International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 17-06-2024, Accepted: 11-07-2024, Published: 03-08-2024
Volume 9, Issue 8, 2024, Page No. 9-16

Microbial partnerships: Exploring gut bacteria in *Eudocima* moths, unravelling differential gut microbiome composition of *E. materna* and *E. phalonia* (Family: Erebidae)

Monika Rathod[1], Ravindra Pathre[2*], Laxmikant Shinde[3], Sachin Jadhav[1], Yogesh Kayande[1]
[1] Department of Zoology, JES College, Jalna, Maharashtra, India
[2] Assistant Professor, Department of Zoology, Arts, Science and Commerce College, Jalna, Maharashtra, India
[3] Assistant Professor, Department of Zoology, JES College, Jalna, Maharashtra, India

Abstract
Eudocima materna and *Eudocima phalonia* is a commonly found moth species of the Erebidae family. At the same time, collection was done at nearby areas and fruit orchards of the Jalna district of Maharashtra. The study of gut microbiota was done on adults (EMA) and larvae (EML) of *Eudocima materna* and adults of *Eudocima phalonia* (EPA). The larva was reared at a laboratory that fed on *Tinospora Cordifolia* locally called gulvel, both the stages of *E. materna* and *E. Phalonia* were dissected in aseptic condition under laminar airflow, and gut samples were collected in liquid nitrogen. After 16s rRNA sequencing, we found that bacterial symbionts vary in both stages of the life cycle of *E. materna*. In adult *E. materna* 49% Wittichii of bacterial species of the genus Sphingomonas are found whereas in larva only 5% wittichii bacteria were observed. Larva of *E. materna* consists of 33% Komagatae bacterial species; on the other hand, the adult consists of 0% *M.* komagatae bacteria. Adult of *E. Phalonia* symbiotes 61% of *A.* woluwensis species of genus Arthobacter, 0% of Wittichii, and *E. materna* has 0% of woluwensis This is how we have compared gut microbiome based on 16s rRNA sequencing of gut bacteria.

Keywords: Erebidae family, *Eudocima materna*, *Eudocima phalonia*, adult stage, larva stage, gut microbiota, 16s rRNA sequencing

Introduction

Insects have co-evolved with diverse microorganisms in their gut, constituting communities that may affect the host's fitness or be helpful for the host's survival. Therefore, the identification of microbes and host physiological functions—such as development, physiology, ecological interactions, evolutionary diversity, and biochemical functions—is closely associated with their gut microbiota (Zhu, Z., Liu, Y., Hu, H., and Wang, 2022) [20]. The symbiotic relationship between insects and their gut bacteria is crucial; evidence shows a close association between particular groups of insects and the community of gut microbiota inhabiting their gut (Dillon & Dillon, 2004; Tang *et al*., 2012; Morrison *et al*., 2009; Mereghetti *et al*., 2017) [3, 11, 13, 15]. This study focuses on the adult stage of *Eudocima materna* (EMA), the larval stage of *Eudocima materna* (EML), and the adult stage of *Eudocima phalonia* (EPA). Research on gut-associated microbiota has highlighted the significant impact of microbes on host physiology. Symbiotic bacteria, in particular, play crucial roles in host development. Understanding the intricate interactions between bacterial communities and their hosts is essential for studying host-microbe relationships (Hammer *et al.*, 2017;Jia *et al*., 2021; Zhao *et al*., 2023) [6, 7, 19].

In many insects, gut bacteria fulfill functional roles by providing essential nutrients that the hosts lack through mutualistic relations. Indigenous bacteria in moths also modulate gene expression related to vital intestinal functions, including mucosal barrier fortification, nutrient absorption, and xenobiotic metabolism (Dillon & Dillon, 2004;Gong *et al*., 2020) [3, 5]. Most insects harbor gut microbiota that specifically participates in host physiology, supporting their polyphagous nature and providing essential nutrition. For instance, the gut microbiota of pest moths, such as *Spodoptera littoralis*, was investigated using classical 16S rRNA gene sequencing and microarray analysis (Tang *et al*., 2012; Mereghetti *et al*., 2017) [11, 15]. Notably, the genera *Clostridium* and *Enterococcus* (specifically *Enterococcus casseliflavus* and *Enterococcus mundtii*) were found to be the most abundant bacteria in the gut of *S. littoralis* (Tang *et al*., 2012) [15].

"This study contributes to our understanding of gut microbiomes across different species of moths in the Erebidae family, shedding light on the intricate relationships between these insects and their gut microbiota composition. The characterization was done using 16S rRNA gene sequencing and metagenomic technology. The moths were collected from nearby farms in the Jalna district of Maharashtra. *E. materna* is a highly damaging pest of fruit orchards (Ravindra Fakirrao Pathre and Sharad Devidasrao Jadhav, 2020) [14]. Additionally, there may be relative contributions from the host plant and other factors related to variation in microbial composition in these two moths. Some studies suggest that host plant fruits release chemical signals to attract moths, which have adapted chemoreceptors for these particular signals (Kayande *et al*., 2023) [8].

