showed a different pattern with a clear cluster for the hindgut, while foregut and midgut microbiotas loosely clustered together.

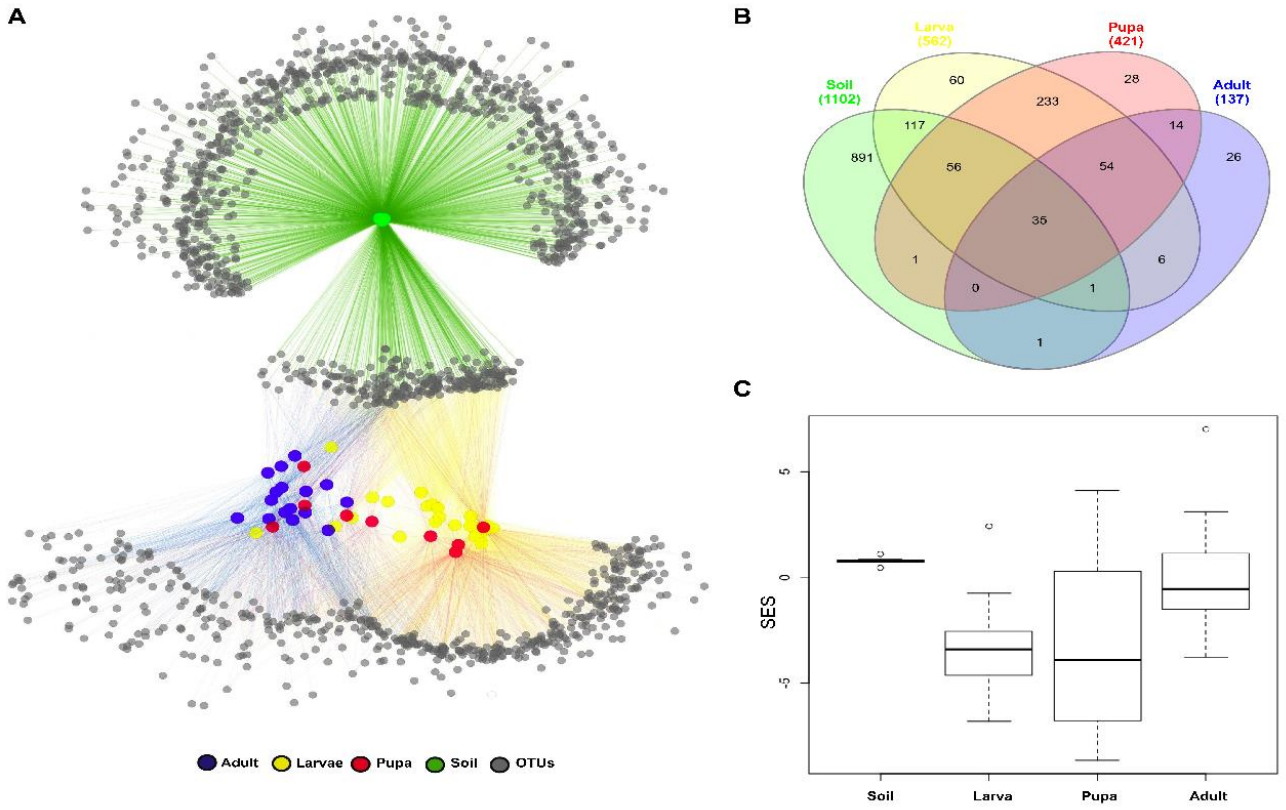

Figure 1 OTU distribution among the different samples. A: Bacterial community network connecting OTUs (grey circles) to the samples (colored circles) in which they were observed. B: Venn diagram showing the shared/specific bacterial OTUs (at 97% similarity) between the different developmental stages and soil. C: Boxplots of the estimated standardized phylogenetic diversity (SES-MPD) in the bacterial communities of rhizospheric soil and Popillia japonica developmental stages.

Based on the correlations of the tested factors (i.e., developmental stages and gut sections) with the NMDS ordinations of the insect-associated bacterial communities, the main factor driving this segregation was the gut section ($R^2 = 0.18$, p-value = 0.003) and to a lesser extent the developmental stage. These results were further supported by the Random Forest (RF) analysis was carried out to investigate the specificity of the microbiota of each sample category by trying to assign each sample to its respective category based on its microbiota. The RF analysis (Supplementary Table S1d) was able to

successfully classify adults and larvae in 100% and 91.7% of the cases, respectively. Conversely, pupae were successfully identified in only 55.6% of the cases. These results suggest that the pupal stage represents a transitional step not only in the development of the insect but also for its associated microbiota. The most important OTUs discriminating between the different developmental stages belonged to the Firmicutes (Clostridiales and Bacilli), Proteobacteria (Alphaproteobacteria) and Actinobacteria (see Supplementary Table S1f). On the other hand, the RF was able to successfully classify the foregut, midgut and hindgut samples in 80%, 82% and 78% of the cases, respectively (supplementary Table S1e). The most relevant OTUs allowing to discriminate between the different gut sections were identified as Firmicutes (Clostridiales) and Proteobacteria (Betaproteobacteria). These results indicate that the different gut sections, as well as larvae and adults, have distinct microbial communities, whereas the pupal stage has not.

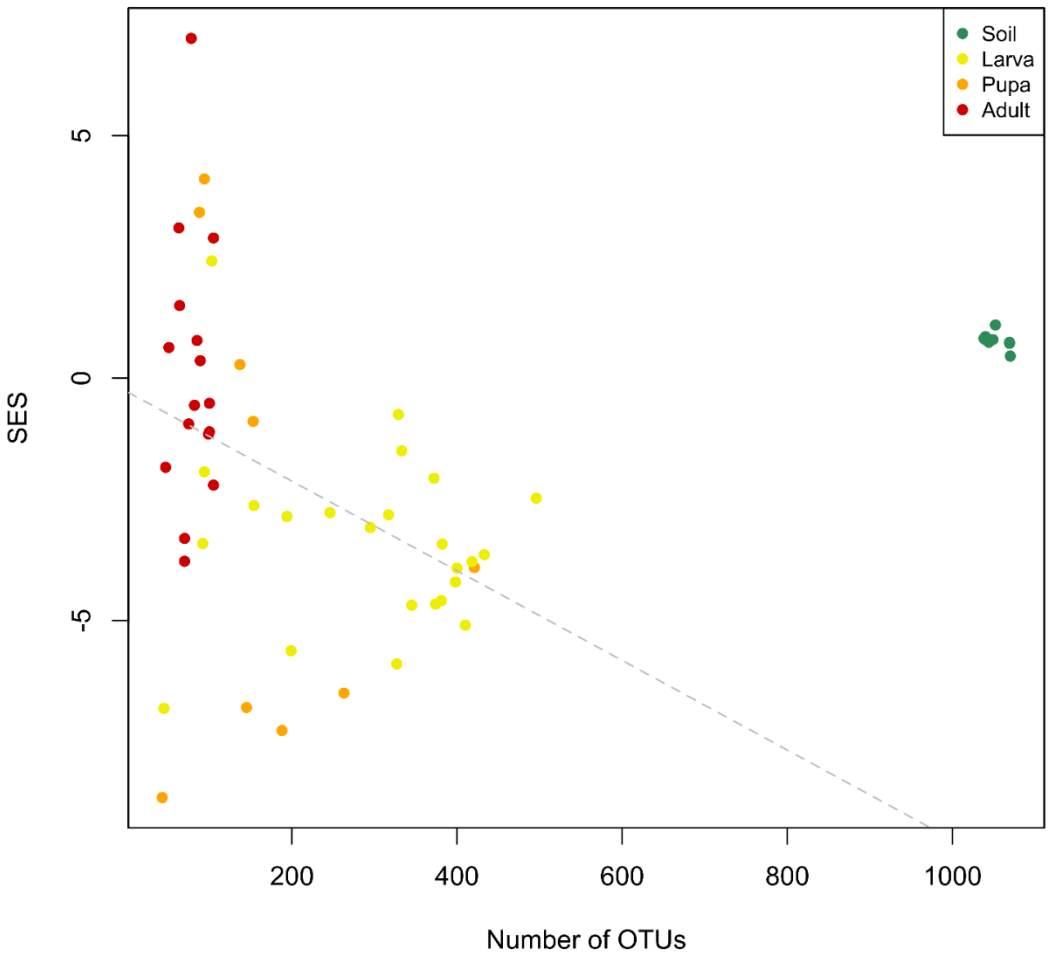

Figure S3: Biplot of the estimated standardized phylogenetic diversity (SES-MPD) and OTUs richness of each community. The dashed grey line represents the linear regression, for the bacterial communities associated with insect samples, of the SES-MPD onto the OTUs richness.

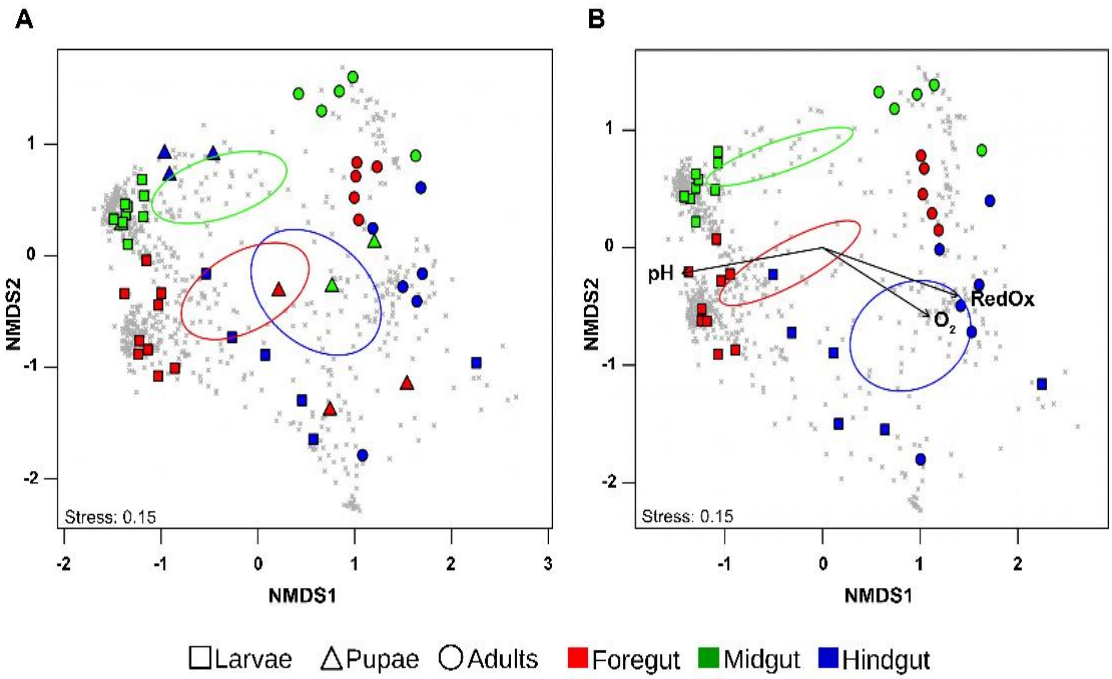

Figure 2: Non-metric multi-dimensional scaling analysis (NMDS) plots displaying sample β-diversity inferred from the OTU table. A: Biplot of the first 2 axes for the NMDS representing correlations between the OTUs abundance in all insect samples and ecological and ontological factors (i.e. developmental stage and gut section). B: NMDS plots showing the correlation between the bacterial OTUs of Adults and larvae and the different Physico-chemical properties (pH, O2 concentration and RedOx potential) of the different gut regions (foregut, midgut, and hindgut). The vectors represent the mean direction and strength of correlation of the different parameters measured (p-value < 0.05). In both figures, shapes indicate the different developmental stages (i.e. square for larvae, triangle for pupae, circle for adults) while colors indicate the gut region (i.e. red for foregut, green for midgut, blue for hindgut)

In order to further investigate the correlation between the Physico-chemical conditions of the gut and microbial composition, we measured pH, O2 concentration and Redox potential in each gut section for both male adults and L3 larvae (see Supplementary Table S1; Fig. S5). While the adult gut constituted a niche with a neutral pH (or at most slightly sub-acidic conditions), the pH in the larval gut increased from neutral in the foregut to alkaline conditions in the midgut and hindgut. Both larval and adult digestive systems were characterized by anoxic conditions, with the exception of the adult foregut where conditions fluctuated from anoxia to microaerophilia. Finally, positive redox potential values were measured in all gut compartments of both larvae and adults, with the exception of the larval hindgut where a decrease in redox potential was measured, underlining the existence of reducing conditions in this region. These three factors were significantly correlated with the microbial composition in the different gut sections. Notably, pH was significantly correlated with the microbiota of larvae (R2 = 0.75,

p-value = 0.001), while O2 concentrations (R2 = 0.54, p-value = 0.002) and redox potential (R2 = 0.74, p-value = 0.001) correlated significantly with the bacterial composition in adult gut regions (Fig 2B).

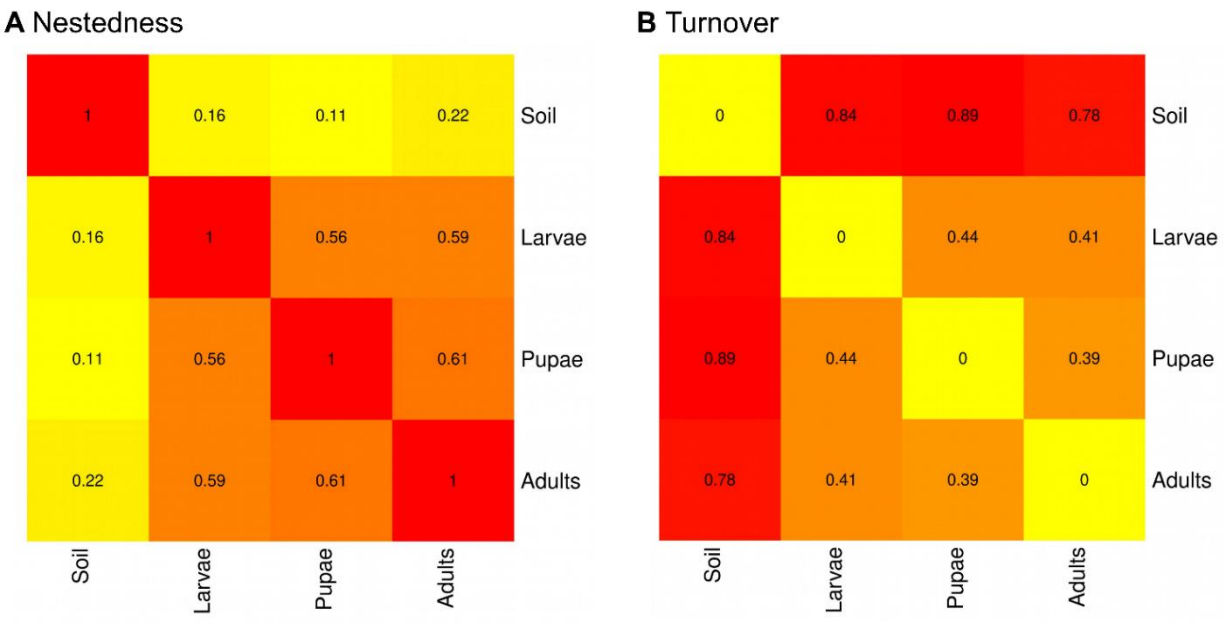

Figure S4: Heatmaps showing the relative pairwise nestedness and turnover values for the different developmental stages and soil

