Bacterial communities in gut of Thai Bumblebee (*Bombus haemorrhoidalis*) and their potential metabolic expression

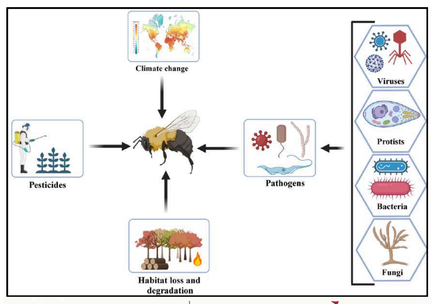
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Abstract

Bumblebees are key pollinators in highland ecosystems. However, population declines driven by pesticide exposure and disease threaten their ecological roles. The gut microbiome also plays a critical role in their health. Despite its importance, information regarding the gut bacterial communities of Thai bumblebees remains limited. This study characterized the gut microbiome of the Thai bumblebee *Bombus haemorrhoidalis* across larval and adult developmental stages using 16S rRNA gene amplicon sequencing. Taxonomic profiling revealed developmental stage–associated differences in community composition. The larval gut microbiota was dominated primarily by the genus *Lactobacillus*, whereas adult guts exhibited greater taxonomic diversity, with dominant phyla including Proteobacteria, Firmicutes, and Bacteroidota. Core genera detected in adults included *Gilliamella*, *Snodgrassella*, and *Apibacter*. Predicted functional profiling suggested stage-associated metabolic differentiation. Larval microbiomes showed relative enrichment of pathways related to amino acid and nucleotide biosynthesis. In contrast, adult microbiomes exhibited greater representation of alternative carbohydrate degradation pathways, phosphotransferase system–associated sugar transport enzymes. These patterns are consistent with increased representation of Gram-negative symbionts and metabolic versatility associated with adult foraging behavior. Collectively, these findings provide foundational insight into the gut microbiome of *B. haemorrhoidalis* and highlight ontogenetic shifts in microbial community structure and functional capacity.

Introduction



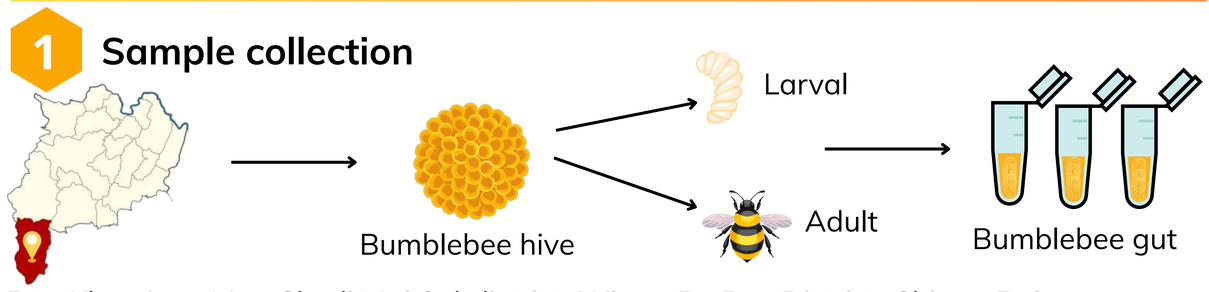
Bumblebees are key pollinators that contribute to ecosystem stability and agricultural productivity. However, their populations have been gradually declining worldwide due to several factors, particularly pathogen infections. In Thailand, research on bumblebees, especially their gut microbiome, remains limited. Because gut microorganisms play crucial roles in host nutrition, immunity, and development, this study investigated the gut microbial diversity and community composition of bumblebees at the larval and adult stages using 16S rRNA gene sequencing. The results provide baseline information that may support future studies on bumblebee health and conservation.

Objectives

- To determine the composition, diversity, and core identity of the bacterial communities in the gut of Thai bumblebees.
- To assess and compare the developmental dynamics of the gut bacterial community between larval and adult stages.
- To evaluate ecological relationships and the functional potential of the gut microbiome in relation to host health.

