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Host-Specific Gut Microbiota in Sympatric Honey Bees: Contrasting Microbial Diversity Between Native *Melipona scutellaris* and Managed *Apis mellifera* in the Atlantic Forest

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Abstract: Bees are essential pollinators with diverse gut microbiomes that vary across species and environments. While the microbiota of managed *Apis mellifera* is well studied, knowledge about native tropical bees such as *Melipona scutellaris* remains limited. Understanding these microbial communities is crucial for pollinator conservation and management. We characterized and compared the intestinal microbiota of worker bees from *A. mellifera* and *M. scutellaris* collected in the Atlantic forest biome. Composite samples of workers from each species were analyzed using high-throughput sequencing of bacterial marker genes, followed by standardized bioinformatic and statistical

approaches to assess taxonomic composition, diversity, and community structure. Results revealed distinct, species-specific gut microbiota profiles. *M. scutellaris* harbored a larger number of core bacterial taxa and exhibited greater phylogenetic diversity, whereas *A. mellifera* showed a more restricted but dominant core community. Only five taxa were shared between species. Phylogenetic diversity was significantly higher in *M. scutellaris* (Faith index: 12.736 vs 7.412, p = 0.009), while Shannon and Pielou indices showed no significant differences. Beta diversity analyses indicated structural differences between species (PERMANOVA: Jaccard p = 0.006; Bray-Curtis p = 0.003; UniFrac weighted p = 0.011; UniFrac unweighted p = 0.008). ANCOM identified *Lactobacillus*, *Anthococcus*, and *Bifidobacterium* as the most differentially abundant taxa, with *Anthococcus* absent in *A. mellifera*. Therefore, despite sharing the same environment, the two bee species maintain structurally distinct gut microbiotas, reflecting host specificity and evolutionary adaptation. These findings provide baseline information on native stingless bee microbiomes and contribute to strategies for conserving and managing tropical pollinators.

Keywords: Gut microbiome; *Melipona scutellaris*; *Apis mellifera*; Metabarcoding; Pollinator conservation

1. Introduction

Bees play fundamental ecological and economic roles as primary pollinators in both natural and agricultural ecosystems, being essential for maintaining biodiversity and ensuring food production. Within this group, species such as Africanized *Apis mellifera* and *Melipona scutellaris* stand out for their ecological relevance and distinct characteristics in behavior, biology, and geographical distribution.

Apis mellifera, an exotic species widely managed in various world regions, is known for its complex social behavior, high productivity, and adaptability (Scott Schneider et al. 2004; De Queiroz Rolim et al. 2018). In contrast, *Melipona scutellaris*, a native Brazilian bee belonging to the tribe Meliponini (stingless bees), represents an important model for studying native pollinators in tropical environments.

Scientific interest in bee gut microbiota has grown significantly, considering that gut dysbiosis can be associated with ecological disturbances and health implications, compromising the role of bees as pollinators (Engel et al. 2012). The intestinal microbiota of *A. mellifera* is relatively well-characterized and known for playing fundamental roles in digestion, immunity, and pathogen protection (Kwong and Moran 2016). However, studies on native bee microbiota, such as *M. scutellaris*, remain scarce, despite these species being essential for maintaining biodiversity in tropical ecosystems (Kwong et al. 2017a; Motta et al. 2018). The relevance of gut microbiota in native bee health is an emerging topic, especially considering increased population disturbances such as colony collapse, pesticide impacts, and environmental changes (Raymann and Moran 2018). Research indicates that microbiota composition can vary significantly between species and environments, suggesting co-evolutionary adaptation to local conditions (Martinson et al. 2012).

Comparative studies between native and exotic bees are essential for understanding microbial community evolution and adaptation to different ecological and physiological conditions. The absence of stingers, morphological differences, foraging strategies, colony organization, and feeding habits—

such as pollen and nectar storage in wax pots—are factors that may uniquely shape *Melipona* spp. gut microbiota composition.

