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Characterization of gut microbiota in *Apis cerana* Across different altitudes in the Peninsular India

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Abstract

Background Honey bees are vital to global ecosystems and agriculture due to their role as key pollinators. The gut microbiota of honey bees is essential for their health, providing nutrition and protection against pathogens. While extensive research has been conducted on Western honey bees, Less is understood about the gut microbiota of *Apis cerana,* an economically important species in South Asia. This study aimed to identify and describe the gut microbiota of *Apis cerana* across different elevations in the Indian peninsula to understand how these bacterial communities adapt to various ecological niches.

Results High-throughput metagenome sequencing of the 16S rRNA gene (V1-V9 region) showed that the core microbiota genera in *Apis cerana* guts across elevations were *Gilliamella, Lactobacillus, Snodgrassella,* and *Frischella. Gilliamella apicola* and *Lactobacillus kunkeei* were identified as the most abundant species. Alpha diversity analysis showed a trend of decreasing species diversity as altitude increased from 200 to 1200 m, with a slight increase observed above 1400 m. Culturable bacterial species identified through 16S rRNA amplification belonged to the *Proteobacteria, Firmicutes, and Actinobacteria phyla.* Different elevations harboured distinct bacterial communities, with some species being unique to certain altitudes.

Conclusions This study provides valuable insights into the diversity and adaptations of *Apis cerana* gut microbiota across various ecological niches in the Indian peninsula. The observed variations in microbial communities at different elevations suggest that environmental factors play a significant role in shaping the gut microbiota of honey bees. Understanding these microbial dynamics could help in developing strategies to improve bee health and address critical questions in host-microbe symbiosis. Furthermore, this research lays the groundwork for future studies on the functional roles of these bacterial communities in *Apis cerana* and their potential applications in beekeeping practices.

Keywords *Apis cerana*, 16S rRNA, Gut microbiota, *Gilliamella*, *Lactobacillus*

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Introduction

Honey bees are social insects important to the global ecosystem and the economy, as they are pollinators of most flowering plants and food crops [1]. In South India, four species of honey bees are well known which includes the Indian honey bee (*Apis cerana*), the western honey bee (*Apis mellifera*), the giant honey bee (*Apis dorsata*) and the dwarf honey bee (*Apis florea*) [2]. Among these species, *A. cerana* is economically reared for honey and other bee products in South India. However, to ensure the long-term sustainability of the honey bee industry, research on factors affecting bee health such as toxins, viruses, parasites, nutrition, and especially their microbiota has intensified due to the recent decline in bee populations [3].

Many studies have shown that bacteria in the bee gut help protect their host from invading pathogens [4]. The gut microbiota of bees is essential for host nutrition, weight gain, endocrine signalling, immune function, pathogen resistance, and even social behaviour [5]. Recently, honey bees have become recognized as effective models for gut microbiota research, helping to explore how gut communities impact their hosts and to understand the general principles that govern gut microbial community composition and dynamics [6]. Therefore, understanding this microbial community will undoubtedly provide new insights into host-microbe interactions, aiding in the improvement of bee health for beekeeping [7]. Initially, studying gut microbes was only possible in a laboratory setting, where traditional culture-based techniques offered an incomplete view of microbial communities [2].

Recently, with the advent of Next-Generation Sequencing (NGS) technologies, full-length gene sequencing has become possible [8]. Only a few studies have been conducted on the gut microbial study of eastern honey bees (*Apis cerana)* as compared to western honey bees (*Apis mellifera*) [9]. *A. cerana* can be found at different altitudes where the flora and climate are suitable.

The eastern honey bee has adapted to diverse environmental conditions across its native range in Asia, including high altitude areas of the Himalayas to the Lowland areas. [10]. The diversity of flora and fauna varies with the altitudes and it will have its influence on the resident gut microbes of the bees adapted to the plain to hilly regions. Although much effort has been made towards understanding honey bee gut microbiota, previous studies have not examined variations of honey bee gut bacterial diversity between altitudes using next-generation sequencing approaches [11]. With the development of next-generation sequencing technologies, sequencing entire genes is now possible. While the V3-V4 hypervariable region of the genome is commonly sequenced, covering the entire gene from the first to the last hypervariable region (V1-V9) would offer more comprehensive detection of bacterial populations [12]. This study focused on the diversity of gut microbiota in *Apis cerana* worker bees based on different altitudes in South India (Tamil Nadu) using Culturable and Non-Culturable approaches through high-throughput metagenome sequencing (V1- V9) to unravel the function of the bacterial community.

Materials and methods

Specimen Collection

Healthy foraging worker honey bee specimen of *Apis cerana* were collected from 18 different locations in Tamil Nadu, India at different elevation from 0–200 m altitude (Chidambaram 11.23°N 79.43°E, Karaikal 10.57°N 79.46°E, Gingee 12.15°N 79.21°E and Virudhachalam 11.32°N 79.19°E), 200–400 m altitude (Edapadi 11.34°N 77.50°E and Pollachi 10.37°N 76.54°E), 400–600 m altitude (Coimbatore 11.00°N 76.55°E, Krishnagiri 12.29°N 78.13°E and Dharmapuri 12.07°N 78.10°E), 600–800 m altitude (Attur 11.38°N 78.37°E, Kodaikanal Foothills 10.23°N 77.32°E and Anamalai 10.28°N 76.51°E), 800–1000 m altitude (Siruvani 10.55°N 76.40°E and Masinagudi 11.30°N 76.42°E), 1000–1200 m altitude (Valparai 10.18°N 76.54°E), 1200–1400 m altitude (Yercaud 11.49°N 78.15°E and Sirumalai 10.11°N 78.00°E) and > 1400 m altitude (Ooty 11.21°N 76.44°E) (Fig. 1) (Table S1).

Ten foraging worker honey bees per hive have been collected from each of the study locations and surface sterilized using 70% ethanol to eliminate the external microorganisms or contaminants [13]. A sterile needle tip was used to stab the bee's thorax, which was then pinned onto wax. Using sterile micro forceps, the entire alimentary canal was carefully pulled out in one smooth motion and each was placed in a 2 mL microcentrifuge tube with 500 µL of PBS [14]. The samples were stored at -80 °C until the DNA extraction process.