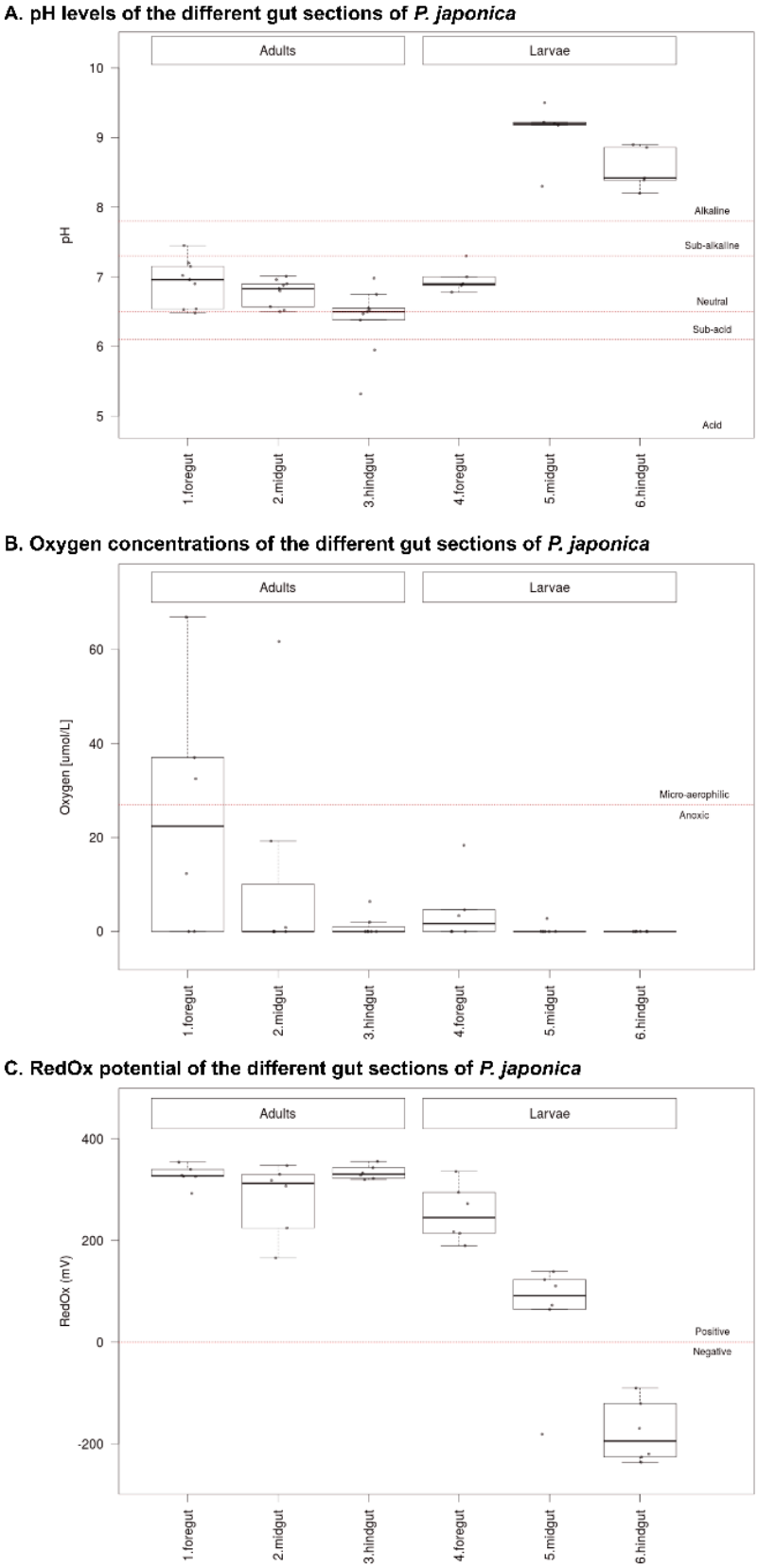

Figure S5: Box-plots displaying the value ranges of the different Physico-chemical properties measured for the different gut sections for both adults and larvae. A: pH, B: Oxygen concentration; C: RedOx potential.

3.3. Taxonomic composition of *P. japonica* gut microbiota

The microbiota associated with different developmental stages of the host and with soil not only differed in terms of bacterial richness and diversity but also concerning bacterial community composition (Fig 3; Fig. 2A, Supplementary Fig. S6). Although Proteobacteria represented the most abundant phylum considering all sample types (35.9% ± SE 4.2%), followed by Firmicutes (32.9% ± SE 5.4%) and Bacteroidetes (15.4% ± SE 3.7%), these proportions changed among the different sample types. Considering larvae (Fig 3B,Fig. S6), the most abundant phylum was Firmicutes with an average of 49.5% ± SE 7.9% (range 26.5% ± SE 5.5% in L2 larvae to 74.5% ± SE 8.7% in L1 larvae), followed by Proteobacteria (31.3% ± SE 5.8% on average; range: 13.9% ± SE 5.1% in L1 larvae to 50.3% ± SE 5.9% in L2 larvae) and Actinobacteria (9.4% ± SE 2.6% on average; range 5% ± SE 2.5% in L1 larvae to 13.9% ± SE 4% in L3 larvae). On the other hand, the most abundant taxa in adults were Bacteroidetes (33.7% ± SE 7.8% on average; 39% ± SE 10.6% in females, 28.3% ± SE 12.9% in males) followed by Firmicutes (29.6% on average; 14.5% ± SE 1.5% in females, 44.8% ± SE 4.1% in males) then Proteobacteria (29.1% on average; 40% ± SE 12.6% in females, 18.2% ± 6.6% SE in males). In pupae, the most abundant phylum was Proteobacteria with 59.7% ± SE 11.5%, followed by Bacteroidetes (19.1% ± SE 9.2%) and Firmicutes (15.4% ± SE 9.9%). It is noteworthy that the proportion of Actinobacteria decreased when passing from soil to adults (going from 24.8% ± SE 1.5% in soil to 6.4% ± SE 1.9% in adults), while the proportion of Bacteroidetes followed the opposite trend, going from 8% ± SE 1.2% in soil to 33.7% ± SE 7.9% in adults (Fig. 3A). Other bacterial taxa present at minor proportions (such as Acidobactria, Chloroflexi, and Nitrospira) followed a trend similar to Actinobacteria, with their proportions decreasing from soil to adults.Looking at the different gut sections (Fig. 3C), we observed similar trends. The relative abundance of Actinobacteria and Proteobacteria decreased from soil to hindgut from 24.2% and 39.6%, respectively, to 1.6% and 17.4%, respectively. On the other hand, the relative abundance of Firmicutes increased from soil to hindgut from 7.3% to 52.3%.

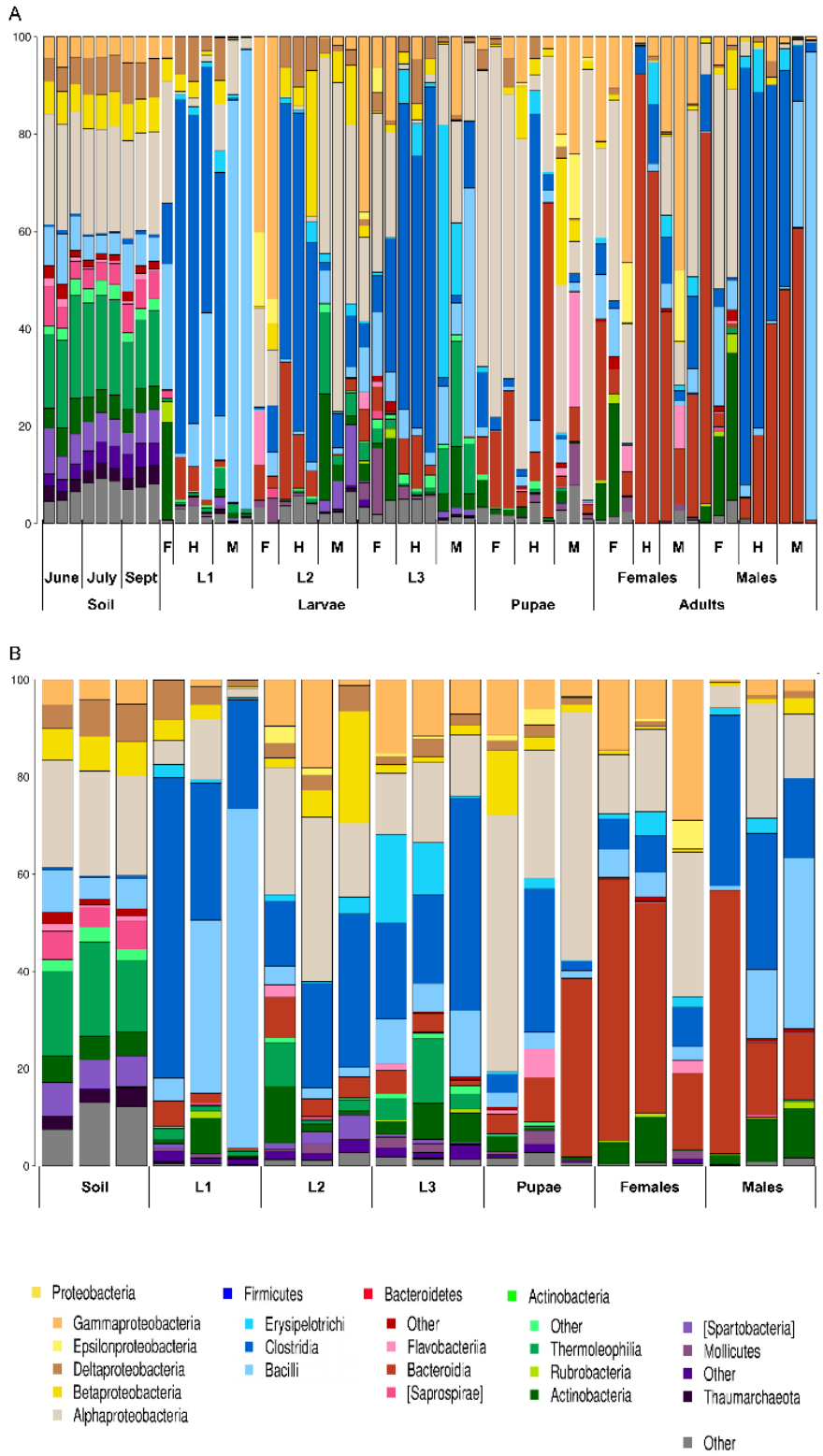

Figure S6: Histograms summarizing the bacterial composition at the order level. the different histograms report only taxa with a relative abundance ≥ 3%. A: The taxa summary at the order level for the different samples. F indicates foregut, M indicates midgut and H indicates hindgut. B the taxa summary at the order level for the different samples grouped by individual pools. Namely, each column corresponds to the samples (foregut, midgut, and hindgut) from the same pooled individuals.

3.4. Spatio-temporal changes in the microbiota taxonomic composition

As mentioned above, 891 OTUs out of the 1,102 “core OTUs” present in the soil was not found in the insect samples, while only 35 “core OTUs” were present in both insects and soil (Fig. 1B). These OTUs belonged predominantly to the Proteobacteria phylum (26 out of the 35 OTUs) with Rhizobiales being the most represented order (8 OTUs). In addition to these 35 OTUs, out of the 630 “core OTUs” found in insects but not in soil, 54 OTUs were shared between all the developmental stages. Proteobacteria, Bacteroidetes, and Firmicutes were the most abundant phyla (28, 10 and 9 OTUs, respectively). Noteworthy, OTUs belonging to the families Rickenellaceae (5 OTUs), Lachnospiraceae (3 OTUs) and Ruminococcaceae (1 OTU) were among the OTUs shared between the insect developmental stages. These families were identified as taxa specifically enriched in the insect guts along the different developmental stages.

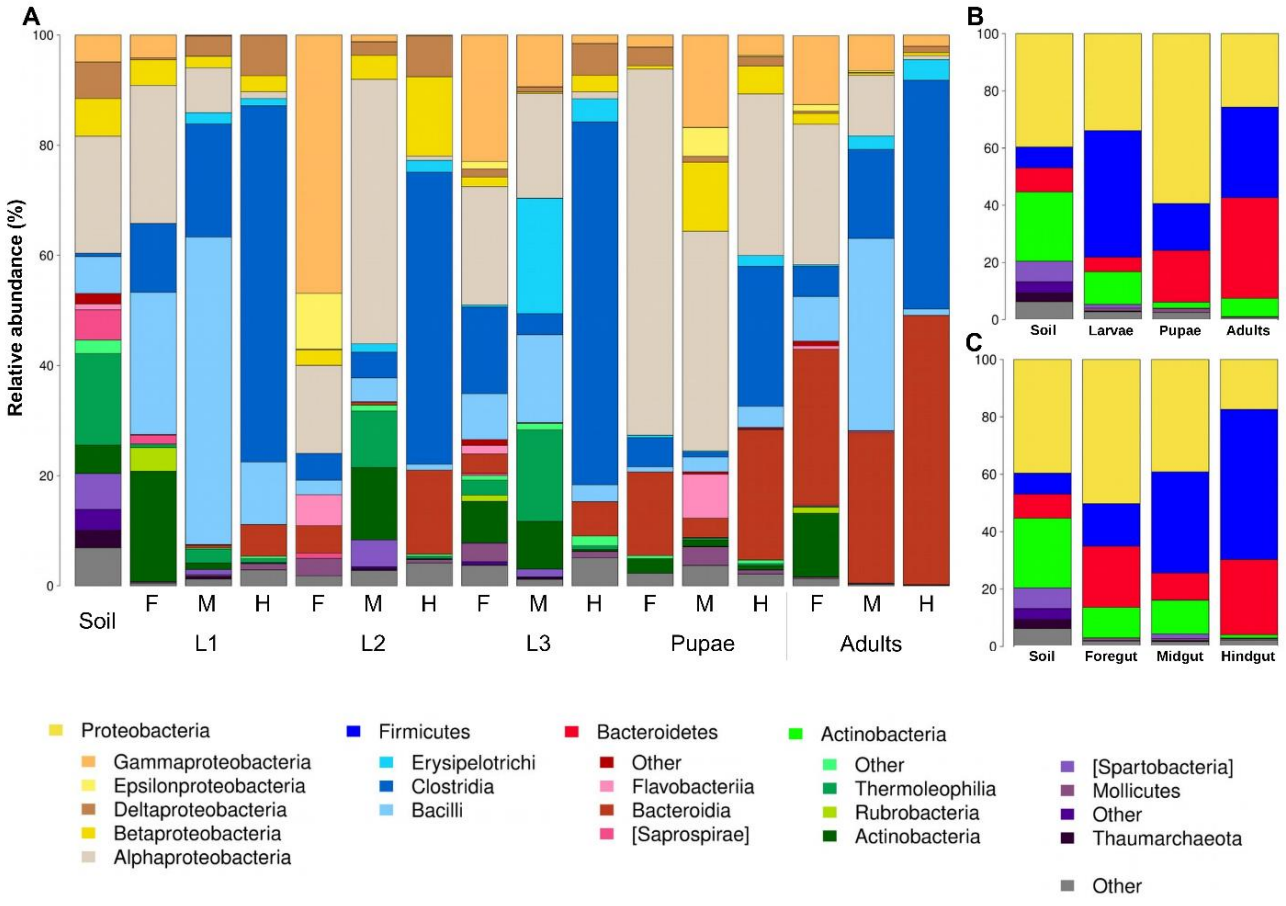

Figure 3: Histograms summarizing the bacterial composition at different taxonomic levels. the different histograms report only taxa with a relative abundance ≥ 3%. A: The taxa summary at the order level for the different samples grouped by category. F indicates foregut, M indicates midgut and H indicates hindgut. B and C the taxa summary at the phylum level for the different samples grouped by developmental stages (B) and by gut section (C).

