Spatio-temporal changes in the gut microbiota of *Popilla japonica* (Coleoptera: Scarabaeidae)

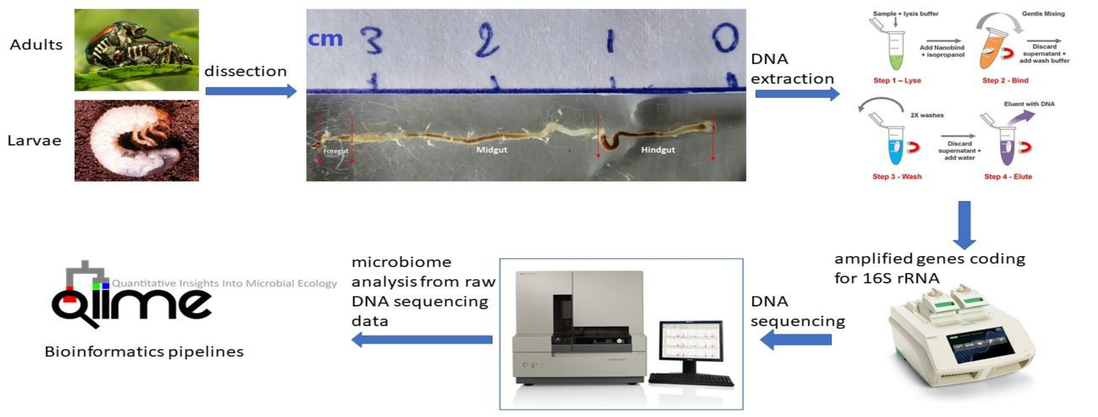
Nizar Goda[1], Bessem Chouaia[1], Elena Crotti[2], Elena Gonella[3], Leonardo Marianelli[4], Matteo Montagna[1]

[1] Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Milan, Italy; [2] Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente , Università degli Studi di Milano, Milano, Italy; [3] Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università di Torino, Grugliasco, Italy; [4]Consiglio per la ricerca e la sperimentazione in agricoltura, Research Centre for Agrobiology and Pedology, Cascine del Riccio, Italy Email: matteo.montagna@unimi.it

INTRODUCTION

The number of invasive insect species is increasing and once established, they have serious impacts on the environment and economy. *Popillia japonica* Newman (EPPO Code: POPIJA) (Coleoptera: Scarabaeidae), commonly known as the Japanese beetle, is a highly polyphagous beetle and an EPPO A2 pest (EPPO, 2006). Native to Japan and the far eastern Russian island of Kuril and has become an established pest in North America, the Azores and more recently in Europe. In 2014, was recorded for the first time on the European mainland when an outbreak was reported within the Ticino Valley Natural Park, Italy (EPPO, 2014). Insect-associated *P. japonica* bacteria have shown to play an important role in their host evolutionary success and adaptation to new environments and food resources. In this study, we investigated the microbiota associated with the three gut regions of different *P. japonica* developmental stages (i.e., larvae, pupae and adults) in order to address the following main biological questions: i) are the microbiotas associated with semaphoronts diverse? ii) are the bacterial communities associated with the three gut regions different? iii) Does the soil has an impact on shaping the bacterial community associated with different developmental stages of *P. japonica* (i.e. larvae, pupae and adults) ?