Materials and methods

1. Sample collection and DNA extraction

We have collected adult *E. materna* from fruit orchards of nearby places in the Jalna district of Maharashtra. Collected adults were carried to the laboratory, and identified morphologically using different keys available. Then after collecting their eggs kept them in a rearing cage until they hatched out of the egg, and fed them on freshly collected leaves of Menispermaceae until we got to the larval stage. Adults and larvae were dissected in aseptic conditions under laminar airflow to prevent external contaminations. Later words guts were collected in liquid nitrogen and kept freezing for further analysis. The samples were then sent to a known laboratory for metagenomics for DNA extraction and 16s rRNA gene amplification and sequencing.

2. 16s rRNA amplification and sequencing the raw sequencing

Data underwent preprocessing and quality control using the DADA2 pipeline in R. Initially, the necessary libraries were loaded, and the working directory was set to access the sequencing files. Forward and reverse reads were then extracted, and sample names were parsed. Subsequently, quality control and error correction were performed to filter out low-quality reads and correct sequencing errors. Dereplication and denoising were conducted to remove redundant sequences and infer amplicon sequence variants (ASVs) using DADA2's algorithm. Following dereplication and denoising, the denoised forward and reverse reads were merged to generate high-quality, overlapping sequences to enhance downstream analyses' accuracy. A sequence table was constructed to represent the abundance of each ASV across samples. Chimeric sequences were identified and removed to ensure dataset integrity. Taxonomy was assigned to the ASVs using a reference database, resolving duplicate taxa names and cleaning the taxonomic table. Finally, a phyloseq object was created to integrate the OTU table, sample metadata, and taxonomic information. Downstream analyses characterized microbial community composition and diversity, visualizing taxonomic composition through bar plots and generating sample-wise taxonomic tables. Alpha diversity was assessed using metrics like Shannon, Simpson, and Chao1 indices, while beta diversity was evaluated through Bray-Curtis or UniFrac distances, visualized using NMDS or PCoA plots. Differential abundance analysis identified taxa significantly differing in abundance between sample groups. Krona visualization explored microbial community taxonomic composition, providing comprehensive insights into microbial community structure and diversity.

3. Microbiome analysis report

Microbiome analysis is crucial for understanding the composition and function of microbial communities in various environments. In this report, we present an analysis pipeline for processing and analyzing microbiome data using the DADA2 and phyloseq packages in R.

3.1 Sample Information

Table 1

Sample ID (As on tube)	Sample Type (Choose from the dropdown)	Species	Genus
EMA	RRSID - 2099	Eudocima Materna	Eudocima
EML	RRSID - 2100	Eudocima Materna	Eudocima
EPA	RRSID - 2101	Eudocima phalonia	Eudocima

3.2 Data Acquisition and Preprocessing

We began by setting up the working directory and loading the required R libraries, including dada2, tidyverse, psadd, vegan and phyloseq. Fastq files containing raw sequencing data were retrieved and processed to extract sample names. Quality control and error correction were performed on the raw sequences to ensure data integrity and accuracy

3.3 Dereplication and Sample Inference

Dereplication was carried out to collapse identical sequences into unique sequences, followed by sample inference using the DADA2 algorithm. This step allowed us to identify and correct errors in sequencing data, resulting in high-resolution sequence variants (ASVs).

3.3.1 Merging Paired Reads

Paired-end reads were merged to generate consensus sequences, reducing spurious sequence variants and improving data quality.

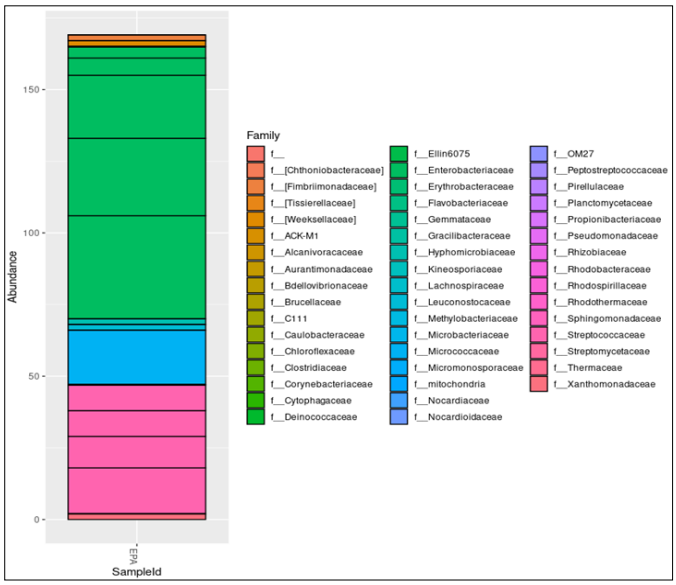
3.3.2 Constructing Sequence Table

A sequence table was constructed to represent the abundance of each ASV across samples, providing a higher-resolution view of microbial community composition compared to traditional OTU tables.