We next performed a TEA (Taxon Enrichment Analysis) to identify which bacterial families were consistently enriched in insects compared to soil (Fig. 4). This analysis showed that among the Firmicutes, the Ruminococcacae was significantly enriched in larvae compared to soil ($P < 0.001$) but there were no differences when comparing the different developmental stages. Similarly, other bacterial families belonging to the Firmicutes and specifically to the order Clostridiales (namely Christensenellaceae and Lachnospiraceae) resulted to be significantly enriched in larvae and generally in insects when compared to soil samples. These families were also enriched in the different compartments of the gut when compared to soil ($P < 0.001$), independent of the insect developmental stages. Other bacterial families, such as Rikenellaceae (Bacteroidetes) and Desulfovibrionaceae (Proteobacteria), were also enriched in larvae compared to soil. These bacteria were also enriched in other portions of the gut but not all of them. Desulfovibrionaceae were also enriched in the midgut and hindgut, while Rikenellaceae were only enriched in the hindgut. Interestingly, all enriched families were absent from the soil samples (supplementary table S3). While this family was not always present in the foregut, Desulfovibrionaceae, Lachnospiraceae, and Ruminococcaceae were present in all midgut and hindgut samples for all developmental stages. Rikenellaceae, on the other hand, were present in all hindgut samples but absent from two midgut samples, namely one L1 and one pupal midgut sample (supplementary table S3)

It is noteworthy that the TEA did not evidence any significantly enriched taxonomic group between the different developmental stages of the insect nor did it evidence enriched taxonomic group between the different gut sections. This is partly supported by the fact that the nestedness component of the alpha-diversity between the different insect developmental stages was relatively high (0.59 on average), indicating that a higher fraction of the microbiotas is shared between the different insect developmental stages than between insects and soil.

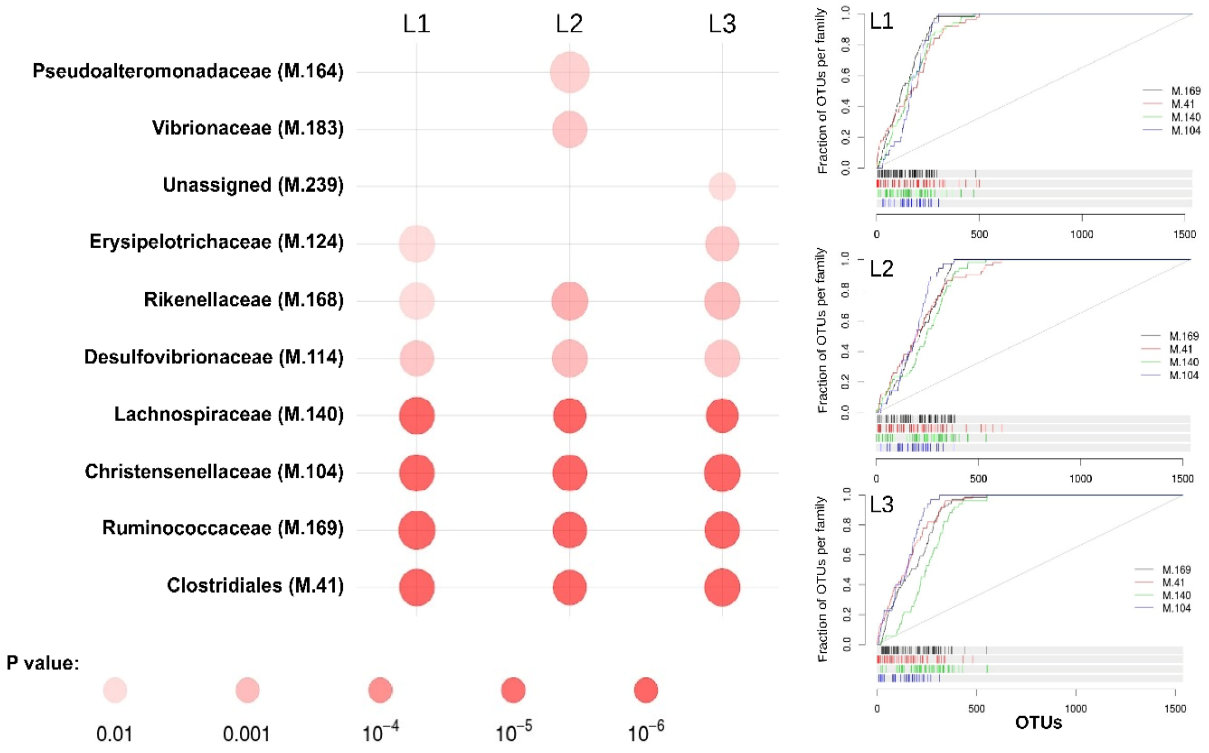

Figure 4: Taxa Enrichment analysis (TEA) carried out on the different larval stages using soil as reference. The main figure indicates the families that were enriched in the different larval stages compared to soil. The color intensity of the circles indicates the p-value while its size indicates the effect size. The panels on the right-hand side are the ROC curves, plotting the ranked OTUs belonging to the enriches families against the totality of the ranked OTUs, represent the rank of the different OTUs belonging to the families Lachnospiraceae (green), Christensenellaceae (blue), Ruminococcaceae (black) and the order Clostridiales (red) in general.

An Indval analysis carried out to identify OTUs specific to a given developmental stage showed that 23 OTUs were unique to larvae, five were associated only with pupae while 13 were specific to adults (See table S2a for supporting information). Members of the Lachnospiraceae family were the most represented OTUs among those unique to both larvae and adults (with nine and five OTUs present respectively).

The same analysis carried out on the different gut sections for each developmental stage gave a different picture. For the pupal stage, there was no OTU specific to a given gut section. For adults, 15 OTUs were found only in the foregut, while 5 OTUs were specific to the hindgut. No OTU was found to be unique to the midgut. On the other hand, in the larvae, only two OTUs were specific to the foregut, while the midgut and hindgut had respectively 105 and 145 specific OTUs. It is noteworthy that three out of the

five OTUs that were unique to the adult hindgut were also found specifically associated with the larvae hindgut. These OTUs belonged to the Rikenellaceae (denovo5575 and denovo143435) and Nitrosomonadaceae (denovo213936) families.

3.5. Phylogenetic relationship of Christensenellaceae associated with *P. japonica*

Bacteria belonging to Christensenellaceae have previously been observed only in humans. To better understand the phylogenetic relationships between members of the Christensenellaceae associated with P. japonica and those associated with humans, we performed a Maximum Likelihood phylogeny using our OTUs and 16S rRNA gene sequences from those isolated from humans (Figure S7). The OTUs associated with the insect formed several clusters distinct from the cluster of human-associated symbionts. Hence the bacteria associated with *P. japonica* belong to different taxonomic groups within the Christensenellaceae family.

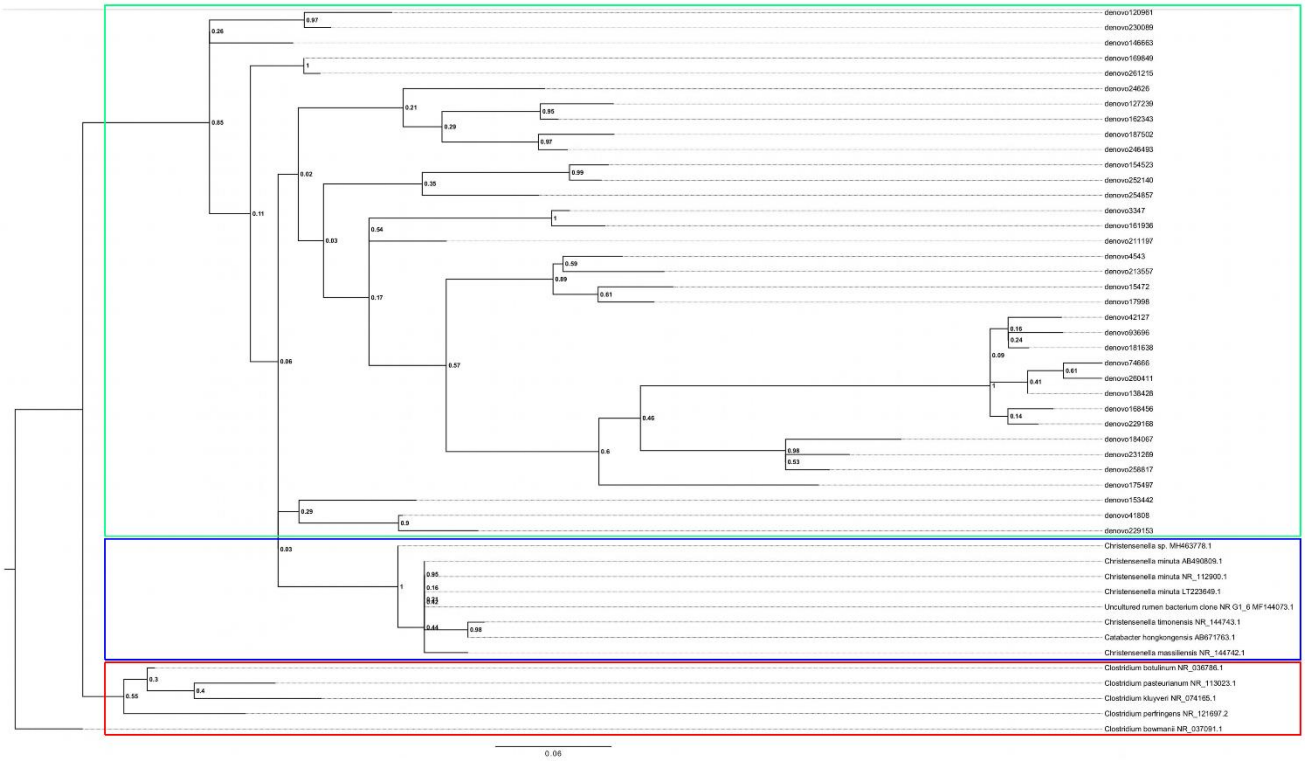

Figure **S7**: Maximum likelihood phylogenetic tree based on the partial 16S rRNA gene sequences. The blue circle indicates the Christensenellaceae group of bacteria associated with the human gut. All other taxa were detected in the present study in association with P. japonica gut sections. The scale bar at the bottom indicates the distance in nucleotide substitution per site. The alphanumeric sequence at each node either the GeneBank accession number or the de novo OTUs

4. Discussion

In this study, we demonstrate that soil bacteria represent an important source for the gut microbiota of *P. japonica* larvae, but as the insect develops, the gut bacterial community experiences important changes in richness, diversity, and composition. Specifically, 37% of the OTUs (209 OTUs) present in larvae derived from the soil microbiota and 35 OTUs present in the soil was maintained throughout all the developmental stages of the insect. In addition, larvae had a higher OTU richness and diversity compared to adults. This is likely linked to the different lifestyles of the two stages: larvae are soil-dwelling and similar in OTU numbers to other soil-dwelling arthropods such as terrestrial isopods (healthy isopods OTUs on average 209; Dittmer et al., 2016), termites (number of OTUs consistently higher than 400; Su et al., 2016) and ants (number of OTUs about 400; Vieira et al., 2017; Zhukova et al., 2017), while the OTU numbers of adults are comparable to those of non-soil-dwelling insects (in 218 insect species, average OTUs 84; Yun et al., 2014). Pupae are an intermediate state between larvae and adults in terms of bacterial taxonomic richness and diversity, representing a bottleneck for bacterial transmission due to metamorphosis. Nonetheless, key bacterial taxa involved in plant material degradation are still transmitted to adults (see below for a detailed discussion). This reduction in both richness and diversity at the pupal stage could be due to a combination of factors both random and deterministic. On the one hand, a reduction in the number of bacterial cells during metamorphosis could have caused a random reduction in the diversity of the microbiota. On the other hand, the observed reduction in microbiota diversity throughout host development could be caused by one (or several) active mechanisms, such as (i) the change of nutrition (or lifestyle) between soil-dwelling larvae and adults, (ii) specific Physico-chemical properties (e.g., the change in gut pH between larvae and adults), and/or (iii) enzymatic activities, among others. As a matter of fact, the observed changes (i.e. decrease in richness and diversity) are not a constant in insect development and other studies monitoring gut microbiota changes throughout development have shown different trends, such as an increase in species richness (Brucker and Bordenstein 2012) or more generally the absence of a clear trend (Oliveira et al., 2018; Gao et al., 2019; Huang et al., 2019). The trend that we observe in *P. japonica* could be explained by its ecology, since soil-dwelling arthropods such as termites and woodlice consistently present higher microbiota richness and diversity (Dittmer et al., 2016; Su et al., 2016; Vieira et al., 2017; Zhukova et al., 2017) due to their proximity to a microbially rich and diverse environment (i.e. soil). On the other, arthropods living in "aerial" ecosystems (i.e. plants and leaves) tend to have a less rich and diverse gut microbiota (Yun et al., 2014; Mereghetti et al., 2019).

Interestingly, the decrease in microbiota richness and diversity throughout the host developmental stages is accompanied by a shift in the phylogenetic community structure. Specifically, larvae and pupae harbor phylogenetically clustered bacterial communities, i.e. consisting of closely related bacterial taxa. In contrast, the adult microbiota is phylogenetically overdispersed, similarly to rhizospheric soil communities. The observation that larvae microbiotas are phylogenetically clustered and at the same time taxonomically rich compared to adults could be explained by a selection of certain taxonomic groups through the gut environment. The phylogenetic overdispersion of the adult gut microbiotas suggests that the pupal stage represents a crucial bottleneck for the gut microbiota in terms of species richness. This might be due to the random survival of bacterial taxa present in the larvae throughout metamorphosis (and its associated gut tissue restructuring) at the pupal stage. However, the fact that a certain number of taxa are maintained throughout the development from larvae to adult but are absent from soil, suggests the existence of a mechanism to specifically maintain essential bacterial partners (e.g., Ruminococcaceae, Lachnospiraceae). In other words, the survival of certain bacterial taxa may not be entirely random. Another possible explanation might be that the adult gut microbiota is renewed by feeding on leaves and flowers in contrast to rhizospheric soil and/or that the Physico-chemical properties of the adult gut are more stable than in larvae (see Fig. S5). Hence, despite the potential existence of a mechanism to maintain and transmit a fraction of the microbiota, other bacterial taxa could still be transient and dependent on the food source (e.g., different parts of the plant, different plant species), as observed in *D. melanogaster* where acetic acid bacteria are always associated with the fly but the presence of other bacterial taxa is dependent on the environment (Adair et al., 2018; Wong et al., 2015).

This study allowed us to identify several factors potentially shaping microbiota composition in *P. japonica*. Specifically, we demonstrate that among the tested factors, microbiota composition varied significantly between different gut sections as well as between insect developmental stages. This strong correlation between different gut sections and microbiota diversity and composition is most likely due to i) differences in the Physico-chemical conditions prevailing in each gut section (Fig S5) as well as ii) biotic factors such as host enzymatic potential and immune response. It is noteworthy that the pupae represent a transitional stage with a reshuffling of the microbiota between the larval and adult stages. In other words, the larvae and adult microbiotas formed clearly distinct clusters, while the pupae microbiota was more dispersed between the larvae and adult clusters. This may have had an impact on the statistical analyses, leading to an apparently weaker effect of the developmental stages on microbiota composition.

Regarding the physicochemical factors, oxygen availability was the most strongly correlated with differences in bacterial community structure between the different gut sections in adults, while intestinal pH was the most strongly correlated factor in larvae. Although both the midgut and hindgut compartments were largely anoxic in adults, the oxygen concentration in the midgut showed a higher degree of variation compared to the more anoxic hindgut. This is likely due to a considerably larger influx of oxygen via the gut epithelium in the case of the midgut, as observed in *Pachnoda ephippiata* (Lemke et al., 2003). This variability in oxygen availability between the different gut compartments may favor bacteria that are more tolerant of such fluctuations. In larvae, the pH in the midgut and hindgut was alkaline, while the foregut had a neutral pH. It is important to note that the larvae are soil-dwellers feeding on fresh roots and decaying soil organic matter (SOM) (Fleming, 1972). In this regard, they are similar to other soil-dwelling macroinvertebrates, including many coleopterans, which feed on SOM and play an important role in its degradation and stabilization (Lavelle et al., 1997; Wolters, 2000). It has been shown that the conditions in the anterior hindgut of the humivorous termite *Cubitermes* spp. (i.e. high alkalinity and oxygen influx) lead to a decrease of the molecular weight of the organic matter (Kappler and Brune, 1999), rendering it more soluble and thus more accessible for digestion in subsequent less-alkaline compartments (Ji et al., 2000; Kappler et al., 2000; Ji and Brune, 2001). Although the complex microbial communities in the guts of humivorous macroinvertebrates are thought to participate in the transformation of ingested SOM (Cazemier et al., 1997; Kane, 1997), detailed information on the composition and activities of the gut microbiota is lacking. In view of the high midgut alkalinity in P. japonica, it is reasonable to assume that at least some of the bacteria in the midgut are tolerant towards high pH conditions since most bacterial taxa are also found in the more neutral gut sections of adults.