This study aimed to characterize and compare the intestinal microbiota of worker bees from *Apis mellifera* and *Melipona scutellaris* sharing the same environment in the Atlantic Forest biome using 16S rRNA gene amplicon sequencing through a metabarcoding approach, providing insights into host-microbiome specificity in tropical bee species.

2. Methods

2.1 Study Design and Sample Collection

Sample collection was conducted in the Beekeeping Sector of the Federal University of Paraíba, Brazil located in the Atlantic Forest biome (Latitude: 6°58'19.77"S, Longitude: 35°43'16.88"W, Altitude: 522 m). Five composite samples were collected for each species, containing an average of 35 worker bees (nurse type) per sample, totaling five distinct colonies of *M. scutellaris* and five distinct colonies of Africanized *A. mellifera* (*A. mellifera scutellata* × sspp).

Collections occurred during the dry season (late November), characterized by high temperatures and minimal rainfall. All bees were collected from the same environmental conditions to minimize environmental variables. During *A. mellifera* colony handling, appropriate personal protective equipment was used. Bees were placed in sterile tubes containing 70% ethanol and stored at -20°C until dissection.

2.2 Sample Preparation and DNA Extraction

Bee intestines were dissected in a sterile environment. Before extraction, specimens were placed on sterile filter paper for 10 minutes for thawing and ethanol evaporation. Dissection involved making a cut in the last abdominal segment of each bee, transferring intestinal contents to sterile microtubes. Each sample consisted of grouped intestines from 35 individuals of the same species.

DNA extraction was performed using a commercial kit (PowerSoil kit, Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA integrity was verified by agarose gel electrophoresis, and concentration was determined using a microvolume spectrophotometer (Colibri LB 915, Titertek-Berthold, Germany).

2.3 Library Preparation and Sequencing

Libraries for 16S rRNA gene metabarcoding were prepared according to Illumina reference protocols (2013). The V3-V4 hypervariable region was amplified by PCR. Reactions (25 µL) consisted of 2.5 µL DNA (5 ng/µL), 5 µL each primer (341F and 805R), and 12 µL 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA).

Thermal cycling conditions were: initial denaturation at 95°C for 3 minutes; 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; final extension at 72°C for 5 minutes.

Amplified products were visualized in 1.5% agarose gel and purified with magnetic beads (AMPure XP, Beckman Coulter, USA). Dual indexes and Illumina sequencing adapters were added using the Nextera XT Index kit (Illumina). After magnetic bead purification, products were quantified by fluorometry (Qubit 2.0, Life Invitrogen, USA).

Libraries were pooled, denatured with NaOH, diluted in hybridization buffer, thermally denatured, and subjected to paired-end sequencing (2×250 cycles) on the Illumina MiSeq platform using 15% PhiX DNA as control.

2.4 Bioinformatics and Statistical Analysis

Raw paired sequences (demultiplexed) were processed in QIIME 2-2020.2 (Bolyen et al. 2019). Reads were filtered, normalized, truncated to 248 base pairs, and processed for chimera removal using DADA2, generating Amplicon Sequence Variants (ASVs) (Callahan et al. 2016). Sequences were aligned using "qiime fragment-insertion sepp" for phylogenetic analyses (Matsen et al. 2012).

Taxonomic composition was identified using a trained Bayesian classifier with 99% similarity threshold against the SILVA database (SILVA-132-99-nb-classifier.qza) through 'qiime feature-classifier classify-sklearn'. No contaminants were detected in negative controls.

Microbial diversity was assessed using Shannon indices (richness and abundance) and Pielou (evenness). Normality tests (Shapiro-Wilk) were applied before statistical comparisons. Normal data were analyzed using ANOVA with Tukey's post-hoc test; otherwise, Kruskal-Wallis followed by Dunn's test were applied ($\alpha = 0.05$) using laercio package version 1.0-0 in R 4.1.0 (Da silva, 2009).