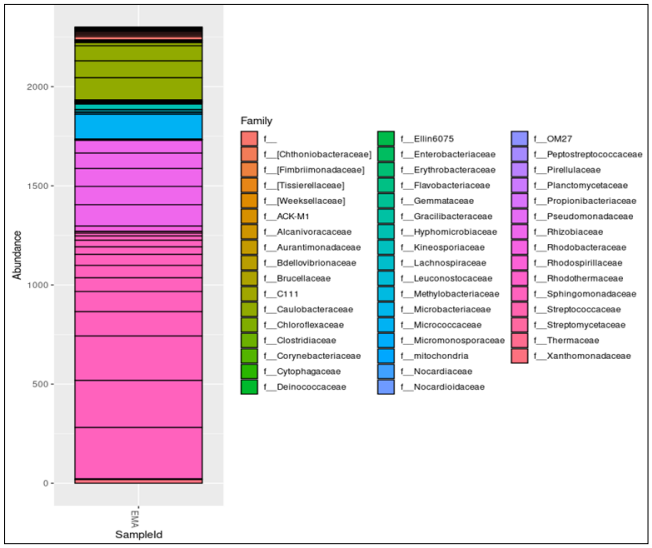
3.3.3 Taxonomy Assignment and Data Filtering

Taxonomic classification was first performed using the Greengenes reference database to assign taxonomic labels to amplicon sequence variants (ASVs). Additionally, species-level assignment was carried out to further characterize microbial communities.

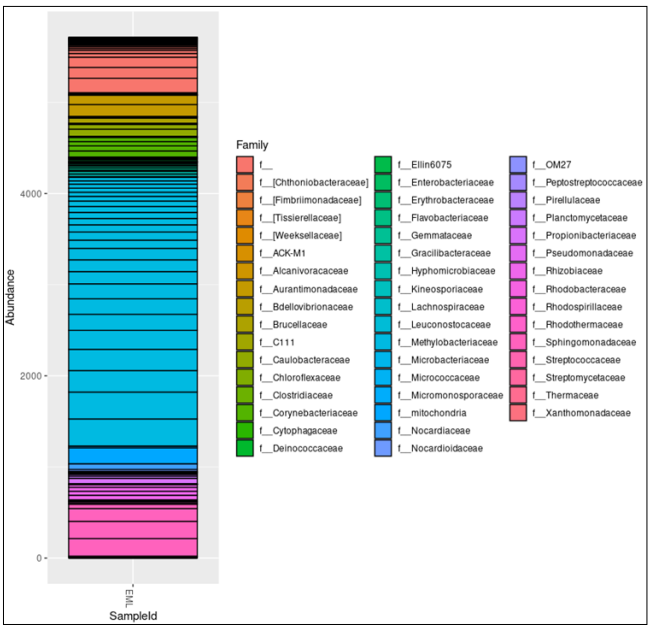
After the taxonomy assignment, data filtering was applied to ensure data quality and completeness. We filtered the data based on the presence of NA values and species information, retaining only the ASVs that had species-level taxonomic information available.



A



B



C

Fig 1: Relative abundance of most predominant families of gut bacteria in (A) *E.pkhalonia* adult, (B) *E.matena* adult, and (C) *E.materna* larva species of Erebidae family

3.4 Data Visualization and Divrsity Analysis

3.5.1 Alpha Diversity

Alpha diversity measures the diversity within individual samples. We calculated various alpha diversity metrics, including

- Shannon diversity index
- Simpson diversity index
- Chao1 estimator

These metrics provide insights into the richness and evenness of microbial communities within each sample.