We further observed differences in microbiota composition at different taxonomic levels (from order to OTU) between the different developmental stages of *P. japonica*. For instance, Actinobacteria decreased in abundance from larvae to adults, while Bacteroidetes increased in abundance. However, no particular taxa were found to be specifically enriched in any of the developmental stages. A similar pattern was observed for the microbiota associated with different gut compartments (foregut, midgut, hindgut): no particular taxon was specifically enriched in any of the compartments. Nonetheless, Proteobacteria decreased from foregut to hindgut, while Firmicutes increased. Actinobacteria were relatively stable between foregut and midgut but decreased in the hindgut.

In contrast, several taxa were found to be significantly enriched between soil and insect gut. Those belonged mainly to the families Ruminococcacae, Christensenellaceae and Lachnospiraceae. Members

of these families are known to degrade cellulose (Flint et al., 2012; Biddle et al., 2013). The fact of finding them enriched in the insect gut may suggest a possible symbiotic relationship where these bacteria help their host degrade and metabolize cellulose, as in the case of the symbiotic association between termites, protists and bacteria (Liu et al., 2013) or woodlice and certain bacterial taxa (Bredon et al., 2018). These bacteria could be important in helping their host metabolize plant roots and leaves and might thus contribute to its success as a polyphagous invasive insect. The bacterial taxa that were enriched in the gut of *P. japonica* have been previously reported in association with various insects but more importantly with ruminants and humans. *Anaerostipes* spp., *Coprococcus* spp. and *Dorea* spp. (members of the Lachnospiraceae family) have all been previously described in association with the human gut (Rainey, 2009) where they are hypothesized to be involved in pectin fermentation. Other members of the Lachnospiraceae family have also been described in association with other insects (Huang and Zhang, 2013; Bourguignon et al., 2018). The Ruminococcaceae family, represented *by Ruminococcus* spp. and *Oscillospira* spp. in *P. japonica*, has also been described in association with humans, ruminants, coleopterans, and termites (Kamagata, 2011; Huang and Zhang, 2013; Bourguignon et al., 2018). *Ruminococcus*, in addition to Bacteroides spp., plays an important role in the fermentation of hemicellulose and the degradation of different plant material through the production of Carbohydrate-Active enzymes (CAZymes) (Jose et al., 2017). CAZymes are very important for the break-down of the different components of lignocellulose (i.e. cellulose, lignin, hemicellulose; Bredon et al., 2018). It is noteworthy that although some insects are able to express some of these enzymes, most of them heavily rely on their associated microorganisms to degrade lignocellulose (Bredon et al., 2018). On the other hand, the role of *Oscillospira* is still unknown and it is hypothesized that it may be involved in lignocellulose degradation (Kamagata, 2011). Rickenellaceae, with the genus *Alistipes*, and Desulfovibrionaceae have also been described in association with the guts of different animals (Koneru et al., 2016; Ruengsomwong et al., 2016), especially termites (Reid et al., 2014; Makonde et al., 2015), where they play an important role in the degradation of cellulose polymers (Ozbayram et al., 2018).

The taxa found to be enriched in insect samples could be preferentially present in insects due to favorable conditions in the gut environment without an actual effect of these bacteria on the insect host. However, the fact that these bacteria were not detected in soil suggests the presence of a more direct transmission mechanism independent of the environmental route. In addition, the consistent presence of these bacteria in the gut regions where plant material is degraded further argues in favor of an active role of these bacteria and not just their presence as transient passengers.

In contrast to the above-mentioned bacterial families which have been observed not only in mammals but also in insects, the family Christensenallaceae had so far been observed exclusively in humans. Although its role in the degradation of nutrients is not yet understood, members of this family (i.e. *Christensenella minuta*) have been shown to play a central role in controlling the Body Mass Index and in helping to shape a "healthy" microbiota in humans and transfected mice (Goodrich et al., 2014). Increased titers of *C. minuta* have also been correlated with longevity in humans (Biagi et al., 2016), while decreased titers were observed during different human diseases (Petrov et al., 2017; Yu et al., 2017). In addition, other bacteria belonging to the genus *Christensenella* have been isolated from diseased humans, although no causality has been established yet (Ndongo et al., 2016). The partial 16S rRNA gene-based phylogeny showed that the Christensenellaceae OTUs found in association with *P. japonica* do not cluster with the taxa associated with humans but rather form different clusters, suggesting that they belong to different taxonomic groups within the Christensenellaceae family (Fig. S7).

Although three biological replicates containing homologous gut regions from five individuals might be limiting, based on the results obtained in this study we can conclude that the gut microbiota of *P. japonica* is highly dynamic across the developmental stages of the insect and changes in microbiota composition strongly correlated with the Physico-chemical properties of the gut. Despite the microbiota high variability, 89 OTUs were maintained from larvae to adults, including 35 OTUs originating from the soil environment. As a future perspective, it would be interesting to investigate if these OTUs represent a stable core microbiota present in all P. japonica populations in different parts of the world or if they are subject to change in different environments. In the first case, this might indicate a more intimate symbiotic relationship potentially maintained via vertical transmission. In the latter case, the variable microbiota would provide a means to investigate the origin of new invasions of this beetle, via a comparative analysis of the local soil and insect gut microbiotas.

Declaration

BC, MM, and LM designed the experiments. BC performed the microbiota and enrichment analyses. MM, GMG and NG performed the statistical analyses. SA performed network analyses. GMz, EG, FP, LM, PFR, and AA performed the sampling. NG dissected the insects and extracted the DNA. FF and FF performed the sequencing. MC, MF, EC, and DD performed the Physico-chemical analyses. BC and MM wrote the manuscript. All authors read and commented on the manuscript.

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Second study: Potentially entomopathogenic nematode isolated from *Popillia japonica* (Coleoptera: Scarabaeidae): bioassay, molecular characterization and the associated microbiota

Summary

The Japanese beetle *Popillia japonica* Newman is an higly invasive pest recently introducted in Europe. In the current study a nematode is isolated from the third larvae instar of *P. japonica* collected in northern Italy. Both BLAST search and the phylogenetic maximum likelihood tree inferred from 18S rRNA sequences confirm the attribution of the isolated nematode to the genus *Oscheius*. The entomopathogenicity of the isolated nematode was tested on larvae of the model organism *Galleria mellonella*, the mortality of the host after five days was more than 50%. Furthermore, the microbiota associated with the isolated nematode wes characterized using a metabarcoding approach. Our results suggest that the bacterial community of the isolated nematode is dominated by bacteria belonging to the genus *Ochrobactrum* (77%), that includes entomopathogenic species. Further studies are needed in order to test the possibility of using this nematode as a biocontrol agent of *P. japonica* in Europe.

1. Introduction

The Japanese beetle (*Popillia japonica* Newman), native to Japan, northern China and the far eastern Russian island of Kuril, was first reported outside its native range in the United States in 1916. Later, it has become an established pest in North America, the Azores and more recently in Europe (Pavesi, 2014; EPPO, 2014 https://gd.eppo.int/reporting/article-3272). In 2014, *P. japonica* was recorded for the first time on the European mainland when an outbreak was reported within the Ticino Valley Natural Park, Italy (Pavesi, 2014). The species is polyphagous and attacks different plants such as *Acer* spp., *Glycine max* (soya bean), *Malus* spp. (ornamental species), *Prunus* spp., *Rosa* spp., *Ulmus* spp. and *Vitis vinifera* (Bragard et al., 2018). The three larval instars attack roots causing plant mortality while adults feed on leaves causing their skeletonization and on the early ripening fruit (e.g., apples, peachs, nectarines) causing severe damages and affecting the quality of fruit (Bragard et al., 2018). In addition, *P. japonica* has an indirect impact on agriculture since in the USA it is reported as a vector of Southern bean mosaic virus and Bean pod mottle virus (Wickizer and Gergerich, 2007). Several methods have been developed to control *P. japonica*. The main strategies involve the use of chemicals to target larval stages and adults (Morris and Grewal, 2011). More environmentally friendly strategies rely on the use of organisms like the parasitoid wasps *Tiphia vernalis* and *Tiphia popilliavora*, which attack overwintering larvae and

newly emerged adults, or predators such as staphylinids and carabids, which attack young larvae and eggs (Potter and Held, 2002). Nematodes represent interesting biocontrol agents since they have a wide host range and can quickly kill the host. They are environmentally friendly and, in association with other biocontrol agents, are important parts of integrated pest management strategies (Grewal et al., 2005). Entomopathogenic nematodes (EPNs) are a group of parasitic nematodes, which have evolved an association with insect pathogenic bacteria, causing the death of the insect host (Dillman et al., 2012). The infective juvenile is the only free-living stage able to attack and colonize the host. Once the colonization occurred, the symbiotic bacteria (*Photorhabdus* spp. and *Xenorhabdus* spp.) harbored in the nematode's intestine are released into the host's hemolymph where they propagate and kill the host by septicemia (Kaya and Gaugler, 1993). A variety of nematodes such as *Steinernema* spp. and *Heterorhabditis* spp. have been observed within the body cavity of *P. japonica* larvae and used as a biocontrol agent against this pest (Cappaert and Smitley, 2002). The commercial species *Heterorhabditis bacteriophora* resulted an efficient biological control agent of *P. japonica* larvae in Italy (Marianelli et al., 2018). Due to the economic impact of *P. japonica*, several studies searching for potential natural enemies have been carried out and are still ongoing, especially in the recently colonized regions. In the USA, the mermithid *Psammomermis polozhentsev*, (Klein et al., 1976) has been reported as the first nematode parasitizing *P. japonica*, and recently a new species *Hexamermis popilliae* (Mazza et al., 2017) was described from *P. japonica* individuals collected in Italy. During a sampling campaign aiming to collect *P. japonica* individuals to study the changes of the associated microbiota throughout the host developmental stages (Chouaia et al. 2019), an individual was found colonized by nematodes. This study aims to identify the isolated nematodes using molecular taxonomy, evaluate its efficiency in suppressing individuals of an insect model organism. Furthermore, we characterized the microbiota associated with the nematode in order to look for potential bacterial symbionts with entomopathogenic activity.

2. Materials and methods

2.1. Collection of the *P. japonica* individuals, nematode isolation and manipulations

Nematodes were obtained from the body cavity of a third-instar larvae specimen of P. japonica collected in Oleggio (45°36′ N, 08°38′ E, 230 m a.s.l. - Novara, Piedmont, Italy). The nematodes were isolated from infected *P. japonica* larvae and then reared using the last instar of Galleria mellonella (Lepidoptera: Pyralidae) as host, following the method described by Kaya and Stock (1997). The parasitized *G. mellonella* cadavers were rinsed in distilled water and placed in modified White' traps (White, 1927) at 24 °C ± 0.5 °C for two weeks. During that time, the emerging infective juveniles (IJs) were collected for

the following experiments. A suspension of the isolated IJs in sterilized distilled water was stored at 10 °C in order to obtain individuals for the in vivo test of "mortality". A sample of the isolated nematodes was stored in absolute ethanol for DNA extraction, molecular identification, and characterization of the associated microbiota.

2.2. DNA extraction and molecular identification of the nematode

After surface sterilization of about 100 nematodes (Montagna et al., 2015), total DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). A fragment of 890 base pairs of the 18S rRNA gene was amplified using the primers 18SF2/18SR2(Montagna et al., 2013) PCR amplification was performed in 25 µL reaction mix containing: 1X Taq reaction Buffer (10 mM Tris-HCl at pH 8.3, 50 mM KCl and 1.5 mM MgCl2), 0.2 mM of each deoxynucleotide triphosphate, 0.5 pmol of each primer, 0.6 U of GoTaq DNA Polymerase and 10 ng of template DNA. PCR thermal profile was as following: an initial denaturation of 3 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 52 °C and 1 min 20 s extension at 72 °C, with a final single extra extension step of 10 min at 72 °C. The obtained amplicon was Sanger sequenced and electropherograms assembled in a consensus sequence using Geneious R10 (Biomatters Ltd). The consensus sequence (accession number: MN263255) was subject to BLAST analysis and compared with sequences available in GenBank. Homologous sequences of close relatives' taxa, according to (Liu et al., 2012) were aligned using MAFFT with a Q-INS-i algorithm that considers the secondary structure of RNA (Katoh et al., 2017). The obtained aligned sequences were tested using jmodeltest2 (Darriba et al., 2012) to select the best model of nucleotide substitutions, which resulted to be the General time-reversible model (GTR) (Tavaré, 1986). The single-locus phylogeny was inferred under Maximum likelihood using PhyML 3.0 (Guindon et al., 2010) and the previously selected nucleotide substitution model, with 100 bootstrap replicates.

2.3. Entomopathogenic activity on *Galleria mellonella*

Given the technical difficulties involved in obtaining and breeding larvae of *P. japonica*, we decided to test the virulence of the nematode by using the last instar larvae of greater wax moth *G. mellonella* (L.), following the procedure described by (Torrini et al., 2015) Briefly, each moth larva was placed in a petri dish (3.5 cm diameter) with two layers of filter paper (Whatman No. 1) and inoculated with nematodes at two concentrations: C1 = 300 nematodes / 250 µl H2O (Treatment 1: T1) and C2 = 400 nematodes / 250 µl H2O (Treatment 2: T2). The control (C) consisted of moth larvae with 250 µl of distilled water. For each assay three replicates were performed, each replicate containing five individuals. Petri dishes

were stored at 24 °C ± 0.5 °C in darkness. The survival number of larvae for each trial was evaluated over a period of five days by counting dead individuals at a 24 h interval. The dead larvae were placed in modified White traps (White, 1927), after death, in order to recover the nematode infective juvenile stages (IJs) from the host cadaver. In order to test for differences between the treatments and the control, data collected on the number of individuals survived after five days were analyzed using the Kruskal-Wallis test in R (version 3.5.1).

2.4. Characterization of the nematode microbiota using 16S rRNA metabarcoding

V1-V2 and V4 regions of 16S rRNA gene were sequenced using Ion Torrent platform (Life Technologies). Firstly, the total DNA of the nematodes was used as template to amplify the V1-V2 region using the primers 27FYM (Frank et al., 2008) and 338R (Amann et al., 1990), and to amplify the V4 region using the primers 515F (Caporaso et al., 2011) and 802R (Claesson et al., 2009) and 806R (Caporaso et al., 2011). The PCR primers were tailed with two different GC rich sequences enabling barcoding in a second amplification. 16S rRNA V1-V2 PCR was performed in 20 µL volume reaction containing 8 µL of HotMasterMix 5 Prime 2.5X (Quanta Bio), 0.4 µL of BSA (20 µg/µL) (Sigma-Aldrich), 1 µL of EvaGreen™ 20X (Biotium), 0.8µL of 27FYM (10 µM) (5' modified with unitail 1 5'-GTGAGAGTTTGATYMTGGCTCAG -3'), 0.8 µL of 338R (10 µM) (5' modified with unitail 2 5'-GCTGCCTCCCGTAGGAGT -3'), and 1 µL (corresponding to 50 ng) of DNA template. Library were prepared accordingly to Chouaia et al. 2019 and sequencing was performed at Life Sciences Department of Trieste University, Italy. The obtained reads were analyzed using QIIME 2 version 2018.11 (Bolyen et al., 2019). After trimming, 16S rRNA sequences were clustered into Operational Taxonomic Units (OTUs) with a similarity cut-off of 97% using the de novo clustering method implemented in the q2-vsearch plugin (Rognes et al., 2016). Chimeras were identified and filtered using the uchime-denovo method implemented in q2-vsearch (Edgar et al., 2011). Taxonomic assignment of OTU representative sequences was performed using q2-feature-classifier (McDonald et al., 2012) and adopting Greengenes 13.8 as reference database. The 16S rRNA reads were deposited in the Sequence Read Archive (SRA) of NCBI with accession number PRJNA564721

3. Results

3.1. Molecular characterization of the nematode

The 897 bp long 18S rRNA fragment amplified from the nematode DNA was assigned to the Rhabditidae family based on a BLAST identity of 100%. The sequence showed an identity of 100% with sequences

from *Oscheius myriophilous* and *Rhabditis myriophila* (synonymized with *O. myriophilous* by Sudhaus (2011)). Interestingly, the sequence identity was high (99.5%) even with *Oscheius microvilli* NJAU-N19 (accession number: KT825913). The phylogenetic Maximum Likelihood tree inferred on the 18S rRNA sequence dataset confirmed the assignment of the isolated nematode to the genus *Oscheius* (bootstrap value of 95%) (Figure 1).