Isolation of DNA

The insect gut samples were processed and DNeasy Blood and Tissue Kit was used to extract DNA. *A. cerana* bees dissected gut were put in 1.5 mL tubes with 200 µL of CTAB lysis buffer and homogenized with a micro pestle. The homogenized biological tissue was subsequently placed in a water bath maintained at a temperature of 65 °C for a duration ranging from 45 min to 1 h. Upon completion of the incubation period, the specimens underwent centrifugation at a speed of 10,000 rpm for 10 min, after which the supernatant was carefully transferred into a fresh 1.5 mL tube. To this tube, a solution of chloroform and isoamyl alcohol in a ratio of 24:1 was introduced, mixed thoroughly via inversion, and subjected to a second round of centrifugation at 12,000 rpm

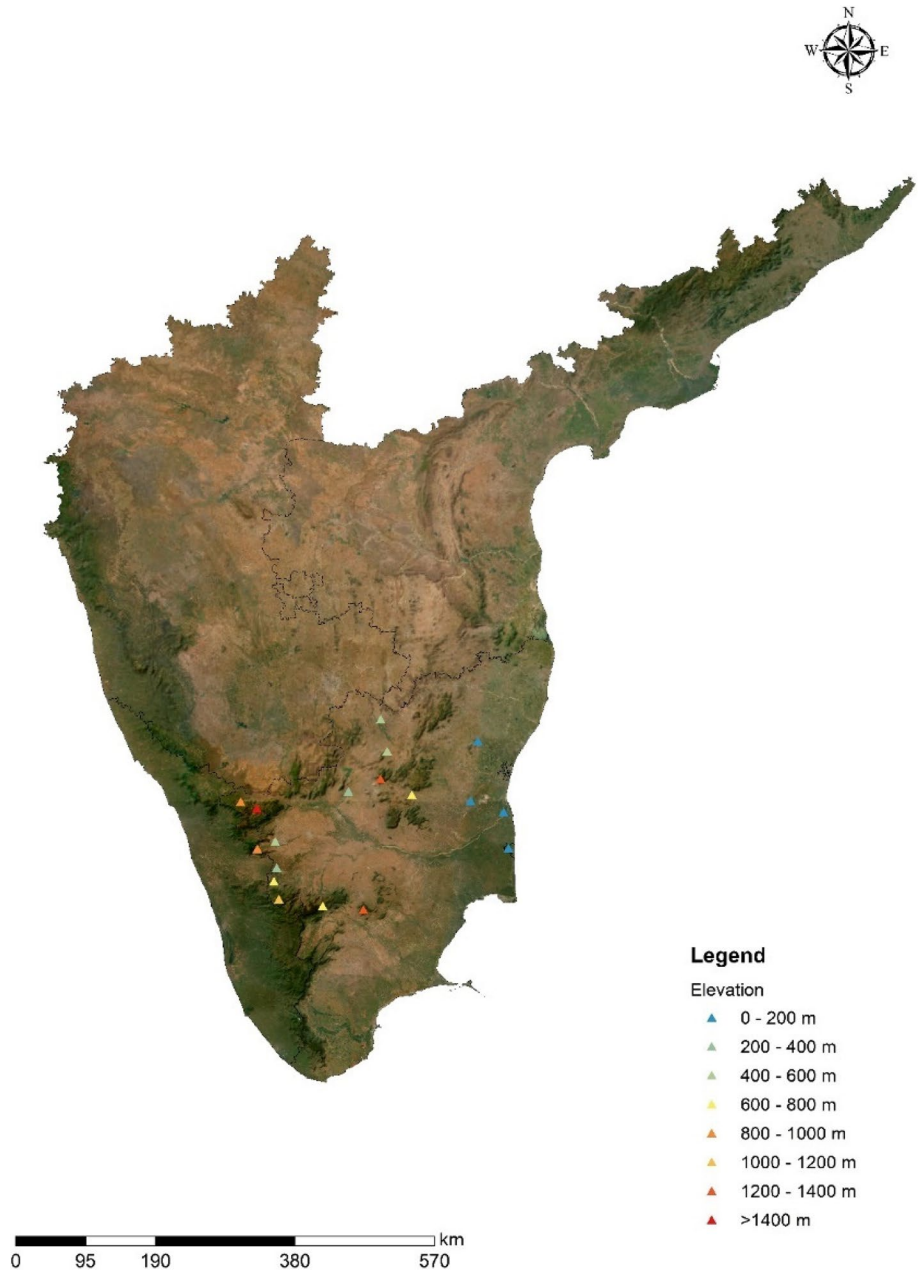


Fig. 1 Map of *Apis cerana* sample collection locations in Tamil Nadu, India

for 12 min. The upper aqueous layer was subsequently extracted and placed into a new 1.5 mL tube, to which 300 µL of isopropanol and 15 µL of sodium acetate were incorporated. This mixture was then preserved overnight at -20 °C. The following day, the samples underwent centrifugation at 12,000 rpm for 12 min, after which the supernatant was meticulously discarded, ensuring the pellet remained intact within the centrifuge tube. The tubes were left to air-dry until all remnants of isopropanol had evaporated, after which the pellet was reconstituted in 50 µL of sterile water and stored at -20 °C for subsequent analysis. The samples were analysed quantitatively and qualitatively using the nanodrop technique and agarose gel electrophoresis (1% Agarose/TAE), respectively.

16S rRNA V1-V9 Amplification and Sequencing

The amplicons derived from the samples were validated through Agarose (1%)/EtBr gel electrophoresis. The PCR products underwent purification utilizing 1.6X Ampure

XP beads (Beckmann Coulter, USA). In this investigation, metagenomic sequencing (V1-V9) was performed by Syngenome (OPC) Private Limited, Coimbatore. A total of 50 ng of DNA from each sample was subjected to PCR amplification employing barcoded 16S rRNA (V1-V9 region) specific primers 8F: 5'-AGAGTTTGA TCCTGGCTCAG-3'; 1492R: 5'-TTCAGCATTGTTCCA TCGGGA-3' and LongAmp (NEB) Taq 2X master mix in accordance with the protocol delineated in the 16S Barcoding Kit 24 V14 (SQK-16S114.24; Oxford Nanopore). The anticipated length of the amplicon is approximately 1500 base pairs for the V1-V9 segment of the 16S rRNA gene and includes 5' tags that enable the ligase-independent incorporation of Rapid Sequencing Adapters. The amplicons extracted from the specimens were validated through Agarose (1%)/EtBr gel electrophoresis. The PCR products underwent purification utilizing 1.6X Ampure XP beads (Beckmann Coulter, USA).

The Nanopore Library was prepared as the barcoded amplicons from each sample were quantified and ~ 50 ng from each sample were pooled. The pooled amplicons were subjected to library clean-up using the 1 × AmPure beads (Beckmann Coulter, USA) supplied in the 16S Barcoding Kit 24 V14. Nanopore sequencing was conducted utilizing the MinION platform (Oxford Nanopore Technologies, Oxford, UK) with R10.4.1 flow cells (FLO-MIN114) adhering to a 72-h sequencing protocol on the MinKNOW software (version 22.03.2, ONT), incorporating live base calling with standard parameters activated.

The sequencing data was executed subsequent to the base-calling and de-multiplexing of Nanopore raw reads utilizing Guppy v6.0.6. Basecalling the MinION flow cell R10.4.1 sequencing data (FAST5 files) with Guppy version 2.3.4, developed by Oxford Nanopore Technologies, produced pass reads in FASTQ5 format exhibiting an average quality score exceeding 7. The sequences corresponding to the adapter and barcode were meticulously trimmed utilizing the Porechop tool, also from Oxford Nanopore Technologies. The sequencing reads underwent size filtration via SeqKit software version 0.10.0, selectively preserving sequences ranging from 1200 to 1950 base pairs for the V1-V9 region.