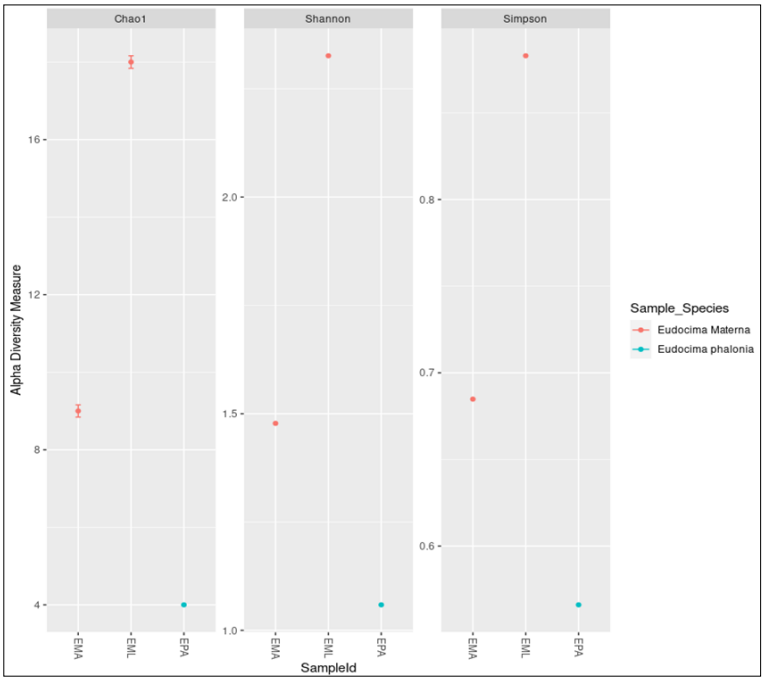


Fig 2: Alpha diversity of Chao1, Shannon, and Simpson indices of gut bacterial communities in EMA (*E.materna* adult), EML (*E.materna* larva), and EPA (*E.phalonia* adult).

3.5.2 Beta Diversity

Beta diversity measures the dissimilarity between microbial communities across samples. We utilized ordination techniques such as NMDS (Non-metric Multidimensional Scaling) and PCoA (Principal Coordinates Analysis) to visualize the beta diversity based on various distance metrics, such as Bray-Curtis dissimilarity and Jaccard distance. These ordination plots allow for the exploration of microbial community composition and structure between samples.

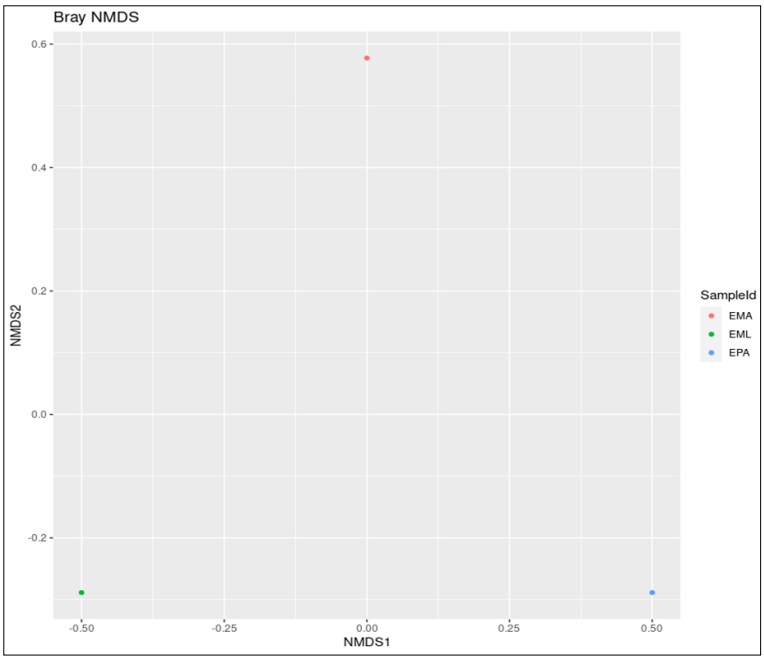


Fig 3: Nonmetric multidimensional scaling (NMDS) analysis of gut bacterial communities in EMA, EML, and EPA. The coloured points in the figure represent a sample and the degree of difference is represented by the distance between points. These ordination plots allow for the exploration of microbial community composition and structure between samples.

Result

4. Identified Gut Bacteria Species

Table 1: *Eudocima materna adult*

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV1	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__komagatae
ASV4	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__organophilum
ASV7	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Corynebacteriaceae	g__Corynebacterium	s__stationis
ASV10	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Propionibacteriaceae	g__Propionibacterium	s__acnes
ASV13	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Stenotrophomonas	s__geniculata
ASV16	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__frigidarium
ASV19	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Enterobacter	s__cloacae
ASV25	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__pseudoalcaligenes
ASV28	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__fragi
ASV31	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Pantoea	s__agglomerans

Table 2: *Eudocima materna* larva

ASVs	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV2	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	f__mitochondria	g__Zea	s__luxurians
ASV5	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__adhaesivum
ASV8	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__komagatae
ASV11	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	s__wittichii
ASV14	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Micrococcaceae	g__Arthrobacter	s__woluwensis
ASV17	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Enterobacter	s__cloacae
ASV20	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	g__Erythromicrobium	s__ramosum
ASV23	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	g__Altererythrobacter	s__indicus
ASV26	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Streptomycetaceae	g__Streptomyces	s__reticuliscabiei
ASV29	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Arenimonas	s__oryziterrae