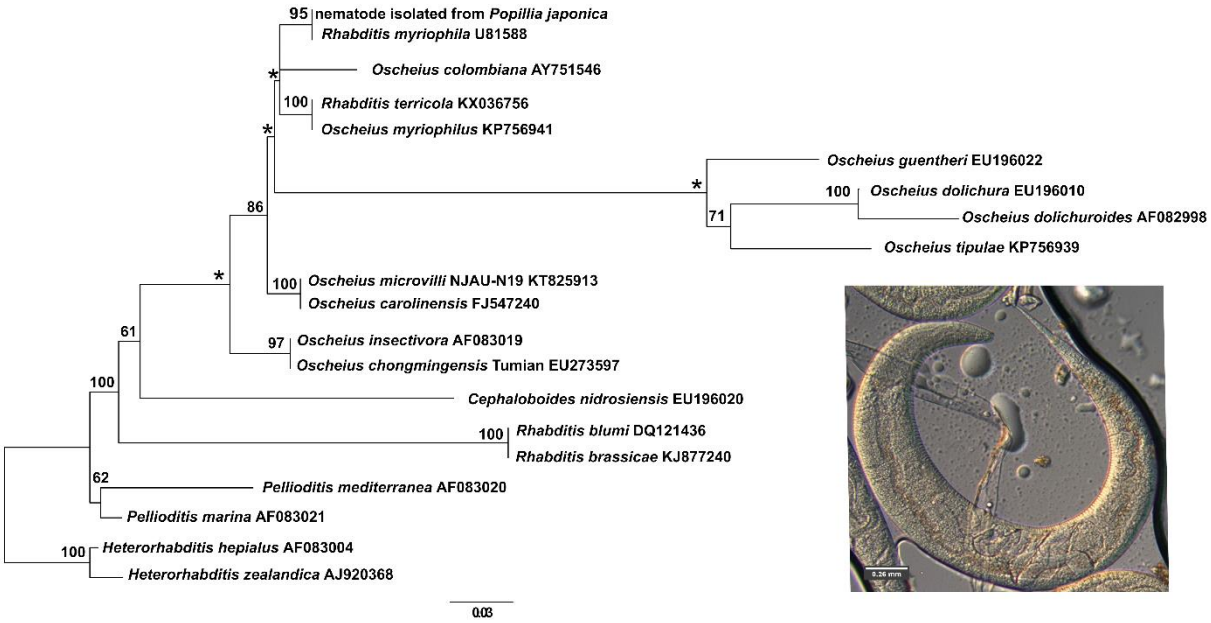

Figure 1: A. Maximum Likelihood tree based on 18S rRNA sequences obtained with PhyML 3.0. Bootstrap values above 50 are shown at the branch points, black asterisks indicate bootstrap values lower than 50. B. The isolated nematode captured with scanning light microscope Olympus BX50 optical with BX-Pol simple polarizing.

3.2. Entomopathogenic activity on *Galleria mellonella*

In order to investigate the possibility to use the isolated nematode as a biocontrol agent, its virulence activity has been evaluated using the model organism *G. mellonella*. After the inoculation of the insects, their survival was monitored for five days. In the end, six and seven larvae (corresponding to 40% and 46% of the individuals involved in T1 and T2, respectively) survived for the two tested nematode concentrations. In contrast, 14 individuals (corresponding to 93%) survived in the control experiment (Figure 2). IJs were recovered from the insect host cadaver using White traps, nine and eight larvae (corresponding to 60% and 54% of the individuals involved in T1 and T2, respectively) mortality for the two tested nematode concentrations. In contrast, one individual (corresponding to 7%) mortality in the

control experiment. The results of the Kruskal-Wallis test indicate a significant difference in term of survival among the groups ($\alpha 2 = 6.79$, df = 2, p-value = 0.0336; Table 1 Post-hoc test (Tukey's HSD) showed a difference between control and both treatments while no difference has been detected between the two treatments (Table 1).

Table 1: Statistical analysis. A) Kruskal-Wallis test among T1, T2 and control groups. B) Tukey HSD tests among the different treatments T1, T2, and control.

A) Kruskal-Wallis test

	Survival score
Chi-square	6.79
df	2
Assymp.sig.	0.0336

B) Post-hoc Tukey HSD

(I) TREATMENTS		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	Upper Bound
T1	T2	-0.333	0.385	0.420	-1.28	0.61
	C	-2.667*	0.385	0.000	-3.61	-1.72
T2	T1	0.333	0.385	0.420	-0.61	1.28
	C	-2.333*	0.385	0.001	-3.28	-1.39
C	T1	2.667*	0.385	0.000	1.72	3.61
	T2	2.333*	0.385	0.001	1.39	3.28

3.3. Taxonomic composition of the microbial community of the nematode

A total of 139,245 bacterial 16S rRNA sequences of the regions V1-V2 and V4 were obtained from the DNA isolated from the nematodes. The microbiota associated with the nematode consisted of 1188 bacterial OTUs, obtained clustering the reads at 97% of sequence similarity. The nematode's bacterial community was characterized by values of Shannon and Pielou indices of 2 and 0.2, respectively indicating low diversity and evenness. The most abundant taxa in the microbiota were represented by the phylum Proteobacteria 99% (1106 OTUs), within this phylum, 50.7% of the 16S rRNA reads were assigned to the family of Brucellaceae (259 OTUs) and 43.6% assigned to unclassified family belong to order Rhizobiales (400 OTUs). At the genus level, the bacterial community associated with the nematode

is dominated by *Ochrobactrum* 49.7% (184 OTUs) followed by an unclassified genus belonging to the order Rhizobiales 43.2% (400 OTUs) and Pseudomonas 4.2% (85 OTUs) (Figure 3).

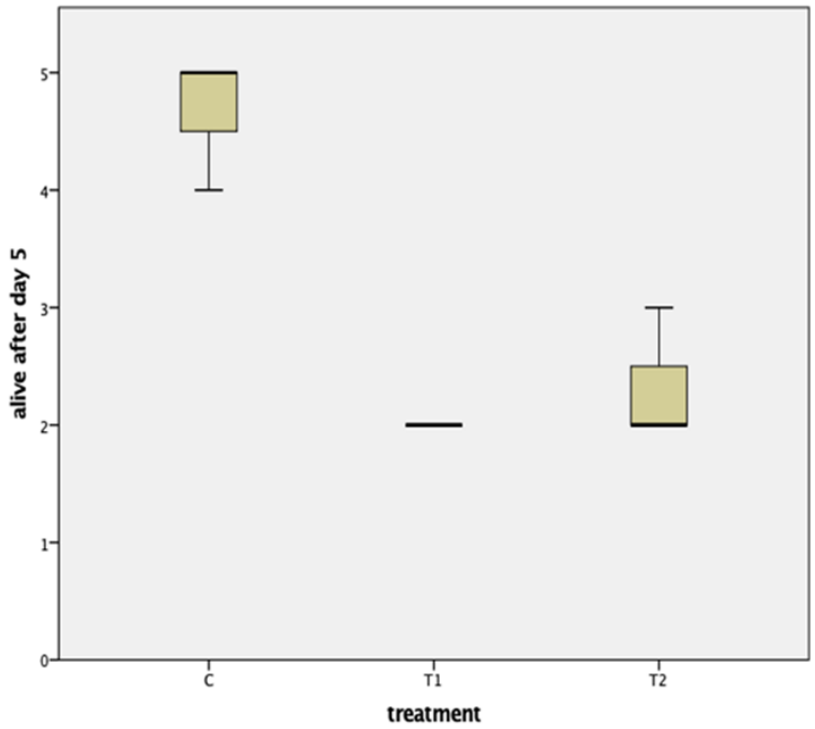

Figure 2: Boxplot of the survival numbers of *G. mellonella* for each treatment after 5 days

A single OTU constitutes 95% of the reads assigned to the unclassified genus in the order Rhizobiales, so we used the representative sequence of this OUT to perform a search with BLAST on the NCBI database and with the classifier tool of RDP (Ribosomal Database Project, https://rdp.cme.msu.edu). Blast analysis assigns the selected sequence to the genus *Ochrobactrum*, while RDP classifier to unclassified Brucellaceae.

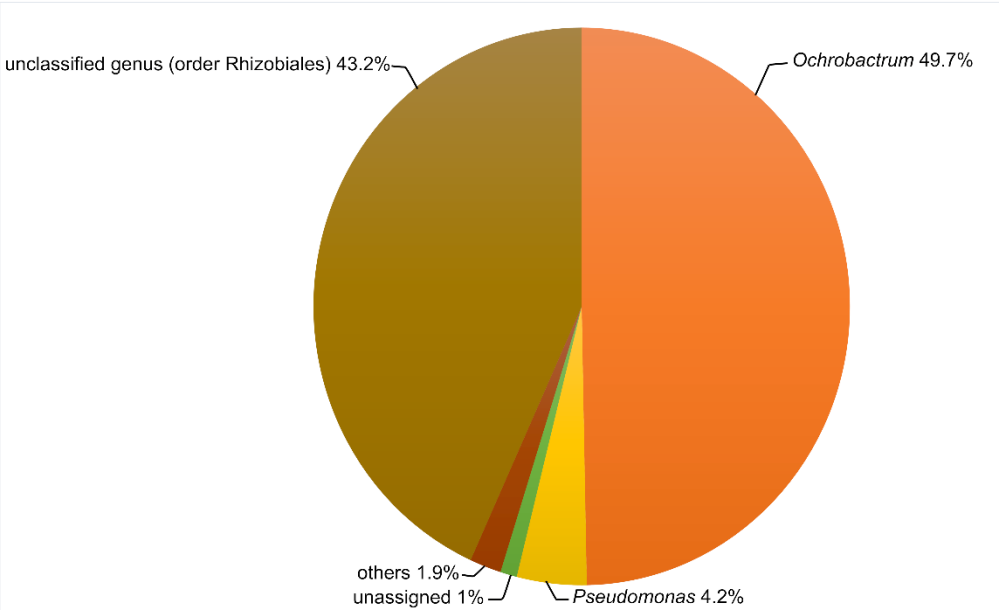

Figure 3 The composition and relative abundances of bacterial genera associated with the nematode isolated from third-instar larvae of *P. japonica*

4. Discussion

The present study reports the detection of a nematode associated with the third instar larvae of *P. japonica*, a polyphagous beetle recently reported from north Italy. Our analyses (i.e., BLAST and phylogenetic inference) clearly assigned the nematode to Rhabditidae family. The obtained sequence showed a similarity of 100% with *Rhabditis myriophila*, which was isolated for the first time from the millipede *Oxidis gracilis* (Poinar, 1986). Based on the phylogenetic tree, the nematode isolated from *P. japonica* clusters, even if with low support value, in a group that includes *Rhabditis myriophila* and *Oscheius colombiana*, the latter previously reported as a necromenic nematode (Stock et al., 2005).

Even considering the low sample size of *G. mellonella* larvae, the significant difference between control and trials (T1 and T2) and the amount of individuals killed by nematodes (more than 50%) let us to consider, in agreement with Dillman et al. (2012), that the isolated nematode is an entomopathogen. But because virulence and mortality were not very high (i.e. mortality within 48h less than 100%), our nematode cannot be considered as entomopathogenic.

In order to further investigate the pathogenicity of the nematode, we characterized the associated bacterial community that resulted in extremely unbalanced (see Pielou index). This microbiota is dominated by bacterial OTUs assigned to the genus *Ochrobactrum*, Gram-negative bacteria belonging to the family Brucellaceae (Holmes et al., 1988). Species in this genus have been defined as opportunistic

pathogens in humans (Lebuhn et al., 2006). In a previous study, *Ochrobactrum anthropi* has been reported to be associated with the nematode *Steinernema scapterisci* (Aguillera and Smart Jr, 1993); in addition, *Ochrobactrum* sp was isolated from dead larvae of *G. mellonella* infected by *Steinernema* sp. and *Heterorhabditis* sp. (Razia et al., 2011). The pathogenic role of bacteria of the genus *Ochrobactrum* was recently demonstrated in the case of *Oscheius chongmingensis* (Fu and Liu, 2019) on *G. mellonella* larvae. Further studies on *P. japonica* larvae must be performed in order to test the possibility to use this nematode as a biocontrol agent of this pest.

Declaration

NG designed and performed the experiments, carried out the microbiota and statistical analyses. MB helped with the microbiota analyses. NG, MM, and MB wrote the manuscript. All authors read and commented on the manuscript.

MM: Matteo Montagna; NG: Nizar Goda; MB: Matteo Brunetti

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Conclusion

In this PhD thesis, two research articles have been presented. The first article deals with the developmental stages and gut microenvironments and how these influence the gut microbiota dynamics in the invasive beetle *P. japonica* Newman (Coleoptera: Scarabaeidae). The results highlight the influence of different developmental stages of *P. japonica* (i.e., larvae, pupae, adults), gut compartments (i.e., foregut, midgut, hindgut) and physicochemical properties (i.e., pH, oxygen concentration, redox potential) in shaping the bacterial community associated with *P. japonica*. The results also reveal that the gut microbiota of *P. japonica* are highly dynamic across the developmental stages of the insect while changes in microbiota composition are strongly correlated with the physicochemical properties of the gut. Despite the microbiota's high variability, in our case, 89 OTUs were maintained from larvae to adults, including 35 OTUs originating from the soil environment. In the future, it would be worthwhile investigating whether these OTUs represent a stable core microbiota present in all *P. japonica* populations in different parts of the world or whether they are subject to change in different environments. In the first case, this might indicate a more intimate symbiotic relationship which is potentially maintained via vertical transmission. In the latter case, variable microbiota would provide a means to investigate the origins of new invasions of this beetle, via a comparative analysis of local soil and insect gut microbiotas.

In the second article, Potentially entomopathogenic nematode isolated from *Popillia japonica* (Coleoptera: Scarabaeidae): bioassay, molecular characterization and the associated microbiota. Our study of the isolated nematode suggests that it engages in entomopathogen activity. Further, it is probable that the bacteria of the genus *Ochrobactrum*, which is dominant in the isolated nematode, contributes to nematode entomopathogen activity, while the genus *Ochrobactrum* has been reported to be associated with the nematode *Oscheius chongmingensis* and described as a pathogenic bacterium. Further studies on *P. japonica* larvae must be performed in order to test the potential to use this nematode as a biocontrol agent against *P. japonica* larvae.

Annexes

Table S1: Summary of the different ecological indices and Random Forest results for each sample. 1a: Ecological indices summary for the different samples. 1b: summary statistics of the comparison of the different alpha diversity values between the different developmental stages. 1c: Standardized phylogenetic evenness results for all the samples. 1d: Results of the Random Forest goodness of prediction for the developmental stages. 1e: Results of the Random Forest goodness of prediction for the gut section. 1f: Top 10 OTU predictors of the Random Forest prediction for the developmental stages. 1g: Top 10 OTU predictors of the Random Forest prediction for the gut sections.