16S rRNA Gut Microbiome Analysis

The processed sequences derived from each dataset were subjected to analysis for thorough taxonomic characterization of microbial assemblages employing the MetONTIIME computational tool. The resultant rRNA sequences were subsequently imported into Qiime2 utilizing the SingleEndFstqManifestPhred33 input schema for the purpose of conducting diversity assessments. The sequences underwent dereplication and were subsequently organized into operational taxonomic units (OTUs). The classification of representative sequences was executed using the BLAST algorithm in combination with Qiime2 (classify-consensus-vsearch), referencing the SILVA full-length 16S ribosomal RNA database (version 138).

The relative abundances of microbial taxa within individual samples were assessed by categorizing the samples subsequent to taxonomic classification encompassing kingdom, phylum, class, order, family, genus, and species levels. The long-read amplicons sequenced via the Nanopore MinION platform were taxonomically categorized utilizing the SILVA database as a reference.

The microbial classifications obtained were compared at different taxonomic levels for the samples. The absolute and relative abundance of the taxa determined at taxonomical levels of Class, Order, Family, Genus, and Species are shown using heatmaps. Core microbiome diversity analysis was performed using the Microbiome Analyst tool for alpha, and beta diversity across the locations.

Culturing and identification of honey bee gut microbes

The gut samples from bees ($n=5$) were homogenized with 100 µl of phosphate-buffered saline (PBS) utilizing a micro pestle. A series of dilutions (1/10, 1/100, and 1/1,000) of this homogenate were subsequently prepared, and 10 µl of each diluted specimen was inoculated onto four distinct culture media: Nutrient agar (NA), Brain Heart Infusion (BHI) agar, De Man, Rogosa and Sharpe (MRS) agar, and Luria Bertani (LB) agar. The samples underwent aerobic incubation at a temperature of 37 °C for a duration of 18 to 24 h. The bacterial colonies that emerged on the agar plates were enumerated and selected based on their phenotypic variations. The distinct colonies were subjected to repeated sub-culturing from master plates to achieve isolation of pure bacterial strains.

The 16S rRNA gene from all isolates was subjected to amplification utilizing the universal bacterial primers 8F and 1492R, thereby encompassing nearly the entirety of the gene's length. The polymerase chain reaction (PCR) protocol commenced with an initial denaturation phase at 94 °C for a duration of 2 min, succeeded by 35 cycles comprising denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. A concluding extension was executed at 72 °C for 10 min. The resultant PCR products were resolved on a 1.5% agarose gel, and the bands were visualized under a UV transilluminator, subsequently documented utilizing a documentation unit (UVITEC CAMBRIDGE). An amplicon size measuring 1.5 kb was achieved, and the PCR products were dispatched to Biokart Pvt. Ltd., Bangalore, for Sanger sequencing. The sequences obtained, formatted as FASTA files for both forward and reverse primers,

were employed for homology assessments through the BLASTn program available at the National Center for Biotechnology Information (NCBI).

Results

Taxonomic composition of the core bacterial community associated with *A. cerana* gut

We conducted a comprehensive profiling of the gut microbiota in 160 worker honey bees from eight different geographical locations across varying elevations in the Indian peninsular region, with biological replication. We analyzed a total of 4,092,574 reads from the V1-V9 region of the 16S rRNA gene, with an average of 511,571 reads per sample (ranging from a minimum of 278,099 to a maximum of 1,038,300 reads). Sequence Read Archive for Metagenomics data were deposited in NCBI public repository (Table S3). The analysis identified the core microbiota genera of the honey bee gut across different elevations as *Gilliamella*, *Lactobacillus*, *Snodgrassella*, and *Frischella (*Fig. 2). The relative abundance of these core microbiota in each sample is summarized in Table 1. In addition to the core microbiota, it also uncovered minor taxa that were common across all regions, such as *Apibacter* and *Klebsiella (*Fig. 3).

At the species level, the eight most abundant bacterial species were identified across all different regions. Among these, *Gilliamella apicola* was the most abundant species within the predominant genus *Gilliamella*, while *Lactobacillus kunkeei* was the most abundant within the dominant genus *Lactobacillus*. Interestingly, the comparison of species diversity across the elevations revealed that *Enterobacter cloacae* and *Enterobacter hormaechei* were unique to the 200 m elevation, distinguished from microbiomes at higher elevations. Additionally, *Lactobacillus kunkeei* was predominantly present at a higher percentage at the 600 m elevation (Fig. 4).

Diversity variation of *A. cerana* gut microbiota across different elevations

The analysis of gut microbiota diversity in *A. cerana* at varying elevations reveals different levels of species richness and evenness, as measured by different alpha diversity indices including Chao1, Shannon, and Observed Features. The data indicates a clear trend of decreasing species diversity as the altitude increases from 200 to 1200 M. The Shannon index for bacterial communities was 3.6, suggestive of richness in bacterial diversity in 200 M altitude. The higher Chao 1 index indicates the higher number of Operational Taxonomic Units (OTU) which relates higher species diversity within the samples of different elevation (Table 2). At 1400 M, species richness is moderate and consistent. Above 1400 M, the increase in species richness and variability, this might be due to under specific ecological factors and