Table 3: *Eudocima phalonia* adult

ASVs	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV3	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__hispanicum
ASV6	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Methylobacteriaceae	g__Methylobacterium	s__komagatae
ASV9	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	s__wittichii
ASV12	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Paracoccus	s__marcusii
ASV15	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans
ASV18	k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__frigidarium
ASV21	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	s__celatum
ASV24	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Erwinia	s__dispersa
ASV27	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__umsongensis
ASV30	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__mendocina

4.1 Adult *E. materna*:

- **49%** *wittichii* genus *Spingomonas*.
- **0%** of *S. komagatae* genus Methyl bacterium
- **21%** s__marcusii genus paracoccus
- **5%** s__frigidarium genus Flavobacterium
- **2%** of bacteria of genus Pseudomonas (s__pseudoalcaligenes and s__mendocina)

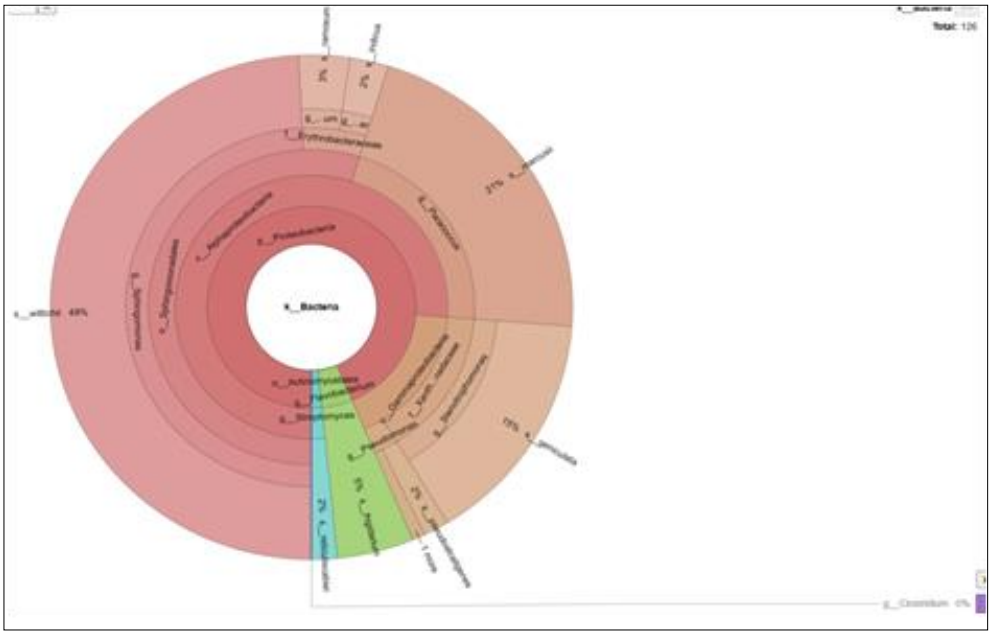


Fig 4 A: *Eudocima materna* adult (EMA)

4.2 Larva of *E. materna*:

- **33%** o *komagatae* genus Methyl bacterium
- **5%** of *wittichi* bacteria genus *Spingomonas*
- **0%** s__marcusii genus paracoccus
- **0.5%** s__cloacae genus Enterobacter
- **0.5%** s__frigidarium genus Flavobacterium
- **0.4%** of bacteria of genus Pseudomonas (s__umsongensis and s__fragi).