Table S1a: Ecological indices summary for the different samples

	Ecological indices								Sample information						
Sample.ID	Reads	Evenness.J	Observed OTUs	chao1	Shannon	mpd.obs	mpd.obs.z	mpd.obs.p	Sample	Stage	Development	Description	Oxygen	pH	Rx
A.1	239121	0.85	1103	1103.0	5.93	1.24	0.72	0.77	Soil	Soil	Soil	S.S03	M.An	NA	NA
A.2	62966	0.66	103	125.8	3.05	1.19	-1.99	0.02	Foregut	L1	Larvae	FL1.2	An	Sac.N	P
A.4	66147	0.61	116	143.1	2.91	1.27	2.59	0.99	Foregut	L2	Larvae	FL2.1	An	Sac.N	P
A.5	63160	0.81	51	96.0	3.18	1.09	-6.56	0.00	Foregut	L2	Larvae	FL2.2	An	Sac.N	P
A.6	281025	0.85	1104	1104.6	5.97	1.23	0.54	0.71	Soil	Soil	Soil	S.S02	M.An	NA	NA
A.7	73473	0.69	168	179.1	3.55	1.17	-2.62	0.01	Foregut	L3	Larvae	FL3.1	An	Sac.N	P
A.8	27298	0.81	92	137.5	3.68	1.16	-3.24	0.00	Foregut	L3	Larvae	FL3.2	An	Sac.N	P
A.9	87421	0.76	209	237.5	4.08	1.16	-2.93	0.00	Foregut	L3	Larvae	FL3.3	An	Sac.N	P
A.10	65649	0.46	308	343.5	2.62	1.09	-6.40	0.00	Foregut	Pupae	Pupae	FP1	NA	NA	NA
A.11	80611	0.26	264	342.6	1.44	1.09	-7.67	0.00	Foregut	Pupae	Pupae	FP2	NA	NA	NA
A.12	50309	0.42	167	181.1	2.14	1.10	-6.71	0.00	Foregut	Pupae	Pupae	FP3	NA	NA	NA
A.13	208802	0.55	131	169.0	2.68	1.18	-2.25	0.02	Foregut	Female	Adult	FF1	M.An	Sac.N	P
A.14	50611	0.69	73	82.0	2.94	1.15	-4.03	0.00	Foregut	Female	Adult	FF2	M.An	Sac.N	P
A.15	70563	0.61	92	162.0	2.74	1.36	6.85	1.00	Foregut	Female	Adult	FF3	M.An	Sac.N	P
A.16	173052	0.40	67	107.0	1.67	1.19	-1.86	0.03	Foregut	Male	Adult	FM1	M.An	Sac.N	P
A.17	150020	0.66	100	127.5	3.04	1.23	0.35	0.66	Foregut	Male	Adult	FM2	M.An	Sac.N	P
A.18	94199	0.63	78	96.0	2.75	1.16	-3.21	0.00	Foregut	Male	Adult	FM3	M.An	Sac.N	P
A.19	92418	0.84	1087	1102.8	5.86	1.24	0.81	0.79	Soil	Soil	Soil	S.L02	M.An	NA	NA
A.20	65945	0.80	536	553.2	5.01	1.17	-2.41	0.00	Midgut	L1	Larvae	ML1.1	An	Sal.Al	P.N

A.21	52620	0.31	271	293.0	1.74	1.17	-2.88	0.00	Midgut	L1	Larvae	ML1.2	An	Sal.Al	P.N
A.22	43506	0.23	234	308.0	1.26	1.12	-5.85	0.00	Midgut	L1	Larvae	ML1.3	An	Sal.Al	P.N
A.23	153143	0.74	406	436.4	4.47	1.17	-2.87	0.01	Midgut	L2	Larvae	ML2.1	An	Sal.Al	P.N
A.24	86996	0.58	388	421.1	3.48	1.19	-1.41	0.07	Midgut	L2	Larvae	ML2.2	An	Sal.Al	P.N
A.25	44219	0.76	399	438.1	4.58	1.17	-2.00	0.03	Midgut	L2	Larvae	ML2.3	An	Sal.Al	P.N
A.26	54507	0.50	336	377.0	2.91	1.16	-3.12	0.00	Midgut	L3	Larvae	ML3.1	An	Sal.Al	P.N
A.27	133142	0.68	446	500.3	4.13	1.20	-0.83	0.20	Midgut	L3	Larvae	ML3.2	An	Sal.Al	P.N
A.28	42291	0.59	444	466.0	3.61	1.15	-3.99	0.00	Midgut	L3	Larvae	ML3.3	An	Sal.Al	P.N
A.29	88155	0.62	155	176.1	3.15	1.23	0.34	0.64	Midgut	Pupae	Pupae	MP1	NA	NA	NA
A.30	64478	0.71	177	204.6	3.68	1.20	-0.90	0.19	Midgut	Pupae	Pupae	MP2	NA	NA	NA
A.31	61468	0.24	57	87.6	0.97	1.05	-8.38	0.00	Midgut	Pupae	Pupae	MP3	NA	NA	NA
A.32	25003	0.53	99	109.1	2.44	1.20	-1.11	0.14	Midgut	Female	Adult	MF1	M.An	Sac.N	P
A.33	4200	0.69	108	139.0	3.21				Midgut	Female	Adult	MF2	M.An	Sac.N	P
A.34	81477	0.57	114	125.0	2.69	1.21	-0.52	0.30	Midgut	Female	Adult	MF3	M.An	Sac.N	P
A.35	65210	0.48	124	138.9	2.34	1.20	-1.06	0.14	Midgut	Male	Adult	MM1	M.An	Sac.N	P
A.36	32241	0.40	110	133.3	1.89	1.27	2.76	1.00	Midgut	Male	Adult	MM2	M.An	Sac.N	P
A.37	95526	0.15	119	134.3	0.70	1.21	-0.54	0.30	Midgut	Male	Adult	MM3	M.An	Sac.N	P
A.38	85127	0.84	1086	1095.7	5.90	1.24	0.79	0.79	Soil	Soil	Soil	S.L03	M.An	NA	NA
A.39	70876	0.79	467	515.0	4.85	1.14	-3.82	0.00	Hindgut	L1	Larvae	HL1.1	An	Sal.Al	N
A.40	69861	0.79	428	439.7	4.77	1.13	-4.31	0.00	Hindgut	L1	Larvae	HL1.2	An	Sal.Al	N
A.41	34469	0.60	405	461.2	3.60	1.15	-3.52	0.00	Hindgut	L1	Larvae	HL1.3	An	Sal.Al	N
A.42	68171	0.69	380	423.6	4.12	1.10	-5.83	0.00	Hindgut	L2	Larvae	HL2.1	An	Sal.Al	N
A.43	46103	0.65	384	425.1	3.85	1.13	-4.65	0.00	Hindgut	L2	Larvae	HL2.2	An	Sal.Al	N
A.44	68380	0.62	428	443.5	3.77	1.12	-5.00	0.00	Hindgut	L2	Larvae	HL2.3	An	Sal.Al	N
A.45	48207	0.76	438	461.2	4.61	1.12	-4.82	0.00	Hindgut	L3	Larvae	HL3.1	An	Sal.Al	N
A.46	66680	0.76	449	486.3	4.65	1.14	-4.37	0.00	Hindgut	L3	Larvae	HL3.2	An	Sal.Al	N
A.47	85606	0.75	488	511.0	4.66	1.15	-3.61	0.00	Hindgut	L3	Larvae	HL3.3	An	Sal.Al	N
A.48	94727	0.48	115	157.2	2.29	1.30	4.11	1.00	Hindgut	Pupae	Pupae	HP1	NA	NA	NA
A.49	118290	0.75	485	507.1	4.63	1.14	-3.90	0.00	Hindgut	Pupae	Pupae	HP2	NA	NA	NA
A.50	98553	0.33	107	170.3	1.53	1.29	3.40	1.00	Hindgut	Pupae	Pupae	HP3	NA	NA	NA
A.51	120548	0.15	89	132.9	0.69	1.25	1.51	0.94	Hindgut	Female	Adult	HF1	An	Sac.N	P
A.52	71254	0.31	88	126.8	1.37	1.28	3.20	1.00	Hindgut	Female	Adult	HF2	An	Sac.N	P

A.53	80937	0.84	1081	1100.0	5.89	1.24	0.79	0.80	Soil	Soil	Soil	S.L01	M.An	NA	NA
A.54	68441	0.45	58	73.0	1.81	1.23	0.65	0.76	Hindgut	Male	Adult	HM1	An	Sac.N	P
A.55	101540	0.49	125	163.2	2.35	1.24	0.77	0.78	Hindgut	Male	Adult	HM2	An	Sac.N	P
A.56	90060	0.51	105	185.1	2.36	1.21	-0.89	0.19	Hindgut	Male	Adult	HM3	An	Sac.N	P
A.57	46457	0.86	1089	1093.3	6.00	1.24	0.72	0.76	Soil	Soil	Soil	S.S01	M.An	NA	NA
A.58	217476	0.83	1099	1100.0	5.80	1.24	0.97	0.84	Soil	Soil	Soil	S.G01	M.An	NA	NA
A.59	185582	0.83	1095	1097.1	5.80	1.24	0.75	0.78	Soil	Soil	Soil	S.G02	M.An	NA	NA
A.60	106769	0.83	1090	1094.5	5.78	1.24	0.86	0.81	Soil	Soil	Soil	S.G03	M.An	NA	NA
A.3	102	Discarded from the analysis							Foregut	L1	Larvae	FL1.1			
A.61	153								Foregut	L1	Larvae	FL1.3			
A.62	61								Foregut	L2	Larvae	FL2.3			
A.63	88								Hindgut	Female	Adult	HF3			

Table S1b: summary statistics of the comparison of the different alpha diversity values between the different developmental stages

Chao1							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Soil	Pupae	1082.501798	7.830083925	203.0319504	115.7137037	21.44811465	0.006
Pupae	Adult	203.0319504	115.7137037	97.39862475	21.82822369	3.396642699	0.0024
Soil	Adult	1082.501798	7.830083925	97.39862475	21.82822369	125.4019062	0.003
Larvae	Adult	338.3247125	135.7024115	97.39862475	21.82822369	6.862847782	0.002
Pupae	Larvae	203.0319504	115.7137037	338.3247125	135.7024115	-2.569658864	0.019
Soil	Larvae	1082.501798	7.830083925	338.3247125	135.7024115	15.93539819	0.0015
Female	Male	98.33746702	12.08211736	96.66841411	27.06110334	0.142029539	0.881
L1	L2	360.256321	138.105805	313.9175706	137.0120867	0.606096265	0.637
L3	L1	342.9620319	129.0763338	360.256321	138.105805	-0.241174867	0.86415
L3	L2	342.9620319	129.0763338	313.9175706	137.0120867	0.422571113	0.73832

Shannon							
Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Soil	Pupae	8.459207841	0.109892337	3.59397799	1.587678914	8.646649424	0.006
Pupae	Adult	3.59397799	1.587678914	3.106080339	1.02723025	0.892734316	0.383
Soil	Adult	8.459207841	0.109892337	3.106080339	1.02723025	14.9472582	0.003
Larvae	Adult	5.436230703	1.328253554	3.106080339	1.02723025	5.783054921	0.002
Pupae	Larvae	3.59397799	1.587678914	5.436230703	1.328253554	-3.254223855	0.0048
Soil	Larvae	8.459207841	0.109892337	5.436230703	1.328253554	6.609100149	0.0015
Female	Male	3.20499638	1.131904389	3.029145641	0.930532568	0.318905817	0.768
L1	L2	4.999210114	2.038305506	5.471288349	0.796233669	-0.56274107	0.66426
L3	L1	5.744973254	0.815326204	4.999210114	2.038305506	0.935048959	0.4725
L3	L2	5.744973254	0.815326204	5.471288349	0.796233669	0.656091588	0.60783

Pielou's evenness

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value	
Soil		Pupae	0.84223784	0.009931439	0.489211091	0.177865044	5.605134566	0.0
Pupae	Adult	0.489211091	0.177865044	0.493008675	0.156630284	-0.053113795	0.967	
Soil	Adult	0.84223784	0.009931439	0.493008675	0.156630284	6.408544651	0.003	
Larvae	Adult	0.678520689	0.147621501	0.493008675	0.156630284	3.703061759	0.002	
Pupae	Larvae	0.489211091	0.177865044	0.678520689	0.147621501	-3.000468782	0.0098	
Soil	Larvae	0.84223784	0.009931439	0.678520689	0.147621501	3.221969633	0.0045	
Female	Male	0.502860707	0.170898481	0.485345984	0.144097502	0.207879222	0.93726	
L1	L2	0.606685953	0.220865572	0.702216051	0.079920336	-1.062189792	0.40163	
L3	L1	0.713329607	0.093644112	0.606685953	0.220865572	1.221183615	0.381	
L3	L2	0.713329607	0.093644112	0.702216051	0.079920336	0.245659527	0.9555	

Table S1c: Phylogenetic evenness results for all the samples

Sample.ID	ntaxa	mpd.obs	mpd.rand.mean	mpd.rand.sd	mpd.obs.rank	mpd.obs.z	mpd.obs.p	runs	TYPE
A.1	1069	1.24	1.224998049	0.01910839	771	0.72	0.771	999	Soil
A.2	94	1.19	1.223392229	0.01911408	21	-1.99	0.021	999	Larvae
A.4	103	1.27	1.220348955	0.019943422	993	2.59	0.993	999	Larvae
A.5	45	1.09	1.223285385	0.019678181	1	-6.56	0.001	999	Larvae
A.6	1070	1.23	1.224238416	0.018109501	708	0.54	0.708	999	Soil
A.7	154	1.17	1.223077556	0.020224879	5	-2.62	0.005	999	Larvae
A.8	92	1.16	1.223978527	0.019339371	1	-3.24	0.001	999	Larvae
A.9	194	1.16	1.216117261	0.019499561	1	-2.93	0.001	999	Larvae
A.10	263	1.09	1.219091439	0.019921445	1	-6.40	0.001	999	Pupae
A.11	188	1.09	1.224790822	0.01698258	1	-7.67	0.001	999	Pupae
A.12	145	1.10	1.224237651	0.018186714	1	-6.71	0.001	999	Pupae
A.13	105	1.18	1.22352891	0.018739785	18	-2.25	0.018	999	Adult
A.14	70	1.15	1.225135277	0.017546134	1	-4.03	0.001	999	Adult
A.15	78	1.36	1.221470351	0.019629761	1000	6.85	1.000	999	Adult

A.16	47	1.19	1.223623057	0.019301206	27	-1.86	0.027	999	Adult
A.17	89	1.23	1.222837043	0.019382224	655	0.35	0.655	999	Adult
A.18	70	1.16	1.222656289	0.018483361	2	-3.21	0.002	999	Adult
A.19	1040	1.24	1.223147954	0.018703168	787	0.81	0.787	999	Soil
A.20	496	1.17	1.218911906	0.020307971	4	-2.41	0.004	999	Larvae
A.21	246	1.17	1.223768896	0.017878027	3	-2.88	0.003	999	Larvae
A.22	199	1.12	1.22530258	0.017911295	1	-5.85	0.001	999	Larvae
A.23	317	1.17	1.22173292	0.019648021	5	-2.87	0.005	999	Larvae
A.24	333	1.19	1.221180993	0.019722925	71	-1.41	0.071	999	Larvae
A.25	372	1.17	1.214662845	0.019853694	30	-2.00	0.030	999	Larvae
A.26	295	1.16	1.221733762	0.019497624	1	-3.12	0.001	999	Larvae
A.27	329	1.20	1.2159206	0.019742335	200	-0.83	0.200	999	Larvae
A.28	418	1.15	1.221719319	0.019201677	1	-3.99	0.001	999	Larvae
A.29	137	1.23	1.223678119	0.018433699	639	0.34	0.639	999	Pupae
A.30	153	1.20	1.216701906	0.01947816	186	-0.90	0.186	999	Pupae
A.31	43	1.05	1.222589994	0.020061834	1	-8.38	0.001	999	Pupae
A.32	99	1.20	1.221660361	0.020469177	137	-1.11	0.137	999	Adult
A.34	100	1.21	1.224283254	0.018700553	303	-0.52	0.303	999	Adult
A.35	100	1.20	1.222754527	0.019544391	143	-1.06	0.143	999	Adult
A.36	105	1.27	1.222795514	0.01858017	996	2.76	0.996	999	Adult
A.37	82	1.21	1.22425593	0.018914097	299	-0.54	0.299	999	Adult
A.38	1040	1.24	1.223644621	0.018418923	787	0.79	0.787	999	Soil
A.39	400	1.14	1.218928915	0.019568467	1	-3.82	0.001	999	Larvae
A.40	381	1.13	1.215869191	0.020770471	1	-4.31	0.001	999	Larvae
A.41	382	1.15	1.223469924	0.020002427	1	-3.52	0.001	999	Larvae
A.42	327	1.10	1.216060006	0.020161597	1	-5.83	0.001	999	Larvae
A.43	345	1.13	1.21990318	0.019605401	1	-4.65	0.001	999	Larvae
A.44	374	1.12	1.218423408	0.019096862	1	-5.00	0.001	999	Larvae
A.45	410	1.12	1.220097489	0.020441644	1	-4.82	0.001	999	Larvae
A.46	398	1.14	1.21908588	0.019115423	1	-4.37	0.001	999	Larvae
A.47	433	1.15	1.217751599	0.019519297	1	-3.61	0.001	999	Larvae
A.48	94	1.30	1.216451261	0.019663502	1000	4.11	1.000	999	Pupae