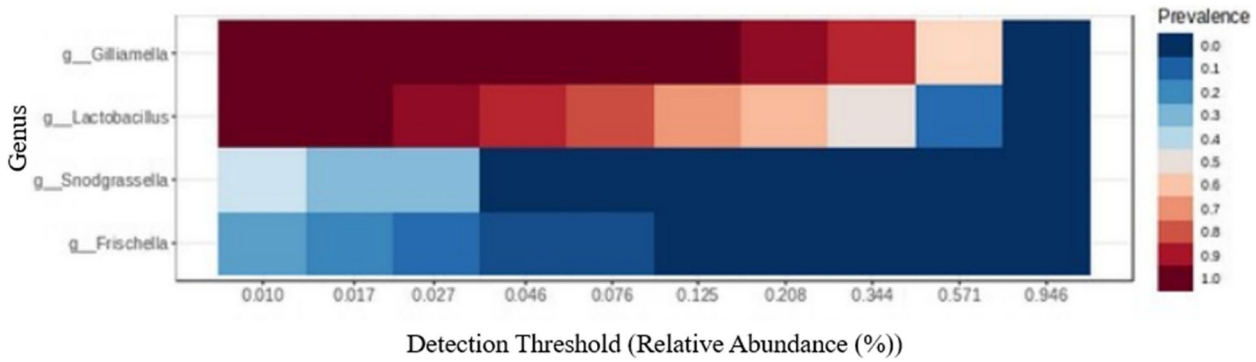
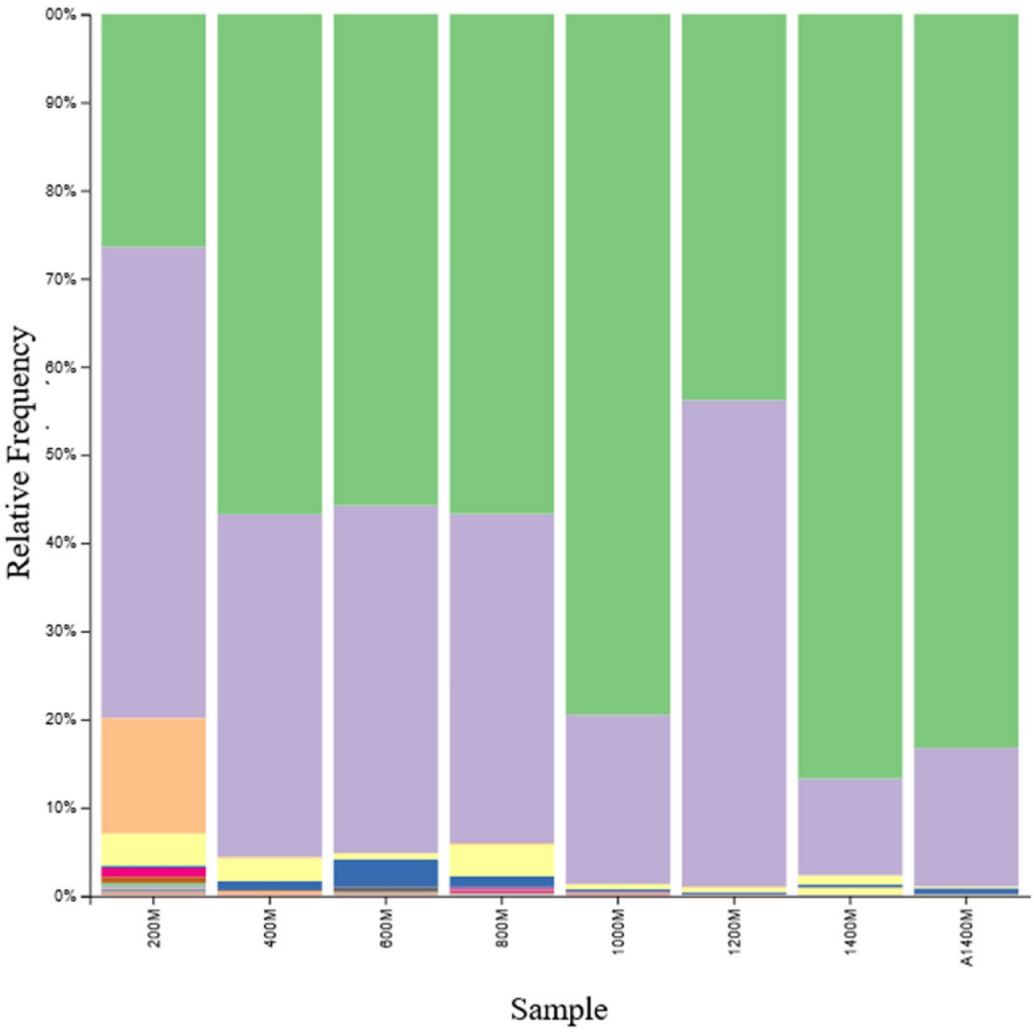


Fig. 2 Heatmap that shows the prevalence of different bacterial genera at various detection thresholds of relative abundance

Table 1 Relative abundance of core microbiome across all elevations

MSL	200 M	400 M	600 M	800 M	1000 M	1200 M	1400 M	Above 1400 M
Species								
Expressed in percentage (%)								
Gilliamella	26	56	55	56	79	43	86	83
Lactobacillus	53	30	39	37	19	55	10	15
Snodgrassella	3	2	0.6	3	0.5	0.5	1	0.1
Frischella	0.2	0.9	3	1.2	0.2	0.2	0.2	0.5



d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Gilliamella
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Enterobacter
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae;g__Snodgrassella
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Frischella
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Klebsiella
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;__
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Paraclostridium
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae;g__Escherichia-Shigella
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Apibacter
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Orbales;f__Orbaceae;g__Orbus

Fig. 3 Bacterial genera associated with *Apis cerana* gut at different elevations—The top 12 taxa in abundance were shown in the bar charts. Each color represents a species, and the height of the color block indicates the proportion of the species in relative abundance