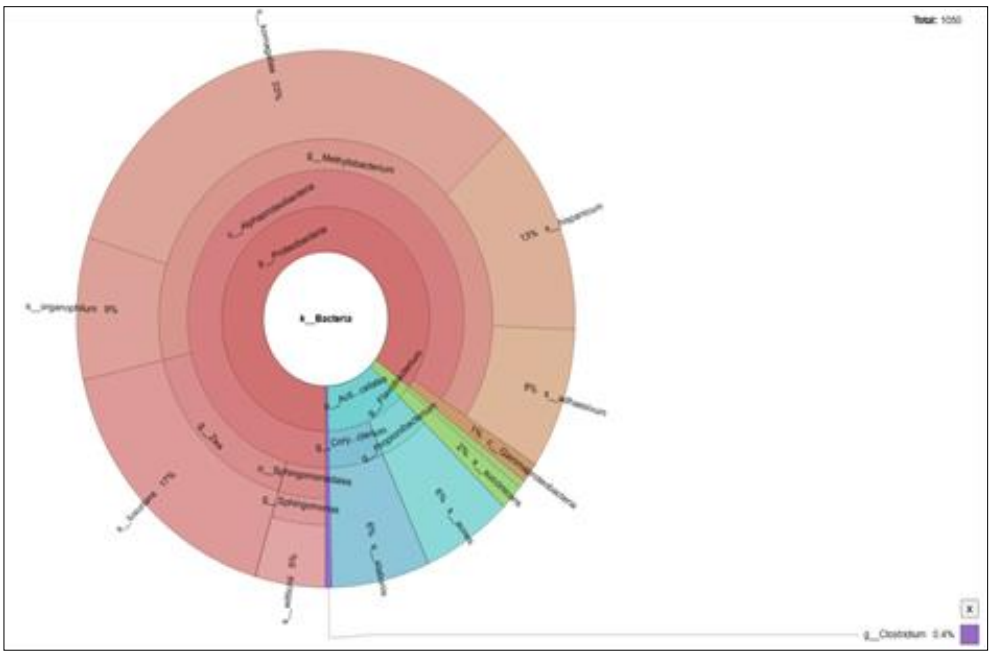


Fig 4 B: Eudocima materna larva (EML) showing significant difference in bacterial taxa at the level of Phylum, Class, Order, Family, Genus and species from inside to outside, diameter and percentage of sections of circles shows relative abundance. Different coloures and shades were used to show taxonomic variations in bacteria

4.3 Adults of *E. Phalonia*

- *E. Phalonia symbionts*
- **61%** are *woluwensis*.genus *Arthobacter.*
- **0%** of *wittichii*. genus *Spingomonas*
- **0%** *s__marcusii* genus *paracoccus*
- **32%** *s__cloacae* genus *enterobacter*

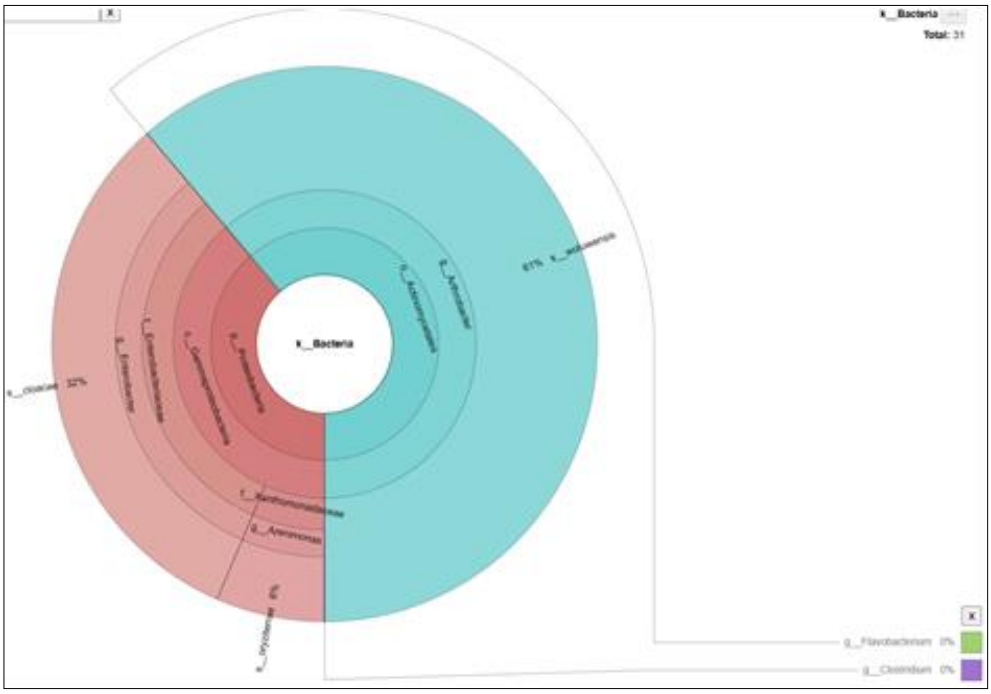


Fig 4 C: *Eudocima phalonia* Adult (EPA)

*Figure 4 A,B, and C Showing significant difference in bacterial taxa at the level of Phylum, Class, Order, Family, Genus and species from inside to outside, diameter and percentage of sections of circles shows relative abundance. Different colors and shades were used to show taxonomic variations in bacteria.