Beta diversity was analyzed qualitatively using Jaccard coefficient (presence/absence) and Unweighted UniFrac (phylogenetic basis), and quantitatively using Bray-Curtis coefficient (abundance) and Weighted UniFrac (phylogenetic basis) in QIIME 2-2020.2. Group differences were tested by PERMANOVA (Anderson 2001).

Core taxa (present in all samples of a species) were identified using 'qiime feature-table core-features'. Differential abundance was tested using ANCOM. Significant ASVs were subjected to variance analysis (ANOVA) after normality checks. Beta diversity visualization was performed with Emperor 2020.2.0 plugin, and alpha diversity graphs were generated in R 4.1.0.

3. Results

3.1 Core Microbiota Characterization

Core microbiota analysis revealed distinct bacterial profiles between species. *M. scutellaris* demonstrated greater microbial complexity with 12 core taxa representing 22% of detected genera, including three *Lactobacillus*-associated taxa (*Lactobacillus* sp., *Lactobacillus* sp. MbHmro5, and uncultured *Lactobacillus* sp.), *Pectinatus* sp., *Acinetobacter*, *Bifidobacterium commune*, *Snodgrassella*, *Anthococcus* (uncultured *Streptococcus* sp.), and members of families Enterobacteriaceae, Prevotellaceae, Acetobacteraceae, and Leuconostocaceae.

A. mellifera core microbiota showed 7 core taxa representing 63% of detected genera: *Lactobacillus*, *Gilliamella*, *Snodgrassella*, *Bifidobacterium*, *Commensalibacter*, and members of families Rhizobiaceae and Orbaceae.

When combined, only five core taxa (5% of genera) were observed across both species: *Lactobacillus*, *Snodgrassella*, *Bifidobacterium*, *Acinetobacter*, and a member of Enterobacteriaceae (Figure 1).

3.2 Alpha Diversity

Microbial diversity comparison revealed significant differences in Faith index (Kruskal-Wallis: $p = 0.009$), indicating *M. scutellaris* exhibited greater phylogenetic diversity (12.736) than Africanized *A. mellifera* (7.412). However, Shannon ($p = 0.601$) and Pielou ($p = 0.075$) indices showed no significant differences, suggesting similar richness and evenness between species. The number of observed ASVs also did not differ significantly ($p = 0.341$), reinforcing that the key differences are related to the phylogenetic microbiota structure (Figure 2).

3.3 Beta Diversity

Dissimilarity analyses demonstrated marked microbial composition differences between species. Qualitative metrics—Jaccard coefficient (PERMANOVA: $p = 0.006$) and unweighted UniFrac ($p = 0.008$)—indicated significant variations in taxa presence/absence. Quantitative metrics—Bray-Curtis coefficient ($p = 0.003$) and weighted UniFrac ($p = 0.011$)—highlighted differences in relative abundance. These results confirm structurally distinct microbial communities between species in both taxonomic composition and abundance distribution (Figure 3).

3.4 Differential Abundance Analysis

ANCOM analysis identified *Lactobacillus* ($W = 13$), *Anthococcus* ($W = 13$), and *Bifidobacterium* ($W = 12$) as taxa contributing most to observed differences. Several taxa showed significant relative abundance variations between species (Supplementary Material S1).

Bifidobacterium (ANOVA: $p = 0.0157$) and *Anthococcus* (ANOVA: $p = 0.0091$) were more abundant in *M. scutellaris*, with *Anthococcus* completely absent in Africanized *A. mellifera*. Other taxa

including *Apibacter* (Kruskal-Wallis: p = 0.0053), *Lactobacillales* (p = 0.0070), and *Commensalibacter* (p = 0.0070) also exhibited significant differences.

Families Rhizobiaceae (p = 0.0082), Neisseriaceae (p = 0.0082), and Enterobacteriaceae (p = 0.0090) showed species-specific prevalence patterns, while *Snodgrassella* (p = 0.0090) and *Gilliamella* (p = 0.0088) exhibited distinct abundance patterns.