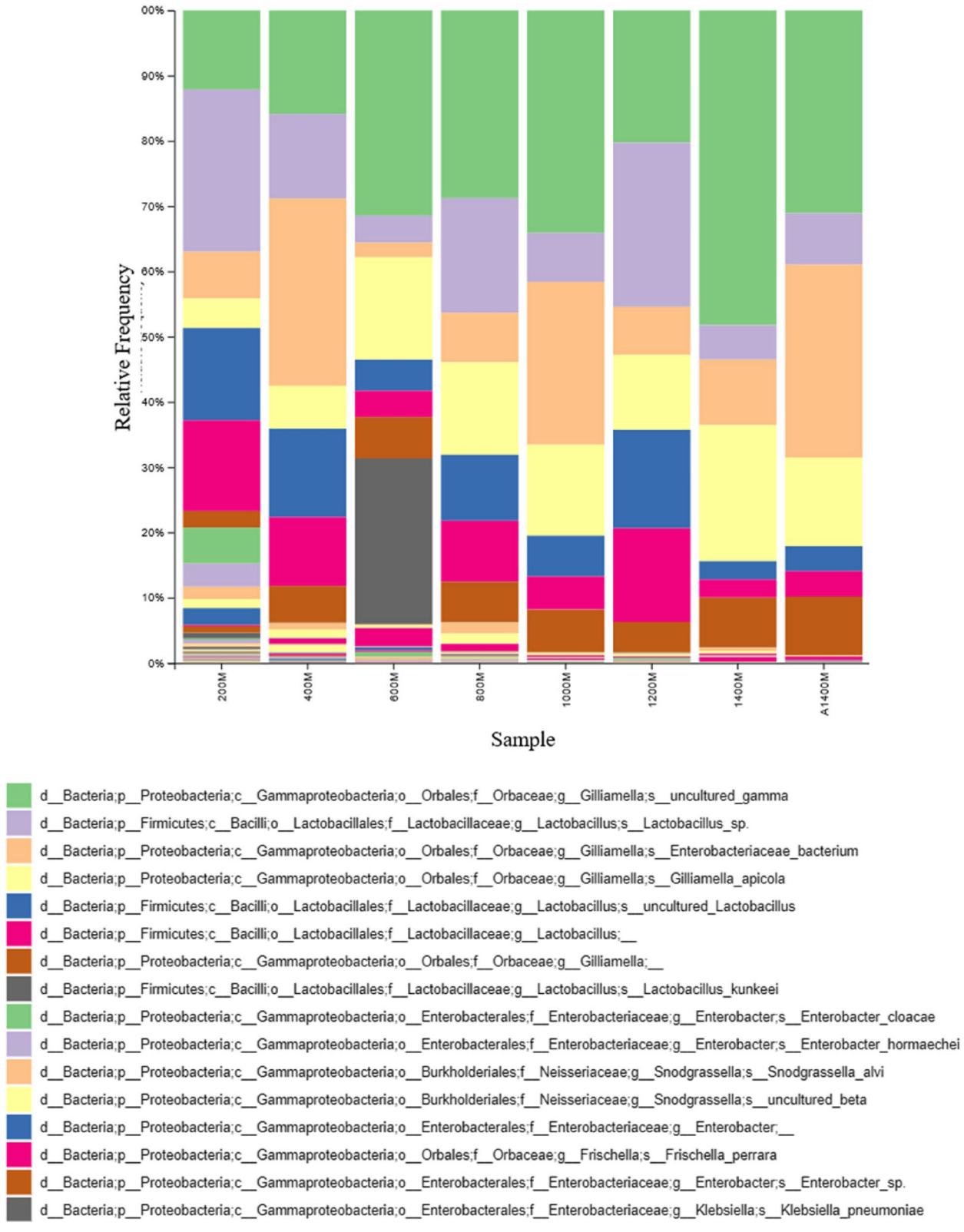


Fig. 4 Bacterial species associated with *A. cerana* gut at different elevations in India Peninsular—The top 16 taxa in abundance were shown in the bar charts. Each color represents a species, and the height of the color block indicates the proportion of the species in relative abundance

Table 2 Number of Operational Taxonomic Units (OTU) detected

Replicates	Phylum	Class	Order	Family	Genus	Species	OTU's
200 M	6	10	30	53	1277	360	360
400 M	5	4	27	43	71	141	141
600 M	6	10	28	44	79	173	173
800 M	5	7	22	36	69	161	161
1000 M	5	6	20	37	60	114	114
1200 M	3	4	9	12	20	51	51
1400 M	6	8	20	34	57	115	115
A1400M	4	6	19	22	35	66	66

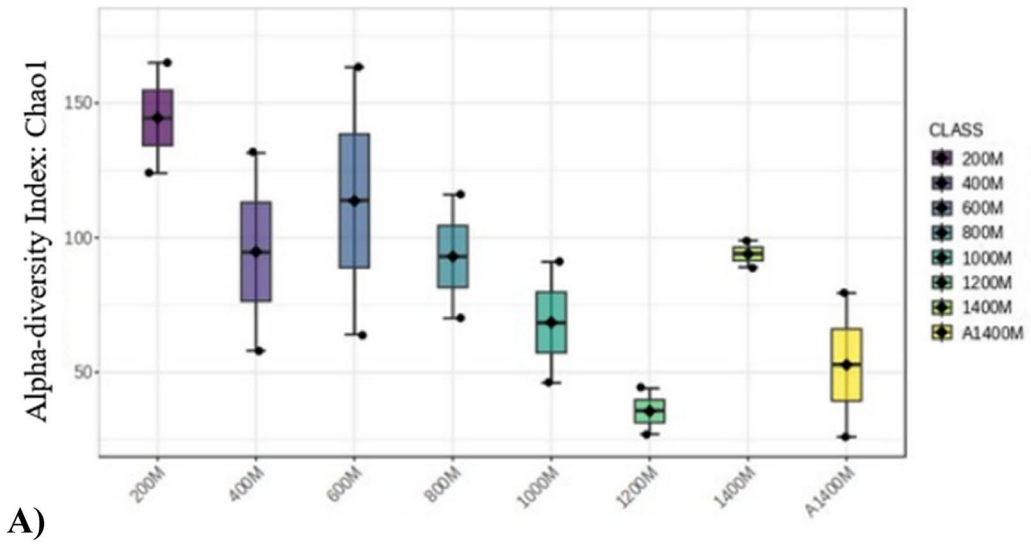
environmental conditions. The rarefaction curves of observed features yield higher number of OTUs on the depth of sequencing for all elevations except at 1200 m, where the curve began to plateau, indicating that the number of OTUs detected was decreased which further confirms the less species diversity in 1200 m (Fig. 5). In Fig. 6 the beta-diversity analysis can be seen. The PCoA plot illustrates how microbial communities from different altitudes cluster or separate in the ordination space. The axes represent the first three principal coordinates (PC1, PC2, and PC3), which explain 58.4%, 29.7%, and 7% of the variation, respectively. Samples from similar altitudes tend to cluster together, indicating that altitude may influence the microbial composition in the honey bee gut. The PERMANOVA (Permutational Multivariate Analysis of Variance) test results show an R-squared value of 0.51297, which indicates that about 51.3% of the variation in microbial community composition can be explained by the altitude factor and a *p*-value of 0.354 explains that the differences observed in the microbial communities across different altitudes are not statistically significant (typically, a *p*-value less than 0.05 would be considered significant). This implies that the ecological factor along with the altitude have a combined effect on the gut microbial composition in honey bees.

Culturable bacterial species identification of *A. cerana* gut through 16S rRNA amplification

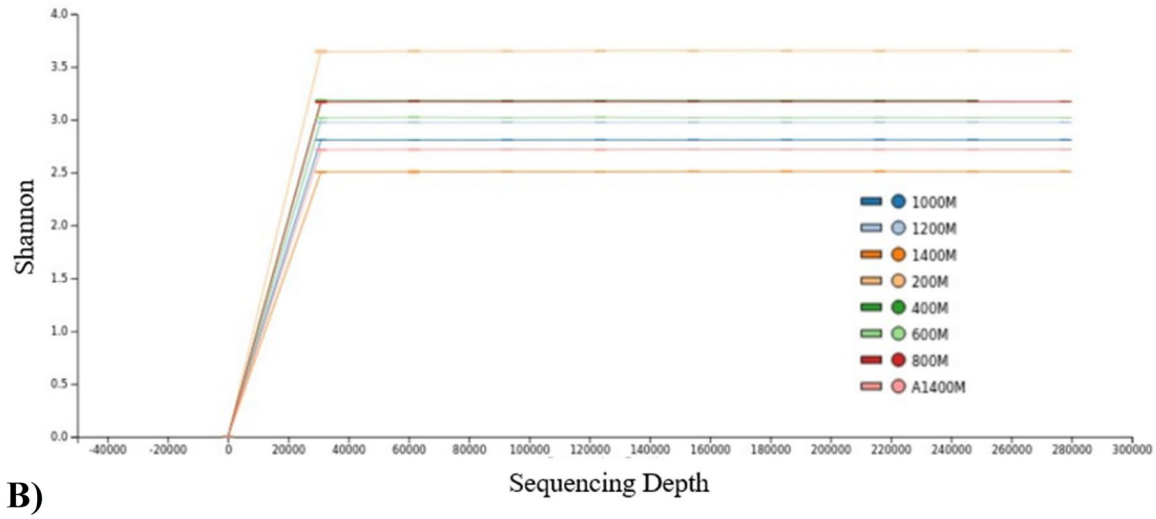
A total of thirty-eight isolates of culturable bacteria were discerned from the gathered populations of A. cerana utilizing 16S rRNA primers, which were classified into three distinct phyla, namely, Proteobacteria, Firmicutes, and Actinobacteria. (Table 3) (Table S2). All sequences generated in the current investigation underwent BLAST analysis at the NCBI to assess their sequence identity (exceeding 95%) and query coverage (surpassing 95%). From the constructed phylogenetic tree (Fig. 7), almost every region irrespective of elevation had culturable *Bacillus sp.* which belongs to the phylum Firmicutes, whereas the *Apilactobacillus kunkeei* and *Pantoea dispersa* belonging to gamma- proteobacteria were found in higher elevation i.e. (> 1400 m MSL). In moderate elevation (600- 1000 m MSL) population carried predominantly *bacillus sp.* under phylum Firmicutes and bacterial isolates belonging to Phyla Gamma-Proteobacteria viz., *Enterobacter sp.* and *Pantoea agglomerans.* Whereas bacteria belonging to phylum Actinobacteria were also found. The gram-positive bacterial isolates (*Bacillus velezensis, Bacillus sp., Bacillus pumilus, Bacillus licheniformis, Bacillus pocheonensis, Brevibacillus sp., Bacillus endophyticus, Bacillus altitudinis and Priestia megaterium)* dominated among the plain population (< 200 m MSL), whereas the higher elevation population was found to have the gram-negative proteobacteria isolates (*Pantoea dispersa* and *Pantoea anthophila*) along with probiotic bacteria i.e. *Apilactobacillus kunkeei* which falls under the phylum Firmicutes was observed. The moderately elevated regions (400 m – 1000 m MSL), *Bacillus sp.* was found predominantly along with the Actinobacteria.