Discussion

Gut microbiota has a different symbiotic association with a host, it might be mutualistic, commensal, parasitic, etc. which may involve different ways in physiology, behaviour, ecology, and evolution of the host. (Wang *et al*., 2020) [16] (Tang *et al*., 2012) [15] Metagenomic analysis to investigate bacterial community provided detailed information on diversity, composition, richness, structure, and function (Zea *et al*., 2019) [17]. In some insects mating attractiveness of the host can also be affected by the gut microbiota, when in D. melanogaster, the composition of *L.plantarum* isolated from the gut of fly mating preferences could be reversed from axenic flies: thus concluding flies mate preferentially with individuals harboring similar microbiota, which can affect the host lineage and leading ultimately to speciation (Engel and Moran 2013) [4]. This study reveals the knowledge of bacterial communities in the gut of lepidopteran moths of the Erebidae family, in which two found species have been taken *Eudocima materna* (Two life stages and larvae) and *Eudocima phalonia* (Adult). Some previous studies on gut microbiota reveal that Lepidoptera has indicated diet influences larval midgut community composition as a key factor (Broderick *et al*., 2004) [1] to evaluate midgut bacterial composition on larvae of Gypsy moth(Family-Erebidae) has been observed to investigate interactions between sources of egg mass and environmental sources such as laboratory-based and wild diet of bacteria on larval midgut community despite that observation says, Midgut Communities Within Gypsy Moth Larvae Become Highly Similar Through Larval Development (Mason & Raffa, 2014) [10]. IN cotton bollworm- *Helicoverpa armigera* four phyla of bacteria (Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes) and two genera of bacteria (Enterobacter and Enterococcus) dominated across all life stages of cotton bollworm (Zhao *et al*., 2023) [19], which was similar to several other lepidopteran pests, which simply supports the observations of this study which shows Proteobacteria and Actinobacteria are dominant phyla in both the species EMA, EML and EPA. *Hyphantria cunea Drury* (Family-Erebidae) larvae has *E. mundtii* and *K. oxytoca* were isolated as the dominant midgut bacterial strains which were similar to *H. cunea* larvae separately fed on an artificial diet and other host leaves, *Enterococcus* is most abundant genus found in *H. cunea* larvae (Chen *et al*., 2023) [2]. If we compare other fruit moths with EMA and EPA, *Carposina sasakii* the peach fruit moth (PFM) and *Grapholita molesta* the oriental fruit moth (OFM) were dominated by the genus *Pseudomonas*, *Gluconobacter*, *Acetobacter*, and *Pantoea* of gut microbiota. *Pantoea* is a highly diverse genus that we have found in EMA species that can cause plant diseases and human diseases but also have functions in habitat restoration and pesticide degradation among the abundant bacteria taxa (Gong & Fruit-feeding Moth, 2020) [5], *Pseudomonas brenneri* plays a prominent role in the removal of heavy metals. This species is significantly more abundant in OFM than PFM we found pseudomonas genera in both EMA and EPA with another species (Gong *et al*. 2020) [5]. The functional role of some of the bacterial genera we have found in EMA, EML, and EPA might be as Observed by (Zhang & Zhang, 2022) [18], the genus's proteobacteria *Methylobacterium* shows the function of Nitrogen fixation, *Sphingomonas* function as Microbe-mediated detoxification of phytotoxins and pesticides, *Propionibacterium* Produce antimicrobial peptides, *Pseudomonas* play role as Anti-phytopathogenic fungi, *Pantoea* Affect oviposition behavior, morphogenesis and development, *Enterobacter* function as Anti-phytopathogenic fungi activity; growth and development, *Stenotrophomonas* isolated from intestine *Diatraea saccharalis* larvae were found to possess cellulolytic activity, *Erwinia* sp. isolated from Lepidoptera insect shows pectinolytic activity (Zhang & Zhang, 2022) [18]. Members of this genus are recognized for their sphingoglycolipid production and their role in the degradation of complex aromatic compounds, strain RW1 of this species is particularly notable for its capacity to metabolize dibenzo-p-dioxin and phenazine-1-carboxylic acid which is found in soil (Moreno-Forero & Van Der Meer, 2015) [12], perhaps it also has been observed in gut of pest insect cotton aphid *Aphis gossypii* which evolved to be a insecticide-resistant by metabolizing imidacloprid due to appearance of *Spingomonas* gut symbiont (Lv e*t al*., 2023) [9].

As per metagenomics data analysis, we found that both the species *E. materna* and *E. phalonia* have different compositions of gut symbionts, there are only a couple of bacterial endosymbionts that were common in both species. On the other hand, larvae and adults of the species *Eudocima materna* have tremendous variations in gut microbiota.

Acknowledgement

This research work was done under the guidance of Dr. R. F. Pathre and funding was supported by Mahatma Jyotiba Phule Research and Training Institute of the Government of Maharashtra. We would like to thank J.E.S College of Jalna for providing us well equipped laboratory and GENESPECTRUM life science LLP Laboratory for sample processing and providing supportive data.