A.49	421	1.14	1.217291644	0.019364028	1	-3.90	0.001	999	Pupae
A.50	88	1.29	1.223917391	0.019465915	999	3.40	0.999	999	Pupae
A.51	64	1.25	1.220561834	0.019925206	938	1.51	0.938	999	Adult
A.52	63	1.28	1.215405951	0.019613898	999	3.20	0.999	999	Adult
A.53	1049	1.24	1.222996139	0.018974483	795	0.79	0.795	999	Soil
A.54	51	1.23	1.222359483	0.018855426	761	0.65	0.761	999	Adult
A.55	85	1.24	1.223088933	0.019097587	784	0.77	0.784	999	Adult
A.56	75	1.21	1.223222475	0.019219002	194	-0.89	0.194	999	Adult
A.57	1069	1.24	1.223292201	0.018280359	755	0.72	0.755	999	Soil
A.58	1052	1.24	1.223008079	0.0199464	841	0.97	0.841	999	Soil
A.59	1044	1.24	1.223453769	0.018702035	783	0.75	0.783	999	Soil
A.60	1038	1.24	1.221209034	0.017738817	807	0.86	0.807	999	Soil

Table S1d: Results of the Random Forest goodness of prediction for the developmental stages

Developmental Stages				
True\Predicted	Adult	Larva	Pupae	Class error
Adult	17	0	0	0
Larva	2	22	0	0.08333333
Pupae	3	1	5	0.44444444

Table S1e: Results of the Random Forest goodness of prediction for the gut section

Tissue				
True\Predicted	Foregut	Midgut	Hindgut	Class error
Foregut	12	2	1	0.2
Midgut	3	14	1	0.22222222
Hindgut	2	1	14	0.17647059

Table S1f: Top predictors of the Random Forest prediction for the developmental stages

OTU	Taxonomy	Mean decrease in accuracy	Standard deviation
denovo128	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Sporomusa;s__	0.024240572	0.006190346
denovo86	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;Other	0.022890248	0.003232444
denovo260	k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__DA101;s__	0.016280397	0.004885278
denovo87	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;s__	0.014643432	0.003207957
denovo8	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__;s__	0.013637245	0.00227108
denovo151	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae;g__Ochrobactrum;s__	0.013514063	0.004185545
denovo178	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__;s__	0.01241389	0.002348696
denovo44	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;g__;s__	0.011356038	0.003135629
denovo118	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea;s__	0.010559095	0.002379024
denovo103	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__garvieae	0.00999746	0.004511545

Table S1g: Top predictors of the Random Forest prediction for the gut sections

OTU	Taxonomy	Mean decrease in accuracy	Standard deviation
denovo107	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__;s__	0.010475506	0.001541429
denovo115	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__;s__	0.009820355	0.00147533
denovo192	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia;s__	0.009113767	0.001699496
denovo187	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other;Other;Other	0.007708588	0.002360486
denovo197	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae;g__;s__	0.007431072	0.002075327
denovo166	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium;s__	0.006434858	0.001414202
denovo53	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Dysgonomonas;s__	0.005906622	0.001748072
denovo112	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae;g__;s__	0.005865706	0.00215959
denovo123	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__;s__	0.005505662	0.00176574
denovo153	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__;s__	0.005375653	0.00188553

Table S2: Indval results indicating the OTUs specific for each developmental stage and gut section. 2a: Indval report for the specific OTUs per each developmental stage 2b: Indval report for the specific OTUs per each gut section for each developmental stage.

Table S2a: Indval report for the specific OTUs for each developmental stage

Adults

OTU	taxonomy	stat	p.value	sig
denovo59022	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	1	0.0002	***
denovo191415	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	1	0.0002	***
denovo208739	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Sporomusa;g__Sporomusa	1	0.0002	***
denovo42733	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	1	0.0002	***
denovo96903	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__garvieae	0.997	0.0003	***
denovo16752	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__garvieae	0.996	0.0012	**
denovo147379	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.994	0.0003	***
denovo58178	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.993	0.0003	***
denovo136898	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__garvieae	0.992	0.0011	**
denovo130402	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.976	0.0005	***
denovo10335	p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;f__Erysipelotrichaceae;f__Erysipelotrichaceae	0.913	0.0097	**
denovo216185	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus;s__garvieae	0.913	0.007	**
denovo137035	p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;f__Leuconostocaceae;f__Leuconostocaceae	0.909	0.0087	**

Pupae

OTU	taxonomy	stat	p.value	sig
denovo28586	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Promicromonosporaceae;g__Xylanimicrobium;g__Xylanimicrobium	0.984	0.0009	***
denovo232450	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;f__Alcaligenaceae;f__Alcaligenaceae	0.978	0.0023	**
denovo73867	p__Spirochaetes;c__[Brachyspirae];o__[Brachyspirales];f__Brachyspiraceae;f__Brachyspiraceae;f__Brachyspiraceae	0.974	0.0013	**
denovo57436	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.949	0.0071	**
denovo23145	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae;g__Desulfarculus;g__Desulfarculus	0.933	0.0096	**

Larvae

OTU	taxonomy	stat	p.value	Sig
denovo226576	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;o__Myxococcales;o__Myxococcales;o__Myxococcales	0.999	0.0002	***

denovo87524	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.998	0.0002	***
denovo2553	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;f__Micromonosporaceae;f__Micromonosporaceae	0.998	0.0002	***
denovo6980	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.996	0.0002	***
denovo100405	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.991	0.0007	***
denovo87797	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.989	0.0009	***
denovo20960	p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;f__Pirellulaceae;f__Pirellulaceae	0.985	0.0004	***
denovo210223	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.983	0.0052	**
denovo138198	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.982	0.0028	**
denovo54307	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.98	0.0002	***
denovo40332	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.966	0.0006	***
denovo40216	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.96	0.0099	**
denovo254912	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;f__Veillonellaceae;f__Veillonellaceae	0.954	0.002	**
denovo254001	p__TM6;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4	0.943	0.0087	**
denovo3805	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales	0.943	0.003	**
denovo73591	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.943	0.0023	**
denovo207564	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.943	0.0025	**
denovo101713	p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__CandidatusXiphinematobacter;g__CandidatusXiphinematobacter	0.943	0.0063	**
denovo138451	p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces;g__Planctomyces	0.943	0.0068	**
denovo252701	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.942	0.001	***
denovo196201	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.939	0.0039	**
denovo217513	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.936	0.0033	**
denovo204875	p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;f__Rhodospirillaceae;f__Rhodospirillaceae	0.935	0.0035	**

Table S2b: Indval report for the specific OTUs per each gut section for each developmental stage

Larvae
Foregut

OTU	taxonomy	stat	p.value	Sig
denovo31511	p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;f__Enterobacteriaceae;f__Enterobacteriaceae	0.965	0.0052	**
denovo68994	p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Vibrio;s__fortis	0.707	0.0092	**

Larvae

Midgut

OTU	taxonomy	stat	p.value	sig
denovo104514	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales	1	1.00E-004	***
denovo20904	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Pseudonocardia;g__Pseudonocardia	0.999	1.00E-004	***
denovo165291	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;o__Myxococcales;o__Myxococcales;o__Myxococcales	0.999	1.00E-004	***
denovo251138	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;g__Dactylosporangium;g__Dactylosporangium	0.999	1.00E-004	***
denovo177896	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Polyangiaceae;f__Polyangiaceae;f__Polyangiaceae	0.998	1.00E-004	***
denovo226576	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;o__Myxococcales;o__Myxococcales;o__Myxococcales	0.998	1.00E-004	***
denovo49029	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium;g__Hyphomicrobium	0.996	1.00E-004	***
denovo49488	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;f__Micromonosporaceae;f__Micromonosporaceae	0.995	1.00E-004	***
denovo1068	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.995	1.00E-004	***
denovo195891	p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter;g__Kaistobacter	0.994	0.0002	***
denovo69066	p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__DA101;g__DA101	0.994	1.00E-004	***
denovo7668	p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;o__MIZ46;o__MIZ46;o__MIZ46	0.993	1.00E-004	***
denovo176985	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;f__Nocardioidaceae;f__Nocardioidaceae	0.993	1.00E-004	***
denovo242177	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Frankiaceae;f__Frankiaceae;f__Frankiaceae	0.993	1.00E-004	***
denovo63026	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia;g__Devosia	0.993	0.0004	***
denovo119600	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;f__Micromonosporaceae;f__Micromonosporaceae	0.993	0.0002	***
denovo130180	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae	0.993	1.00E-004	***
denovo185751	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;g__Labrys;g__Labrys	0.992	0.0002	***
denovo195898	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium;g__Mesorhizobium	0.992	0.0002	***
denovo12776	p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;f__Pirellulaceae;f__Pirellulaceae	0.992	1.00E-004	***
denovo96024	p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;f__Xanthomonadaceae;f__Xanthomonadaceae	0.992	1.00E-004	***
denovo185131	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.992	1.00E-004	***
denovo164667	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;o__Gaiellales;o__Gaiellales;o__Gaiellales	0.991	0.0007	***
denovo25187	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales	0.99	0.0003	***

denovo65601	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.988	1.00E-004	***
denovo162734	p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;f__Rhodospirillaceae;f__Rhodospirillaceae	0.988	0.0012	**
denovo92124	k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__CandidatusNitrososphaera;s__SCA1170	0.987	1.00E-004	***
denovo168599	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium;g__Pedomicrobium	0.987	1.00E-004	***
denovo59578	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;f__Micrococcaceae;f__Micrococcaceae	0.986	1.00E-004	***
denovo206106	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;o__Gaiellales;o__Gaiellales;o__Gaiellales	0.986	1.00E-004	***
denovo68704	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales	0.986	0.0008	***
denovo221095	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;f__Bradyrhizobiaceae;f__Bradyrhizobiaceae	0.985	0.0002	***
denovo81621	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.984	1.00E-004	***
denovo20960	p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;f__Pirellulaceae;f__Pirellulaceae	0.984	1.00E-004	***
denovo128536	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales	0.984	0.0002	***
denovo2553	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;f__Micromonosporaceae;f__Micromonosporaceae	0.984	0.0006	***
denovo47194	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales	0.984	0.0007	***
denovo233993	p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__CandidatusXiphinematobacter;g__CandidatusXiphinematobacter	0.983	1.00E-004	***
denovo161667	p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;g__Bacillus	0.983	1.00E-004	***
denovo27206	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;f__Phyllobacteriaceae;f__Phyllobacteriaceae	0.983	0.0002	***
denovo67301	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium;g__Hyphomicrobium	0.982	0.0003	***
denovo43013	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales	0.981	1.00E-004	***
denovo159667	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;g__Rhodoplanes	0.981	0.0007	***
denovo180124	p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter;g__Kaistobacter	0.981	1.00E-004	***
denovo131411	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;f__Nocardioidaceae;f__Nocardioidaceae	0.98	0.0007	***
denovo81154	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Cellulomonas;s__xylanilytica	0.979	0.0005	***
denovo113106	p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus;g__Amaricoccus	0.976	1.00E-004	***
denovo233806	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nakamurellaceae;f__Nakamurellaceae;f__Nakamurellaceae	0.976	0.0009	***
denovo149616	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.976	0.0003	***
denovo84573	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;f__Nocardioidaceae;f__Nocardioidaceae	0.976	0.0002	***
denovo255772	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;f__Nocardioidaceae;f__Nocardioidaceae	0.974	0.0002	***
denovo196969	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales;o__Rhizobiales	0.973	0.0002	***
denovo119602	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;g__Rhodoplanes	0.973	0.0005	***
denovo21254	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;g__Rhodoplanes	0.972	0.0007	***
denovo164050	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides;g__Nocardioides	0.97	0.0019	**
denovo151856	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.97	1.00E-004	***

denovo74879	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas;g__Balneimonas	0.969	0.0006	***
denovo195654	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium;g__Hyphomicrobium	0.966	0.0007	***
denovo133571	p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae;f__Gaiellaceae;f__Gaiellaceae	0.957	0.0007	***
denovo28600	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales	0.953	0.0007	***
denovo103546	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae;f__Haliangiaceae;f__Haliangiaceae	0.945	0.0023	**
denovo183166	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae;f__Micromonosporaceae;f__Micromonosporaceae	0.943	0.0003	***
denovo99985	p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__A17;g__A17	0.943	0.0002	***
denovo254001	p__TM6;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4	0.942	0.0006	***
denovo135040	p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;f__Sinobacteraceae;f__Sinobacteraceae	0.941	1.00E-004	***
denovo175036	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;o__Myxococcales;o__Myxococcales;o__Myxococcales	0.941	0.0024	**
denovo62001	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae;g__Pseudonocardia;g__Pseudonocardia	0.94	0.0088	**
denovo205967	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae;f__Kineosporiaceae;f__Kineosporiaceae	0.94	0.0012	**
denovo68095	p__Proteobacteria;c__Betaproteobacteria;c__Betaproteobacteria;c__Betaproteobacteria;c__Betaproteobacteria;c__Betaproteobacteria	0.94	1.00E-004	***
denovo184693	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptosporangiaceae;f__Streptosporangiaceae;f__Streptosporangiaceae	0.939	0.0087	**
denovo215187	p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111;f__C111;f__C111	0.938	0.005	**
denovo101713	p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__CandidatusXiphinematobacter;g__CandidatusXiphinematobacter	0.938	0.0008	***
denovo122337	p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;f__Isosphaeraceae;f__Isosphaeraceae	0.937	0.0002	***
denovo198574	k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__CandidatusNitrososphaera;s__SCA1170	0.936	0.007	**
denovo204875	p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;f__Rhodospirillaceae;f__Rhodospirillaceae	0.935	0.0007	***
denovo29015	p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;f__Rhodobacteraceae;f__Rhodobacteraceae	0.935	0.0016	**
denovo43886	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Conexibacteraceae;f__Conexibacteraceae;f__Conexibacteraceae	0.934	0.0029	**
denovo162818	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales	0.934	0.0049	**
denovo10568	p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;o__Myxococcales;o__Myxococcales;o__Myxococcales	0.934	0.0045	**
denovo104399	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales;o__Actinomycetales	0.932	0.0013	**
denovo3571	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae;f__Xanthobacteraceae;f__Xanthobacteraceae	0.932	0.0007	***
denovo160052	p__Firmicutes;c__Bacilli;o__Bacillales;f__Thermoactinomycetaceae;f__Thermoactinomycetaceae;f__Thermoactinomycetaceae	0.93	0.0025	**
denovo167561	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae	0.929	0.0004	***
denovo46413	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.926	0.0013	**
denovo137837	p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus;s__chondroitinus	0.926	0.0027	**
denovo45210	p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus;g__Paenibacillus	0.923	0.0069	**
denovo23784	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae;f__Peptostreptococcaceae;f__Peptostreptococcaceae	0.921	0.0014	**
denovo207860	p__Actinobacteria;c__Actinobacteria;o__Micrococcales;o__Micrococcales;o__Micrococcales;o__Micrococcales	0.914	0.0038	**

denovo163582	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;f__Comamonadaceae;f__Comamonadaceae	0.907	0.0041	**
denovo222762	p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Paenibacillus;g__Paenibacillus	0.905	0.0029	**
denovo138451	p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces;g__Planctomyces	0.88	0.0023	**
denovo258073	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;g__Methylobacterium	0.878	0.0087	**
denovo117538	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae;f__Hyphomicrobiaceae	0.877	0.0015	**
denovo158921	p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae;g__Turicibacter;g__Turicibacter	0.876	0.0055	**
denovo242617	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Kaistia;g__Kaistia	0.875	0.0088	**
denovo241705	p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;f__Xanthomonadaceae;f__Xanthomonadaceae	0.871	0.0056	**
denovo86047	p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales;o__Solirubrobacterales	0.868	0.0081	**
denovo253642	Unassigned	0.865	0.0039	**
denovo79763	p__Firmicutes;c__Bacilli;o__Bacillales;f__Thermoactinomycetaceae;f__Thermoactinomycetaceae;f__Thermoactinomycetaceae	0.862	0.0087	**
denovo194434	p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas;s__mexicana	0.816	0.0019	**
denovo232450	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;f__Alcaligenaceae;f__Alcaligenaceae	0.816	0.0019	**
denovo6720	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium;g__Agrobacterium	0.815	0.0041	**
denovo30581	p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;f__Coxiellaceae;f__Coxiellaceae	0.815	0.0085	**
denovo159854	p__TM6;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4;c__SJA-4	0.815	0.0076	**
denovo98547	p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae;g__Methylobacterium;s__adhaesivum	0.813	0.0026	**
denovo63618	p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;f__Coxiellaceae;f__Coxiellaceae	0.812	0.0088	**