Discussion

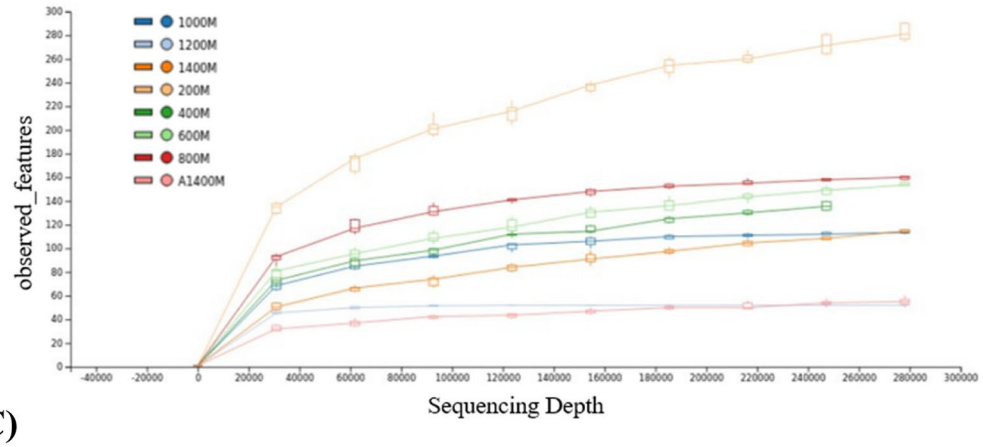
This study characterizes the microbiota of the honey bee species *A. cerana* collected from different elevations ranging from 200 to 1400 m altitudes in Tamil Nadu, which analyses the abundance and diversity of bacterial communities present in the gut and reveals the potential adaptations of gut microbiota to different ecological niches. The core microbiome genera abundance across different elevations in Tamil Nadu aligns with previous studies, confirming that Gilliamella and Lactobacillus are dominant members of the core gut bacteria with prevalences of 60.5% and 32.25%, respectively [15]. Additionally, Snodgrassella and Frischella were identified as relatively abundant genera, though with lower prevalences of 1.3% and 0.8%, respectively. The expandable nature of the *Gilliamella* pan-genome may lead to adaptability in diverse environmental conditions, resulting in high species diversity [16, 17]. Moreover, the abundance



A)



B)



C)

Fig. 5 Alpha-Diversity indices: **A**) Chao1, **B**) Shannon and **C**) Observed Features—represents bacterial diversity variation among the elevations

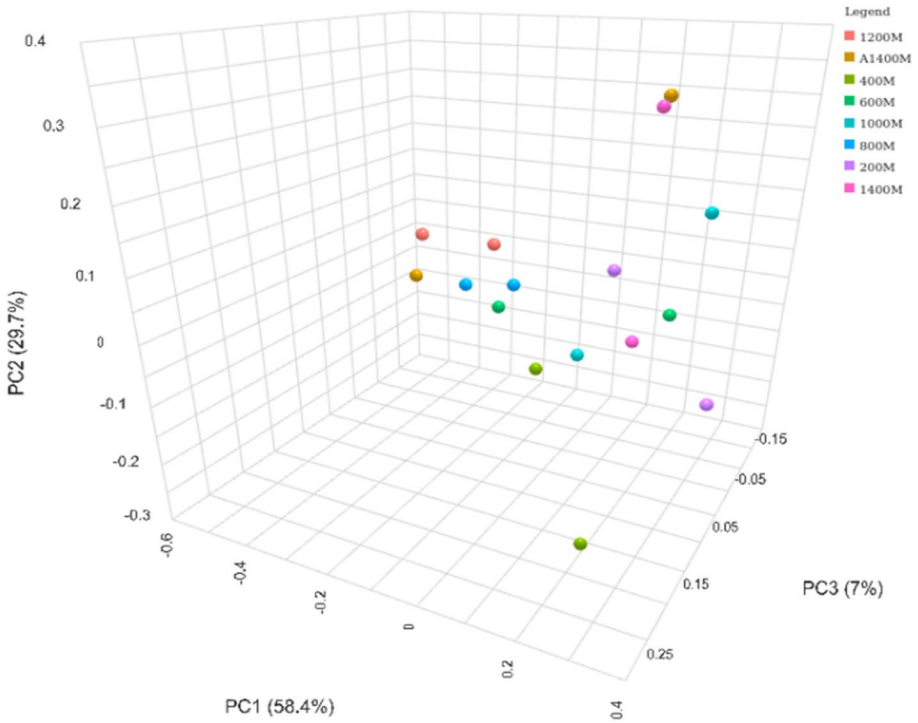


Fig. 6 Beta-diversity analysis revealed variations in bacterial communities across different locations. This analysis, which used Principal Coordinates based on Bray–Curtis dissimilarity, showed that different shades of each color represent the distribution of microbiota in the respective locations

plays a ubiquitous role in the gut of honey bees. *Gilliamella* colonizes the *Snodgrassella* layer to produce a thick biofilm, with the ability to break down polysaccharides and establish a nutritional connection with other symbionts and the host, while *Snodgrassella* acts as an oxidizer of carboxylic acids and induces a immune response in honey bee [18]. The dominant species present in all elevations was *Gilliamella apicola* which aids in the digestion of pollen by breaking down complex sugars and degradation of pectin, providing the bee with essential nutrients [19].