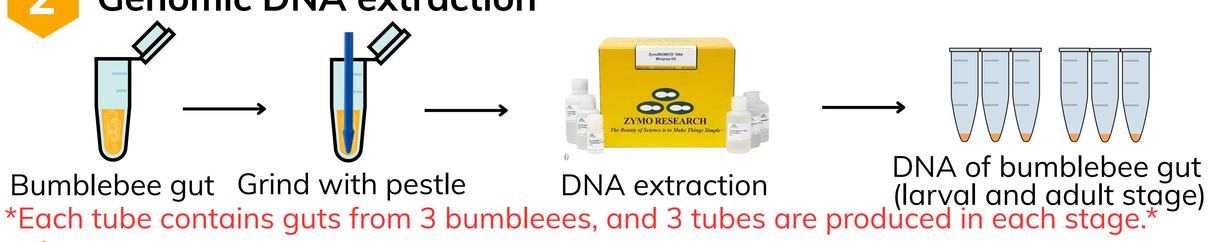
Methodology

1 Sample collection



Ban Khun Lao, Mae Chedi Mai Subdistrict, Wiang Pa Pao District, Chiang Rai.

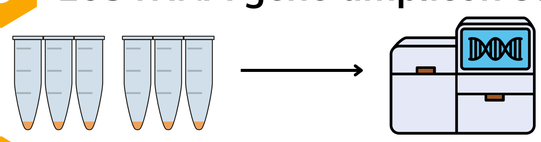
2 Genomic DNA extraction



Bumblebee gut → Grind with pestle → DNA extraction → DNA of bumblebee gut (larval and adult stage)

Each tube contains guts from 3 bumbleees, and 3 tubes are produced in each stage.

3 16S rRNA gene amplicon sequencing



Next-Generation Sequencing (NGS) → Illumina MiSeq

4 Bioinformatic and statistical analyses

QIIME2 → Taxonomy, Alpha & Beta diversity

R Studio → Data visualization

PICRUSt2 → Functional prediction

Result

1 Alphararefraction curve

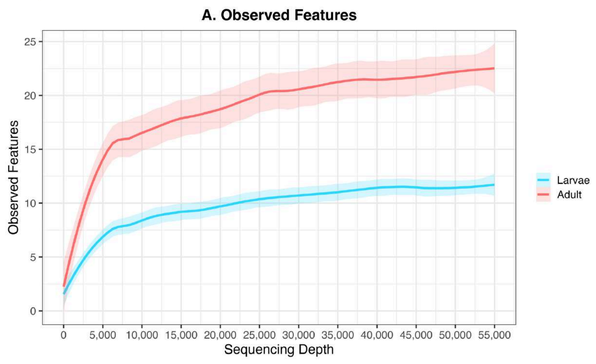


Figure1: Rarefaction curves showing observed microbial features in larvae and adult samples across sequencing depths.

2 Taxonomy

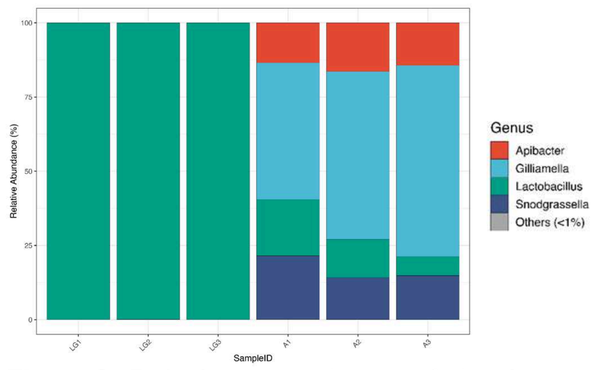


Figure2: Relative abundance of dominant bacterial genera across larval and adult gut samples.

3 Alpha diversity

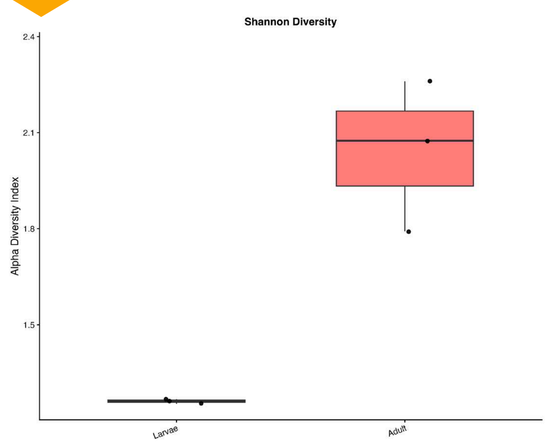


Figure3: Alpha diversity of microbial communities in larval and adult gut samples measured using the Shannon diversity index.