Larvae Hindgut OTU	taxonomy	stat	p.value	Sig
denovo133835	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.997	1.00E-004	***
denovo122447	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.997	1.00E-004	***
denovo95170	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.995	1.00E-004	***
denovo82487	Unassigned	0.993	1.00E-004	***
denovo153025	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.993	1.00E-004	***
denovo261856	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.993	1.00E-004	***
denovo99993	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desulfovibrio;g__Desulfovibrio	0.993	1.00E-004	***
denovo205006	Unassigned	0.992	1.00E-004	***
denovo149993	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Dehalobacter_Syntrophobotulus;g__Dehalobacter_Syntrophobotulus	0.992	1.00E-004	***
denovo2301	p__Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus;g__Opitutus	0.991	1.00E-004	***
denovo42127	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.991	1.00E-004	***

denovo248751	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.99	1.00E-004	***
denovo175690	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.989	1.00E-004	***
denovo39568	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.988	0.0002	***
denovo55062	p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__PSB-M-3;g__PSB-M-3	0.987	1.00E-004	***
denovo138428	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.984	0.0002	***
denovo126908	Unassigned	0.984	1.00E-004	***
denovo197620	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.984	1.00E-004	***
denovo5575	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.983	1.00E-004	***
denovo144495	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.982	1.00E-004	***
denovo242921	p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;f__Coriobacteriaceae;f__Coriobacteriaceae	0.982	1.00E-004	***
denovo68179	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae;f__Dehalobacteriaceae;f__Dehalobacteriaceae	0.981	1.00E-004	***
denovo236976	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.981	1.00E-004	***
denovo18020	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.98	1.00E-004	***
denovo259853	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.98	0.0002	***
denovo41808	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.979	1.00E-004	***
denovo94998	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.978	1.00E-004	***
denovo118186	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;f__Veillonellaceae;f__Veillonellaceae	0.978	1.00E-004	***
denovo62947	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.976	1.00E-004	***
denovo188794	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Dysgonomonas;g__Dysgonomonas	0.974	0.0004	***
denovo131285	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae;g__Dehalobacterium;g__Dehalobacterium	0.973	1.00E-004	***
denovo199228	p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;f__Erysipelotrichaceae;f__Erysipelotrichaceae	0.972	1.00E-004	***
denovo87524	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.972	0.0006	***
denovo88917	p__Cyanobacteria;c__4C0d-2;o__YS2;o__YS2;o__YS2;o__YS2	0.971	1.00E-004	***
denovo146932	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.971	1.00E-004	***
denovo108129	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.971	0.0004	***
denovo185329	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.969	0.0002	***
denovo143435	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.968	0.0002	***
denovo213936	p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae;f__Nitrosomonadaceae;f__Nitrosomonadaceae	0.968	1.00E-004	***
denovo158950	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.967	0.0002	***
denovo112684	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.967	1.00E-004	***
denovo222251	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.967	0.0003	***
denovo200280	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;f__Desulfovibrionaceae;f__Desulfovibrionaceae	0.966	1.00E-004	***

denovo229153	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.966	1.00E-004	***
denovo160517	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.963	0.0002	***
denovo124201	p__Deferribacteres;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae;g__Mucispirillum;g__Mucispirillum	0.962	0.0002	***
denovo147469	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.959	0.0005	***
denovo169849	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.956	0.0002	***
denovo136551	p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;f__Erysipelotrichaceae;f__Erysipelotrichaceae	0.955	0.0002	***
denovo127793	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.954	1.00E-004	***
denovo148356	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.953	1.00E-004	***
denovo153882	p__Proteobacteria;c__Deltaproteobacteria;c__Deltaproteobacteria;c__Deltaproteobacteria;c__Deltaproteobacteria;c__Deltaproteobacteria	0.952	1.00E-004	***
denovo78067	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Dysgonomonas;g__Dysgonomonas	0.952	0.0013	**
denovo151480	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.952	0.0002	***
denovo73359	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.951	1.00E-004	***
denovo117712	p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus;g__Bacillus	0.951	0.0012	**
denovo259057	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.948	0.0005	***
denovo217233	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.948	1.00E-004	***
denovo126955	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus;g__Coprococcus	0.947	0.0037	**
denovo153442	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.947	0.0004	***
denovo25672	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;f__Desulfovibrionaceae;f__Desulfovibrionaceae	0.947	1.00E-004	***
denovo59301	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.944	1.00E-004	***
denovo25640	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.944	0.0013	**
denovo13127	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.944	0.0012	**
denovo173388	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.943	0.0002	***
denovo230089	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.941	0.0002	***
denovo163925	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;f__Desulfovibrionaceae;f__Desulfovibrionaceae	0.941	0.0011	**
denovo137378	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;g__Ruminococcus	0.94	1.00E-004	***
denovo184318	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.939	1.00E-004	***
denovo67331	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.938	0.0004	***
denovo216333	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Proteiniclasticum;g__Proteiniclasticum	0.937	0.0003	***
denovo46870	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.936	1.00E-004	***
denovo17998	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.936	1.00E-004	***
denovo142822	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.935	0.0012	**
denovo184067	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.934	1.00E-004	***

denovo123216	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.934	0.0002	***
denovo187502	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.932	0.0019	**
denovo120961	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.932	0.0004	***
denovo135849	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.929	0.0003	***
denovo24626	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.928	0.0003	***
denovo129712	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.928	0.0007	***
denovo130706	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.927	0.0035	**
denovo127239	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.925	1.00E-004	***
denovo55168	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.924	0.0008	***
denovo9734	p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;f__Xanthomonadaceae;f__Xanthomonadaceae	0.921	1.00E-004	***
denovo88585	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.92	0.0003	***
denovo173993	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;f__Desulfovibrionaceae;f__Desulfovibrionaceae	0.92	0.0026	**
denovo145783	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.92	0.0005	***
denovo198782	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.92	0.0036	**
denovo109754	p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];f__[Mogibacteriaceae];f__[Mogibacteriaceae]	0.919	0.0002	***
denovo247950	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Pelotomaculum;g__Pelotomaculum	0.918	0.0014	**
denovo165044	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.918	0.0005	***
denovo200812	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.918	0.0007	***
denovo177004	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.917	0.0008	***
denovo96838	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__PW3;g__PW3	0.916	0.0008	***
denovo207564	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.912	0.0044	**
denovo107253	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.912	0.0046	**
denovo258817	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.911	0.0021	**
denovo196201	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.91	0.0051	**
denovo244579	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.91	0.0005	***
denovo3347	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.91	0.0014	**
denovo234535	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes;g__Anaerostipes	0.91	0.0007	***
denovo149112	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.909	0.0035	**
denovo43084	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.909	0.0009	***
denovo181638	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.908	0.0035	**
denovo6850	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.908	0.0007	***
denovo225907	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.907	0.0021	**

denovo128743	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.906	0.0026	**
denovo150305	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.905	0.0004	***
denovo113691	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Dysgonomonas;g__Dysgonomonas	0.904	0.0023	**
denovo220410	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.902	1.00E-004	***
denovo261215	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.899	0.0029	**
denovo257270	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.897	0.0039	**
denovo40332	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.896	0.0008	***
denovo87563	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.896	0.0045	**
denovo182100	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.895	0.0056	**
denovo54307	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.892	0.0005	***
denovo254912	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;f__Veillonellaceae;f__Veillonellaceae	0.889	0.0034	**
denovo244668	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;f__Oxalobacteraceae;f__Oxalobacteraceae	0.887	0.0021	**
denovo93372	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.886	0.0044	**
denovo197872	Unassigned	0.886	0.0019	**
denovo231777	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira;g__Oscillospira	0.885	0.0038	**
denovo40216	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.882	0.0028	**
denovo114424	p__Tenericutes;c__Mollicutes;o__RF39;o__RF39;o__RF39;o__RF39	0.881	0.0036	**
denovo56209	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.881	0.003	**
denovo231269	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.881	0.0091	**
denovo240210	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.881	0.0013	**
denovo140365	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.88	0.0015	**
denovo236364	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae;g__Desulfosporosinus;s__meridiei	0.879	0.0023	**
denovo212762	p__Cyanobacteria;c__4C0d-2;o__YS2;o__YS2;o__YS2;o__YS2	0.877	0.001	***
denovo4543	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.875	0.0017	**
denovo108343	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;f__Ruminococcaceae;f__Ruminococcaceae	0.874	0.0012	**
denovo57436	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.873	0.0017	**
denovo168776	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.872	0.0008	***
denovo69720	p__Cyanobacteria;c__4C0d-2;o__YS2;o__YS2;o__YS2;o__YS2	0.868	0.0026	**
denovo192196	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.867	0.0031	**
denovo156767	p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;f__Erysipelotrichaceae;f__Erysipelotrichaceae	0.867	0.0038	**
denovo175497	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.864	0.0065	**
denovo253981	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.859	0.0093	**

denovo18391	Unassigned	0.858	0.0029	**
denovo115642	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.855	0.0053	**
denovo252701	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.85	0.005	**
denovo229168	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae;f__Christensenellaceae;f__Christensenellaceae	0.837	0.0064	**
denovo164524	p__Firmicutes;c__Clostridia;o__Clostridiales;o__Clostridiales;o__Clostridiales;o__Clostridiales	0.832	0.0053	**
denovo159133	p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;f__Lachnospiraceae;f__Lachnospiraceae	0.821	0.0052	**

Adult

Foregut

OTU	taxonomy	stat	p.value	Sig
denovo27158	p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Brevibacillus;g__Brevibacillus	0.998	0.0009	***
denovo52639	p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;f__Caulobacteraceae;f__Caulobacteraceae	0.997	0.0008	***
denovo72506	p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;g__Sphingomonas	0.996	0.0004	***
denovo80767	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia;g__Delftia	0.982	0.0023	**
denovo238230	p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;g__Sphingomonas	0.922	0.0037	**
denovo112746	p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter;g__Rubrobacter	0.913	0.002	**
denovo126712	p__Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter;g__Rubrobacter	0.913	0.002	**
denovo175295	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__Janibacter;g__Janibacter	0.913	0.002	**
denovo197632	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter;g__Achromobacter	0.913	0.002	**
denovo28466	p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;f__Intrasporangiaceae;f__Intrasporangiaceae	0.913	0.0037	**
denovo248414	p__Cyanobacteria;c__4C0d-2;o__MLE1-12;o__MLE1-12;o__MLE1-12;o__MLE1-12	0.912	0.0018	**
denovo250095	p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329;o__Ellin329;o__Ellin329;o__Ellin329	0.912	0.0019	**
denovo237078	p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;f__Caulobacteraceae;f__Caulobacteraceae	0.911	0.01	**
denovo206270	p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter;g__Achromobacter	0.91	0.0051	**
denovo175703	p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Caulobacter;g__Caulobacter	0.909	0.0037	**

Adult

Hindgut

OTU	taxonomy	stat	p.value	sig
denovo117074	p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;f__Desulfovibrionaceae;f__Desulfovibrionaceae	0.894	0.0018	**
denovo143435	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.894	0.0018	**

denovo213936	p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae;f__Nitrosomonadaceae;f__Nitrosomonadaceae	0.894	0.0018	**
denovo5575	p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;f__Rikenellaceae;f__Rikenellaceae	0.894	0.0018	**
denovo13451	p__Euryarchaeota;c__Methanobacteria;o__Methanobacteriales;f__Methanobacteriaceae;g__Methanobrevibacter;g__Methanobrevibacter	0.865	0.0075	**

Table S3: presence-absence matrix of the enriched families for each sample.

Type	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil
Stage	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil
Development	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil
Description	S.G01	S.G02	S.G03	S.L01	S.L02	S.L03	S.S01	S.S02	S.S03
Sample	A.58	A.59	A.60	A.53	A.19	A.38	A.57	A.6	A.1
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0	0	0	0	0	0	0	0	0
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	0	0	0	0	0	0	0	0	0

Type	Foregut	Hindgut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	L1	L1	L1	L1	L1	L1	L1
Development	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae
Description	FL1.2	HL1.1	HL1.2	HL1.3	ML1.1	ML1.2	ML1.3
Sample	A.2	A.39	A.40	A.41	A.20	A.21	A.22
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	0	1	1	1	1	1	0
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	1	1	1	1	1	1	1

Type	Foregut	Foregut	Hindgut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	L2	L2	L2	L2	L2	L2	L2	L2
Development	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae

Description	FL2.1	FL2.2	HL2.1	HL2.2	HL2.3	ML2.1	ML2.2	ML2.3
Sample	A.4	A.5	A.42	A.43	A.44	A.23	A.24	A.25
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	1	1	1	1	1	1	1	1
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	0	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	1	1	1	1	1	1	1	1

Type	Foregut	Foregut	Foregut	Hindgut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	L3	L3	L3	L3	L3	L3	L3	L3	L3
Development	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae	Larvae
Description	FL3.1	FL3.2	FL3.3	HL3.1	HL3.2	HL3.3	ML3.1	ML3.2	ML3.3
Sample	A.7	A.8	A.9	A.45	A.46	A.47	A.26	A.27	A.28
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	1	0	1	1	1	1	1	1	1
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	0	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	1	0	1	1	1	1	1	1	1

Type	Foregut	Foregut	Foregut	Hindgut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae
Development	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae	Pupae
Description	FP1	FP2	FP3	HP1	HP2	HP3	MP1	MP2	MP3
Sample	A.10	A.11	A.12	A.48	A.49	A.50	A.29	A.30	A.31
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	1	1	1	1	1	1	1	0	1
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	1	1	1	0	1	1	1	0	1

Type	Foregut	Foregut	Foregut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	Female	Female	Female	Female	Female	Female	Female	Female
Development	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
Description	FF1	FF2	FF3	HF1	HF2	MF1	MF2	MF3
Sample	A.13	A.14	A.15	A.51	A.52	A.32	A.33	A.34
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	1	1	1	1	1	1	1	1
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	1	0	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0	0	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	1	0	0	0	0	1	1	0

Type	Foregut	Foregut	Foregut	Hindgut	Hindgut	Hindgut	Midgut	Midgut	Midgut
Stage	Male	Male	Male	Male	Male	Male	Male	Male	Male
Development	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
Description	FM1	FM2	FM3	HM1	HM2	HM3	MM1	MM2	MM3
Sample	A.16	A.17	A.18	A.54	A.55	A.56	A.35	A.36	A.37
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	1	0	1	0	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	1	1	1	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0	1	0	1	1	1	1	1	1
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	0	1	0	0	1	1	0	0	0