Lactobacillus species are one of the core microbiome in our study. The abundance of *Lactobacillus* may be due to fructose-based diets of the honey bee, as these microorganisms are frequently located in environments abundant in fructose, including floral structures and the exteriors of fruits. [20]. A well-known Fructophilic Lactic Acid Bacterium (FLAB), *Lactobacillus kunkeei*, is predominantly found in 600 m elevation has a function of showing resistant against various pathogens infecting honey bees, such as *Paenibacillus larvae*, *Ascosphaera apis, Nosema ceranae*, etc. [5, 21] due to antibacterial activity via the production of antimicrobial peptides or protein. *L. kunkeei* plays a major role as a gut microbe in honey bee against Sac Brood virus in *A. cerana*. Parallelly helps in protection against bee pathogen *Serratia marcescens* and increases bee survival rates by inhibiting the proliferation of these pathogens in the gut [22]. It is the most important probiotic genus in the digestive system of honey bees, playing crucial roles in carbohydrate metabolism [23] and defending the hosts by producing antimicrobial substances (like organic acids, diacetyl, benzoate, and bacteriocins) and triggering immune responses [4, 5, 15, 24].

The plains exhibited greater species diversity, including *Bombella apis*, *Klebsiella oxytoca*, and *Serratia marcescens*. The *Bombella apis* associated with larvae, was the most prevalent in fifth-instar larvae. This colonization resulted in an increase in the pupation rate, emergence rate, and overall survival of *A. cerana* larvae [1]. The colonization of gut bacteria can be affected by environmental (non-core) bacteria, artificial nutrition, and the extent and nature of host exposure. Higher frequencies of *Enterobacteriaceae*, related OTUs were found in the guts of *A. cerana* [25]. The *Enterobacter* genus is mainly involved in lactose fermentation. *K. oxytoca, S. marcescens and E. cloacae* isolates display antimicrobial resistance [26].

Bacillus sp. are commonly found in the gut of honey bees and helps in digestion, the lipopeptide surfactin produced by *Bacillus*, was effective in inhibiting pathogens such as *Paenibacillus larvae*, *Ascosphaera apis*, and *Nosema ceranae* [27]. Further, it shows antagonistic

Table 3 Identified gut bacteria from populations of *Apis cerana* at different elevation

Altitude	Sample ID	Bacteria Name	Phylum
200 m MSL	Chidambaram	*Bacillus velezensis*	*Firmicutes*
	Karaikal	*Bacillus sp.*	*Firmicutes*
	Karaikal	*Bacillus pumilus*	*Firmicutes*
	Virudhachalam	*Bacillus licheniformis*	*Firmicutes*
	Gingee	*Bacillus pocheonensis*	*Firmicutes*
	Karaikal	*Brevibacillus sp.*	*Firmicutes*
	Karaikal	*Bacillus endophyticus*	*Firmicutes*
	Karaikal	*Bacillus altitudinis*	*Firmicutes*
	Karaikal	*Bacillus sp.*	*Firmicutes*
	Karaikal	*Priestia megaterium*	*Firmicutes*
400 m MSL	Edapadi	*Micrococcus yunnanensis*	*Actinobacteria*
	Edapadi	*Staphylococcus epidermidis*	*Firmicutes*
	Edapadi	*Pantoea sp.*	*Proteobacteria*
	Edapadi	*Enterobacter cloacae*	*Proteobacteria*
	Edapadi	*Bacillus licheniformis*	*Firmicutes*
	Pollachi	*Bacillus haynesii*	*Firmicutes*
	Pollachi	*Bacillus safensis*	*Firmicutes*
600 m MSL	Krishnagiri	*Bacillus safensis*	*Firmicutes*
	Coimbatore	*Paenibacillus urinalis*	*Firmicutes*
	Coimbatore	*Actinobacterium*	*Actinobacteria*
	Krishnagiri	*Bacillus glycinifermentans*	*Firmicutes*
800 m MSL	Kodaikanal Foothills	*Bacillus sonorensis*	*Firmicutes*
	Kodaikanal Foothills	*Micrococcus sp.*	*Actinobacteria*
	Attur	*Bacillus sp.*	*Firmicutes*
1000 m MSL	Masinagudi	*Enterobacter cloacae*	*Proteobacteria*
	Masinagudi	*Pantoea agglomerans*	*Proteobacteria*
	Siruvani	*Terribacillus sp.*	*Firmicutes*
	Siruvani	*Terribacillus aidingensis*	*Firmicutes*
1200 m MSL	Valparai	*Bacillus sp.*	*Firmicutes*
	Valparai	*Bacillus sonorensis*	*Firmicutes*
1400 m MSL	Sirumalai	*Bacillus aerius*	*Firmicutes*
	Yercaud	*Bacillus cereus*	*Firmicutes*
	Yercaud	*Bacillus cereus*	*Firmicutes*
	Yercaud	*Bacillus haynesii*	*Firmicutes*
	Yercaud	*Bacillus paramycoides*	*Firmicutes*
A1400 m MSL	Ooty	*Apilactobacillus kunkeei*	*Firmicutes*
	Ooty	*Pantoea dispersa*	*Proteobacteria*
	Ooty	*Pantoea anthophila*	*Proteobacteria*

effect on *Paenibacillus*, a causative agent of American foulbrood disease [11], it indicates that the *Bacillus sp.* is found irrespective of elevation as it is important bacterial population in honey bee's crucial gut health. *Actinobacteria* which is found in moderately elevated region exhibited strong antimicrobial activity against various pathogens, including plant pathogenic bacteria (*Ralstonia solanacearum, Xanthomonas campestris*), an insect pathogen (*Beauveria bassiana*, P. larvae), plant fungal pathogens (*Fusarium oxysporum*, *Botrytis cinerea*) and human food-borne bacteria (*S. aureus*), [17, 28, 29].

Our analysis found *Pantoea sp.* in various locations. *Pantoea* species are gram-negative bacteria belonging to the Enterobacteriaceae family, typically associated with plants as either epiphytes or pathogens. Over the past decade, *Pantoea* species have been increasingly recognized as re-emerging pathogens responsible for various diseases in rice plants [30]. These bacteria may enter the