After identifying the different abundant species using ANCOM, we compared each group using ANOVA followed by Tukey test and Kruskal-Wallis followed by Dunn's tests, depending on the normality of data distribution, which was analyzed using Shapiro-Wilk test. Significant differences in gut bacterial composition were observed between *Melipona scutellaris* and *Apis mellifera scutellata x spp*. Among the taxa analyzed, *Bifidobacterium* was significantly more abundant in *Melipona* (p = 0.0157). Similarly, *Apibacter* (p = 0.005 / 0.0027), *Anthococcus* (p = 0.0091), *Lactobacillales* (p = 0.007 / 0.0035), *Commensalibacter* (p = 0.007 / 0.0035), *Rhizobiaceae* (p = 0.008 / 0.0041), *Snodgrassella* (p = 0.009 / 0.0045), *Neisseriaceae* (p = 0.008 / 0.0041), *Enterobacteriaceae* (p = 0.009 / 0.0045), *Frischella* (p = 0.007 / 0.0035), *Gilliamella* (p = 0.0088 / 0.0044), and *Orbaceae* (p = 0.02 / 0.02) were also found at significantly higher relative abundance in *Melipona scutellaris*. In contrast, no significant differences were detected for *Staphylococcus* (p = 0.136 / 0.068), *Lactobacillus* (p = 0.38), and *Rosenbergiella* (p = 0.40) (Supplementary Material S2).

Overall, these findings indicate that most of the differential taxa identified exhibited higher relative abundance in *Melipona scutellaris*, reinforcing species-specific patterns in the gut microbiota of stingless bees compared to honey bees.

4. Discussion

4.1 Species-Specific Microbiota Structure

The striking differences in microbial community structure and composition between *A. mellifera* and *M. scutellaris*, cohabiting the same environment, suggest specific host adaptations. This disparity likely reflects differences in ecological niches, including diet variations, foraging behavior,

and nest characteristics (Kwong and Moran 2016; Kwong et al. 2017b). The microbiota of *M. scutellaris* showed higher diversity, with more rare and conditionally abundant taxa, suggesting a more flexible microbial community possibly influenced by tropical environments and native floral resources. Similar findings were reported by Menezes *et al.* (2015) in stingless bees (*Melipona fasciculata*), where environmental drivers shaped gut microbial diversity. In contrast, *A. mellifera* maintained a less phylogenetically diverse community, consistent with studies showing that managed honeybee populations often display reduced microbiota variability due to homogenized diets and anthropogenic pressures (Soares et al. 2021; Dimov 2022).

The larger number of core taxa in *M. scutellaris* suggests a more diverse and possibly more resilient bacterial community, reflecting greater ecological complexity in gut microbiota symbiotic relationships (da S. Sant'ana et al. 2020). Multiple *Lactobacillus* strains and representatives of families Prevotellaceae, Acetobacteraceae, and Leuconostocaceae—typical of fermentable sugar-rich environments—may relate to distinct feeding habits, especially consumption of pollen and nectar stored in pots (Anderson et al. 2013).

The *A. mellifera* core microbiota, although less diverse, concentrated in highly frequent taxa such as *Gilliamella* and *Snodgrassella*, consistent with described microbial cores for *Apis* genus (Engel and Moran 2013; Raymann and Moran 2018). Low overlap between species' bacterial cores highlights distinct ecological and physiological pressures shaping microbial communities, possibly related to phylogeny, diet, or nest microenvironment (Moran et al. 2012).

4.2 Phylogenetic Diversity Implications

Despite similar taxonomic richness and abundance distributions (evidenced by non-significant Shannon, Pielou indices, and ASV numbers), *M. scutellaris* showed significantly higher phylogenetic diversity (Faith's index). This suggests microorganisms in *M. scutellaris* are more phylogenetically distant, indicating greater evolutionary diversity among bacterial lineages (Lozupone et al. 2012).