4 Beta diversity

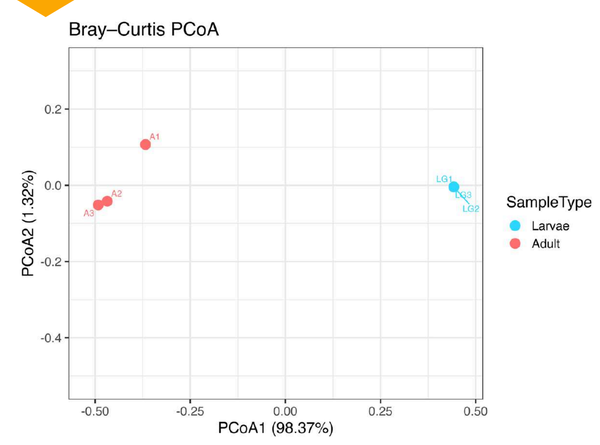


Figure4: Bray–Curtis PCoA comparison of microbial community composition between larval and adult gut samples.

5 Functional prediction

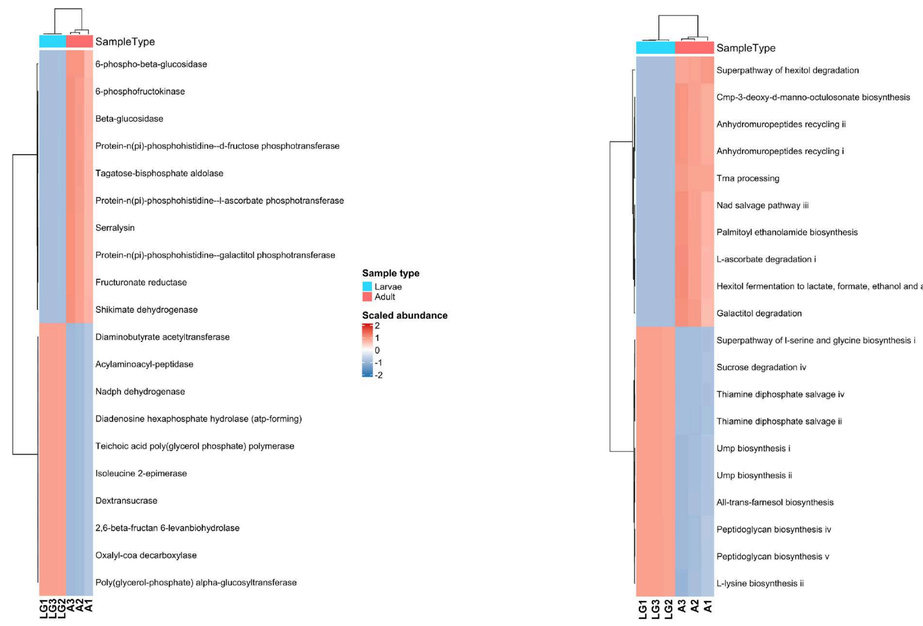


Figure5: Predicted enzyme functions inferred from 16S rRNA gene data, illustrating differences in metabolic enzyme abundance between larval and adult gut microbiomes.

Figure6: Predicted metabolic pathways highlighting stage-associated differences in functional potential between larval and adult gut microbiomes.

Conclusion

- This study provides baseline insight into the gut microbiome of Bombus haemorrhoidalis.
- Distinct taxonomic patterns were observed between larval and adult gut communities, with larvae dominated by Lactobacillus and adults showing greater microbial diversity.
- Functional predictedl profiling indicated stage-associated metabolic differentiation between developmental stages.
- These findings contribute to understanding bumblebee gut microbiome ecology and may support future studies on bumblebee health and conservation.

References

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