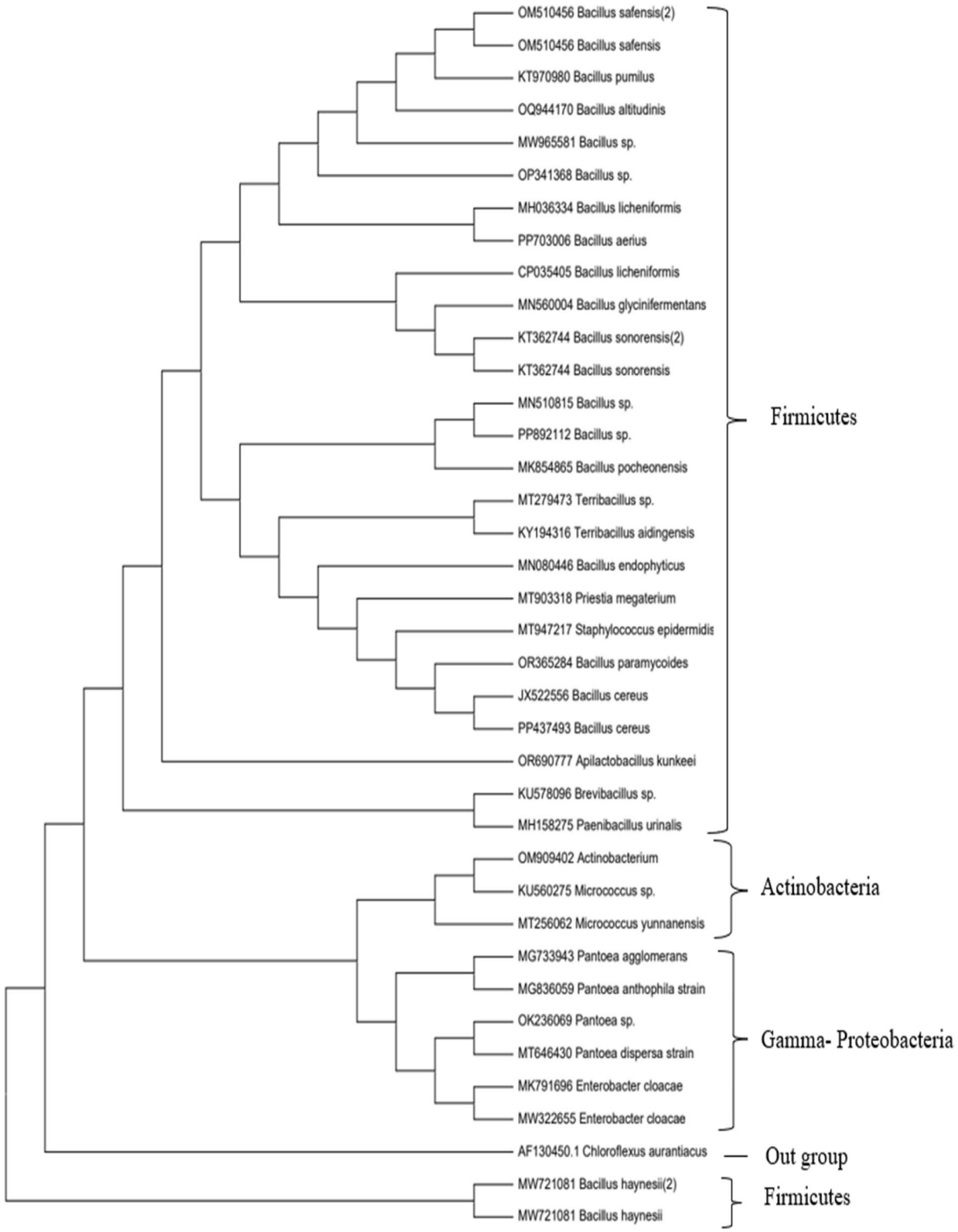


Fig. 7 Phylogenetic dendrogram constructed utilizing the nucleotide sequences of the 16S ribosomal DNA gene. (Maximum Likelihood analysis) using MEGA 11

bee gut during their foraging activities [13]. The gut bacterial community of honey bees exhibit notable seasonal variations. According to Li et al. (2022) [27], non-core bacteria become dominant during winter, while core gut bacteria significantly decrease, this observation supports with the result of abundant *Bacillus sp*. in plains compared to higher altitudes as the temperature decreases. Studying these bacterial networks provides insights into bee diseases and the interactions between hosts and microbes, which can help enhance honey bee health and lead to the discovery of new biotechnologically valuable molecules and enzymes [31].

This study emphasizes that the diversity of honey bee gut microbiota is influenced by a combination of biotic and abiotic factors, offering valuable insights into the underlying ecological dynamics. Biotic factors, such as consistent diets and mechanisms of microbial transmission, play a crucial role. For example, uniform floral resources across altitudes result in similar dietary inputs, fostering comparable microbial profiles in the bees' guts. Additionally, unique social behaviours in honey bees, including trophallaxis the exchange of food within the colony and grooming, promote horizontal microbial transfer. These processes help homogenize microbial communities among individuals, even in differing environmental conditions.

In contrast, abiotic factors like temperature fluctuations and humidity levels have a substantial impact on gut microbiota composition and often surpass the influence of altitude. For instance, lower temperatures or variations in habitat diversity at higher elevations may alter the external microbial environment, indirectly affecting gut microbial communities. Moreover, human activities such as pesticide use, habitat disruption, and pollution introduce additional stressors, reshaping the microbial landscape and further influencing microbial diversity and composition [32]. The interplay of these biotic and abiotic factors highlights the complex ecological forces shaping gut microbiota in *Apis cerana* and underscores the importance of considering both internal and environmental variables when evaluating bee health and microbiota dynamics. Our results align with previous studies on the role of the gut microbiome in bee health, highlighting elevation gradient as a contributing factor [33]. These diverse compositions of the honey bees gut microbiota may be due to the different geographic origins, genetic background and food sources influenced by distinct living conditions. This study explores how the abiotic factor of temperature, compared to the biotic factor of food source, affects the organization of the gut bacterial community across different elevation gradients. The study found that honey bee gut microbes at low altitudes exhibit greater diversity, likely influenced by environmental factors of temperature and food sources prevailing in that region. Conversely, at higher altitudes, the gut microbiota is dominated by core bacteria like Gilliamella and Lactobacillus, with less microbial diversity. This suggests that environmental factors, rather than food habitat alone, significantly impact gut microbial diversity. This highlights the importance of abiotic factors like temperature and environmental interventions in shaping the gut microbiota across different altitudes.

Conclusion

The gut microbiota of social bees is an emerging model to study the genomic diversity and evolution of honey bee-associated bacterial communities. Considering the uniqueness and stability of the bee gut microbiome, it should be regarded as a fundamental aspect of bee biology. Gaining deeper insights into the native bacterial diversity will not only reinforce the honey bee's role as a key model for studying gut microbiota, but could also contribute to solving global issues like bee population decline and developing climate-resilient agriculture by improving pollination services. Understanding this microbial community will undoubtedly provide new insights into enhancing bee health and addressing critical, unresolved questions in host-microbe symbiosis. In addition, genetic modification of these bacteria could allow the researchers to potentially engineer them for the benefit of the host. Exploring native bacterial diversity will strengthen the honey bee's significance as a model for gut microbiota research while contributing to solutions for global challenges such as declining bee populations and advancing climate-resilient agriculture through enhanced pollination.

Supplementary Information

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Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.

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Authors' contributions
SM conceived the project and imputed it into the project idea and its execution, KH experimented, and analysed data. VRS performed sample collections. MS visualised and investigated the study. KH wrote the original draft. KH, SM, MS, NS, VRS, SS and MD wrote the manuscript. SS reviewed the manuscript. All authors read and approved the final manuscript for publication.

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Data availability
The raw data supporting the conclusions of this article are available in the NCBI public repository and are included in the supplementary information files (Table S2, Table S3 and OTU Comparison table).

Declarations

Ethics approval and Consent to participate
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Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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