This phylogenetic diversity may relate to specific ecological or physiological factors, such as foraging strategies, stored food types, or nest microenvironment (Soares et al. 2025a). Greater phylogenetic diversity may also reflect higher microbiome functional plasticity, providing potential advantages in digestion, immune defense, and environmental stress adaptation. Lower phylogenetic diversity in *A. mellifera* suggests more conserved, specialized microbiota consistent with previous *Apis* genus studies (Engel et al. 2012).

Host-microbiome specificity reinforces that biological characteristics are determinant in community structuring, as both species inhabit the same environment yet show phylogenetic microbiota differences (Kwong et al. 2017b).

The results demonstrate that both *Melipona scutellaris* and *Apis mellifera* harbor gut microbial communities dominated by core bacterial phyla typical of social bees, including *Snodgrassella*, *Gilliamella*, *Lactobacillus*, and *Bifidobacterium*. However, *M. scutellaris* presented unique bacterial associations, including higher abundances of *Lactobacillus* and other Firmicutes taxa ($p < 0.05$), while *A. mellifera* maintained a more stable presence of *Snodgrassella alvi* and *Gilliamella apicola* ($p < 0.05$). These findings reinforce the existence of species-specific microbiota structures, as observed by Kwong and Moran (2016), who highlighted that while core taxa are conserved, their relative abundance is strongly shaped by host identity and evolutionary history.

4.3 Community Composition Differences

Beta diversity analysis confirmed consistent, statistically significant differences in intestinal microbial composition between species. PERMANOVA results across different metrics indicate distinctly structured bacterial communities in each species under similar environmental conditions, suggesting intrinsic host factors outweigh environmental ones (Moran et al. 2012).

Qualitative metrics (Jaccard and unweighted UniFrac) indicated differences in taxa presence/absence, reflecting possible physiological or behavioral barriers to microbial colonization

(Powell et al. 2014). Quantitative metrics (Bray-Curtis and weighted UniFrac) confirmed these differences extend to relative abundance and bacterial community phylogenetic composition (Lozupone et al. 2007).

These significant differences in microbial richness and phylogenetic composition between both species reinforces host´s role as a primary filtering mechanism in microbiota selection and evolution (Soares et al. 2025b).

These findings have important implications for understanding microbial ecology in tropical bees, with potential applications in conservation programs, health management, and genetic improvement.

4.4 Functional Implications of Differential Taxa

Greater abundance of *Bifidobacterium* and *Anthococcus* in *M. scutellaris*, with *Anthococcus* completely absent in *A. mellifera*, suggests potentially species-specific symbiotic relationships. *Bifidobacterium* and *Lactobacillus* genera are known for beneficial functions including polysaccharide fermentation, immune system modulation, and pathogen protection (Olofsson and Vásquez 2008; Zheng et al. 2020).

Anthococcus absence in *A. mellifera* may indicate differences in food substrate composition, intestinal colonization mechanisms, or immunological factors limiting establishment. Differences in other taxa (*Apibacter*, *Lactobacillales*, *Commensalibacter*) and families (Rhizobiaceae, Neisseriaceae, Enterobacteriaceae) suggest host-dependent microbial selection influenced by distinct physiological and behavioral characteristics.

In addition, the enrichment of Lactobacillaceae and Bifidobacteriaceae in *M. scutellaris* suggests enhanced carbohydrate metabolism and protective functions against fungal and bacterial pathogens, consistent with the functional predictions (Bleau et al. 2020; Miller et al. 2021). Conversely, the dominance of *Gilliamella* and *Snodgrassella* in *A. mellifera* reflects specialization in pollen digestion and nitrogen metabolism, functions essential for colony productivity but potentially

less flexible in response to environmental fluctuations. These functional contrasts highlight that microbiota composition may directly influence host resilience and ecological adaptation, aligning with evidence that gut symbionts are crucial mediators of bee health and colony survival (Raymann and Moran 2018).