Experimental design

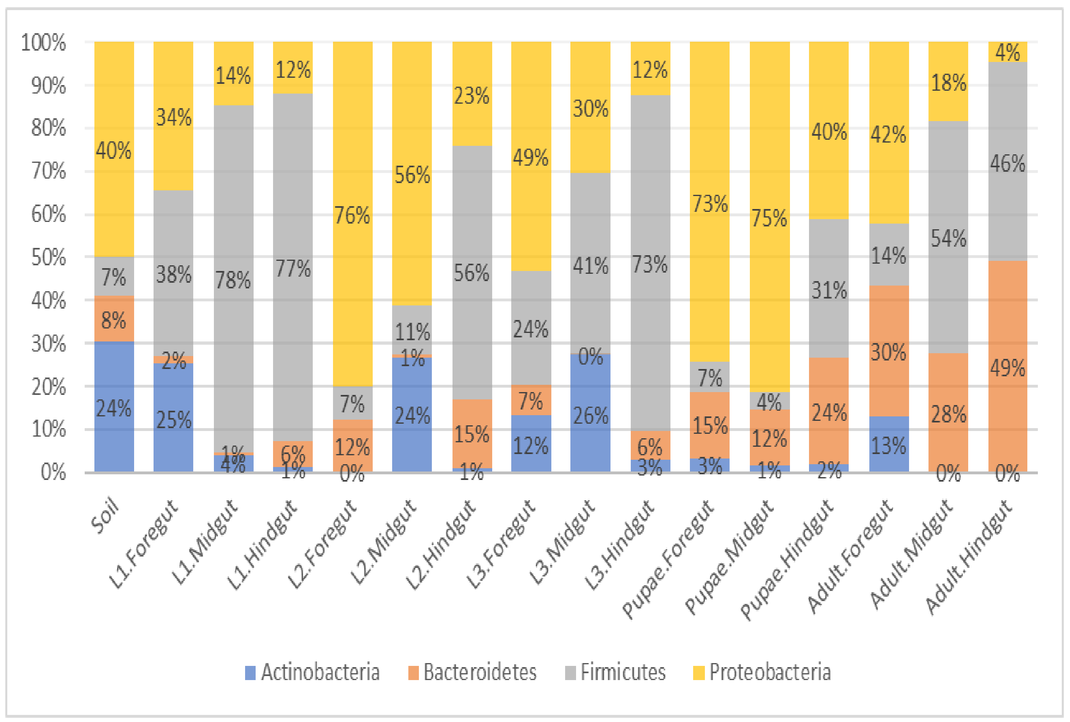


Sampling and sample preparation: insects were sampled in Oleggio (Lombardy, Italy) in the period 2016-2017. The different larval instars (i.e., L1, L2, L3), pupae and adults were dissected under sterile conditions to extract the gut. The organ was then split in its three compartments (i.e., foregut, midgut and hindgut). A sample, from which the DNA is extracted, consists of homologous gut regions extracted from five individuals at the same developmental stage.

DNA extraction and sequencing: Total DNA from each sample was extracted using a modified phenol–chloroform protocol (Mereghetti et al., 2017). The DNA was used a template for amplification by PCR of the bacterial 16S rRNA hypervariable V4 using primers 515F and 806R (Caporaso et al., 2011).

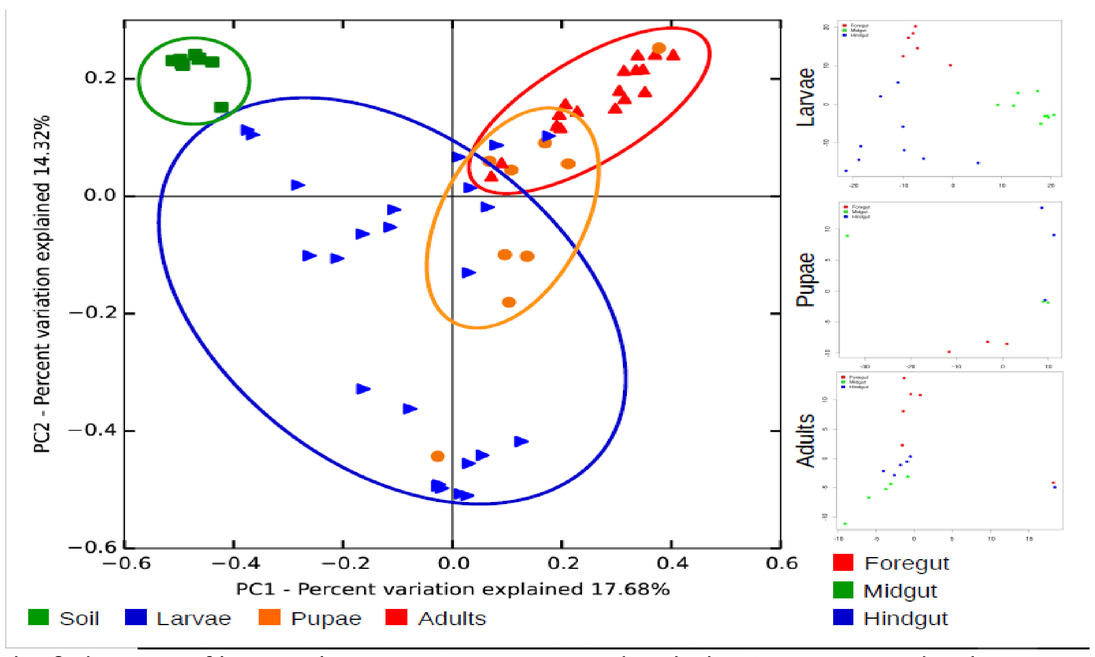
16S rRNA profiling and analysis: after library preparation, the amplicons were sequenced using the Ion Torrent platform at University of Trieste (Trieste, Italy). The resulting reads was analyzed using QIIME pipelines. The obtained OTU table was subjected to statistical analysis addressed the aims of the study. These analyses were carried out by the vegan package implemented in R packages.