Digital Reference for Data Visualization (fig. 5A, 5B and 5C)

E:\16s rRNA\Species_Visualization.html

References

1. Broderick NA, Raffa KF, Goodman RM, Handelsman J, Al BET, Icrobiol APPLENM. Census of the Bacterial Community of the Gypsy Moth Larval Midgut by Using Culturing and Culture-Independent Methods. Applied and Environmental Microbiology,2004:70(1):93–300.
2. Chen H, Hao D, Chen C, Sun Y, Yu X. Effects of midgut bacteria in Hyphantria cunea (Lepidoptera: Erebidae) on nuclear polyhedrosis virus and Bacillus thuringiensis (Bacillales: Bacillaceae). Journal of Insect Science, 2023, 23(2).
3. Dillon RJ, Dillon VM. The Gut Bacteria of Insects: Nonpathogenic Interactions. Annual Review of Entomology,2004:49:71–92.
4. Engel Philipp, Nancy A Moran. "The Gut Microbiota of Insects - Diversity in Structure and Function." FEMS Microbiology Reviews,2013:37(5):699–735. doi:10.1111/1574-6976.12025.
5. Gong Qiang, Li Jun Cao, Li Na Sun, Jin Cui Chen, Ya Jun Gong, De Qiang Pu, *et al*. "Similar Gut Bacterial Microbiota in Two Fruit-Feeding Moth Pests Collected from Different Host Species and Locations." *Insects*,2020:11(12):1–13. Doi:10.3390/insects11120840.

6. Hammer Tobin J, Daniel H Janzen, Winnie Hallwachs, Samuel P Jaffe, Noah Fierer. “Caterpillars Lack a Resident Gut Microbiome.” Proceedings of the National Academy of Sciences of the United States of America,2017:114(36):9641–46. doi:10.1073/pnas.1707186114.
7. Jia Y, Jin S, Hu K, Geng L, Han C, Kang R, *et al*. Gut microbiome modulates Drosophila aggression through octopamine signaling. Nature Communications, 2021, 12(1).
8. Kayande YR, Pathre RF, Jadhav SS. “Review On Diversity, Host Plant Relationship And Seasonal Abundance Of Fruit Piercing Moths And Importance Of Molecular Taxonomy” (Lepidoptera: Hetercocera). International Journal of Research and Analytical Reviews,2023:10(2):165–170.
9. Lv N, Li R, Cheng S, Zhang L, Liang P, Gao X. The gut symbiont Sphingomonas mediates imidacloprid resistance in the important agricultural insect pest Aphis gossypii Glover. BMC Biology,2023:21(1):1–15.
10. Mason CJ, Raffa KF. Acquisition and structuring of midgut bacterial communities in gypsy moth (Lepidoptera: Erebidae) larvae. Environmental Entomology,2014:43(3):595–604.
11. Mereghetti V, Chouaia B, Montagna M. New insights into the microbiota of moth pests. International Journal of Molecular Sciences, 2017, 18(11).
12. Moreno Forero SK, Van Der Meer JR. Genome-wide analysis of Sphingomonas wittichii RW1 behaviour during inoculation and growth in contaminated sand. ISME Journal,2015:9(1):150–165.
13. Morrison M, Pope PB, Denman SE, McSweeney CS. Plant biomass degradation by gut microbiomes: more of the same or something new? Current Opinion in Biotechnology,2009:20(3):358–363.
14. Ravindra Fakirrao Pathre, Sharad Devidasrao Jadhav. Some fruit piercing moths (Lepidoptera: Heterocera) from Jalna district of Maharashtra, India. Journal of Entomological Research,2020:44(1): 131–133.
15. Tang X, Freitak D, Vogel H, Ping L, Shao Y, Cordero EA. Complexity and Variability of Gut Commensal Microbiota in Polyphagous Lepidopteran Larvae. PLoS ONE,2012:7(7): 1–9.
16. Wang X, Sun S, Yang X, Cheng J, Wei H, Li Z, *et al*. Variability of Gut Microbiota Across the Life Cycle of Grapholita molesta (Lepidoptera : Tortricidae). Frontiers in Microbiology,2020:11:1–13.
17. Zea L, Mansoor M, Zaheer A, Sajjad M, Naqqash T, Batool T, *et al*. Saudi Journal of Biological Sciences Original article A comparative study of bacterial diversity based on culturable and culture-independent techniques in the rhizosphere of maize. Saudi Journal of Biological Sciences,2019:26(7):1344–1351.
18. Zhang X, Zhang F. Diversity and Functional Roles of the Gut Microbiota in Lepidopteran Insects. Microorganisms,2022:10(6):1–15.
19. Zhao C, Wang L, Zhang K, Zhu X, Li D, Ji J, *et al*. Variation of Helicoverpa armigera symbionts across developmental stages and geographic locations. Frontiers in Microbiology, 2023, 14.
20. Zhu Z, Liu Y, Hu H, Wang GH. Nasonia-microbiomeassociations: a model for evolutionary hologenomics research. Trends Parasitol,2022:39(2):10 1–112.