Our study combined univariate tests and compositional analysis (ANCOM) to strength statistical robustness, providing multiple evidence lines for microbial differentiation between species. While traditional tests detect individual taxa relative abundance variations, ANCOM considers data compositional structure holistically, offering more reliable perspectives on microorganisms' relative importance in a community context. Despite that, our study has several limitations. The data lacks temporal analysis and the identification of environmental factors related to the taxa differences. Furthermore, a 16S rRNA metabarcoding study lacks functional information, shedding light on metabolic pathways. For such purposes, we highlight the possibility of integrating high-throughput gut metabolomic analysis approaches for complete functional inferences.

5. Conclusions

The structurally distinct intestinal microbiotas with marked differences in taxonomic composition, phylogenetic diversity, and key taxa relative abundances between Africanized *A. mellifera* and *M. scutellaris* bees reinforces the host's central role in gut microbiota modulation and highlight the need to consider phylogenetic, ecological, and behavioral factors when investigating pollinator microbial ecology. Beyond expanding knowledge about tropical bee symbiotic interactions, this work provides a foundation for future functional and biotechnological investigations aimed at native and introduced species conservation and sustainable management.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

All authors have read and approved the contents of this manuscript, and consent the communications on their behalf by the corresponding author.

Availability of Data and Materials

The 16S rRNA gene sequencing datasets generated and analyzed during this study are available in the NCBI Sequence Read Archive (SRA) repository under BioProject accession number PRJNA PRJNA732391. The dataset supporting the conclusions of this article will be included within the article and its additional files.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

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Priscylla Carvalho Vasconcelos: design of the work, article revising.

Núbia Michelle Vieira da Silva: design of the work, acquisition of data.

Adriana Evangelista Rodrigues: conception and design of the work.

Vanessa L. Hale: conception, analysis, and interpretation of data, article drafting, and revising.

Patrício Borges Maracajá: conception, design of the work, interpretation of data, article drafting and revising.

Celso José Bruno de Oliveira: conception, design of the work, interpretation of data, article drafting and revising.

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References

Anderson KE, Sheehan TH, Mott BM, et al (2013) Microbial ecology of the hive and pollination landscape: Bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (Apis mellifera). PLoS One 8:. https://doi.org/10.1371/journal.pone.0083125

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Bleau N, Bouslama S, Giovenazzo P, Derome N (2020) Dynamics of the Honeybee (Apis mellifera) Gut Microbiota Throughout the Overwintering Period in Canada. Microorganisms 8:1146. https://doi.org/10.3390/microorganisms8081146

Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

da Silva, L. J. *laercio-package: Duncan test, Tukey test and Scott-Knott test. R Package Version 1.0–1* (/CRAN. R-project, 2009).

da S. Sant'ana R, de Carvalho CAL, Oda-Souza M, et al (2020) Characterization of honey of stingless bees from the Brazilian semi-arid region. Food Chem 327:. https://doi.org/10.1016/j.foodchem.2020.127041

De Queiroz Rolim MB, Prestes de Andrade G, De Queiroz Rolim AM, et al (2018) Generalidades sobre o mel e parâmetros de qualidade no Brasil: revisão. Med Veterinária 12:73.

https://doi.org/10.26605/medvet-v12n1-2154

Dimov SG (2022) Environmental and Anthropogenic Influence on the Core Beneficial Honeybee Gut Microbiota—A Short Communication from Bulgaria. Bacteria 1:88–95. https://doi.org/10.3390/bacteria1020008

Engel P, Martinson VG, Moran NA (2012) Functional diversity within the simple gut microbiota of the honey bee. Proc Natl Acad Sci 109:11002–11007. https://doi.org/10.1073/pnas.1202970109

Engel P, Moran NA (2013) Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. Gut Microbes 4:60–65. https://doi.org/10.4161/gmic.22517