Bacterial diversity associated with beetle (α-diversity)



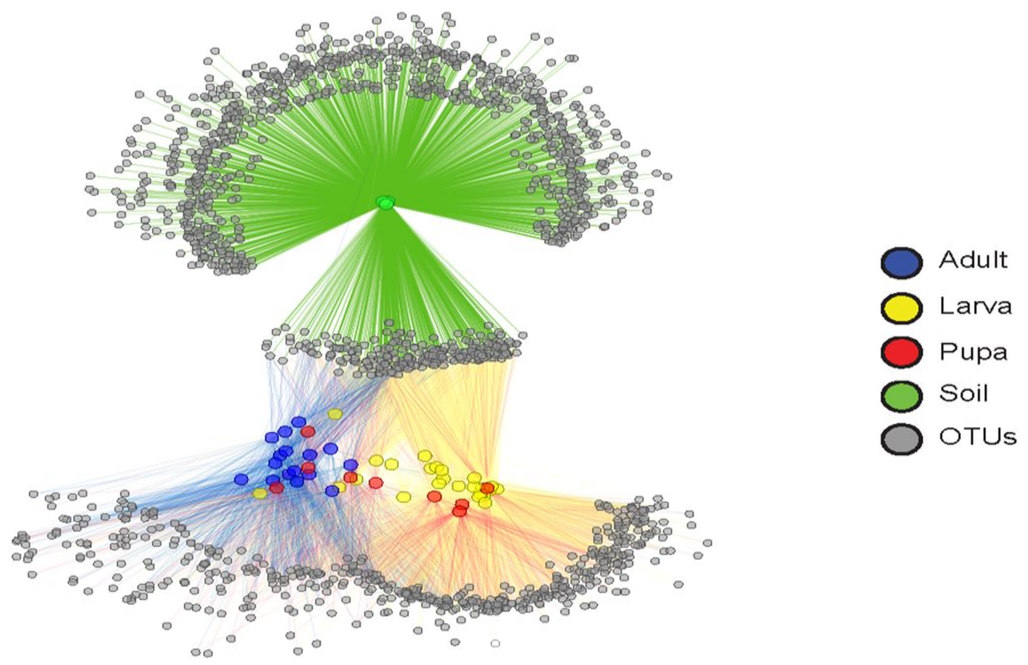
P. japonica harbors a complex and diverse gut microbiota (average 262 OTUs). Larvae resulted to harbor an high diversity of bacteria (289 OTUs), while the hindgut resulted to be the most diverse gut region (average 258 OTUs). Our preliminary analysis showed the absence of a core microbiota shared among the developmental stages. At phylum level, Proteobacteria resulted the most abundant taxon (~37%) followed by Firmicutes, Bacteroidetes and Actinobacteria (~36%, 14% and 9% respectively). At the genus level, *Ochrobactrum* dominated pupae foregut (~50%), *Sphingomonas* dominated both pupae midgut and pupae hindgut (respectively ~26% and ~20%) and *Bacteroides* dominated both Pupae hindgut and adult foregut (~20%).

β-diversity



The β-diversity of bacterial communities associated with the *P. japonica* and soil were investigated through a principal coordinates analysis (PCoA) carried on the Bray-Curtis dissimilarity matrix. The first two components explain a total of 32% of the variation (1st component, 17.68%; 2nd component, 14.3%). The PCoA analysis carried on the different samples showed that, based on their microbial composition, larvae clustered together, well separated from adults, on the other hand, pupae were spread in the continuum from larvae to adults . ANOSIM analysis supports statistically significant differences in bacterial community composition between soil and developmental stages (Unweighted UniFrac, ANOSIM R = 0.662, p = 0.001).

Network analysis



In order to investigate the impact of soil on shaping the bacterial community associated with different developmental stages of *P. japonica* (i.e. larvae, pupae and adults) , Bipartite network analysis for OTUs interaction was carried out. Our preliminary analysis showed that soil resulted to be the highest in term of OTUs number, few of them shared with different developmental stages. Analysis showed also that, based on their microbial composition, larvae clustered together, well separated from adults, on the other hand, pupae were spread in the continuum from larvae to adults.

Conclusion

In this study we demonstrates that : i) the developmental stage (i.e., larvae vs adults) have a great impact on shaping the bacterial community associated with *P. japonica;* ii) bacterial communities associated with soil and insects are different; iii) no core microbiota seems to be shared between the different developmental stages. Further investigation are required to characterize the microbiotas associated with adults from native and invasive areas in order to test for the presence of a stable core microbiota and check if there is a congruence between population genetic structure and similarity/dissimilarity in the microbiota structure.

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