Kwong WK, Medina LA, Koch H, et al (2017a) Dynamic microbiome evolution in social bees. Sci Adv 3:e1600513. https://doi.org/10.1126/sciadv.1600513

Kwong WK, Medina LA, Koch H, et al (2017b) Dynamic microbiome evolution in social bees. Sci Adv 3:e1600513. https://doi.org/10.1126/sciadv.1600513

Kwong WK, Moran NA (2016) Gut microbial communities of social bees. Nat Rev Microbiol 14:374–384. https://doi.org/10.1038/nrmicro.2016.43

Lozupone CA, Hamady M, Kelley ST, Knight R (2007) Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol 73:1576–1585. https://doi.org/10.1128/AEM.01996-06

Lozupone CA, Stombaugh JI, Gordon JI, et al (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230. https://doi.org/10.1038/nature11550

Martinson VG, Moy J, Moran NA (2012) Establishment of characteristic gut bacteria during development of the honeybee worker. Appl Environ Microbiol 78:2830–2840. https://doi.org/10.1128/AEM.07810-11

Matsen FA, Hoffman NG, Gallagher A, Stamatakis A (2012) A format for phylogenetic placements. PLoS One 7:e31009. https://doi.org/10.1371/journal.pone.0031009

388 Menezes C, Vollet-Neto A, Marsaioli AJ, et al (2015) A Brazilian social bee must cultivate fungus to
389 survive. Curr Biol 25:2851–2855. https://doi.org/10.1016/j.cub.2015.09.028
390 Miller DL, Smith EA, Newton ILG (2021) A Bacterial Symbiont Protects Honey Bees from Fungal
391 Disease. MBio 12:2020.01.21.914325. https://doi.org/10.1128/mBio.00503-21
392 Moran NA, Hansen AK, Powell JE, Sabree ZL (2012) Distinctive Gut Microbiota of Honey Bees
393 Assessed Using Deep Sampling from Individual Worker Bees. PLoS One 7:e36393.
394 https://doi.org/10.1371/journal.pone.0036393
395 Motta EVS, Raymann K, Moran NA (2018) Glyphosate perturbs the gut microbiota of honey bees.
396 Proc Natl Acad Sci 115:10305–10310. https://doi.org/10.1073/pnas.1803880115
397 Olofsson TC, Vásquez A (2008) Detection and Identification of a Novel Lactic Acid Bacterial Flora
398 Within the Honey Stomach of the Honeybee Apis mellifera. Curr Microbiol 57:356–363.
399 https://doi.org/10.1007/s00284-008-9202-0
400 Powell JE, Martinson VG, Urban-Mead K, Moran NA (2014) Routes of Acquisition of the Gut
401 Microbiota of the Honey Bee Apis mellifera. Appl Environ Microbiol 80:7378–7387.
402 https://doi.org/https://doi.org/10.1128/AEM.01861-14
403 Raymann K, Moran NA (2018) The role of the gut microbiome in health and disease of adult honey
404 bee workers. Curr Opin Insect Sci 26:97–104. https://doi.org/10.1016/j.cois.2018.02.012
405 Scott Schneider S, DeGrandi-Hoffman G, Smith DR (2004) The African honey bee: factors
406 contributing to a successful biological invasion. Annu Rev Entomol 49:351–376.
407 https://doi.org/10.1146/annurev.ento.49.061802.123359
408 Soares KO, Da Rocha TF, Hale VL, et al (2025a) Comparing the impact of landscape on the gut
409 microbiome of Apis mellifera in Atlantic Forest and Caatinga Biomes. Sci Rep 15:1–9.
410 https://doi.org/10.1038/s41598-025-85114-y
411 Soares KO, de Oliveira CJB, Martínez Villegas LE, et al (2025b) Are you my mother? When host
412 genetics and gut microbiota tell different phylogenetic stories in the Africanized honey bee

hybrid (Apis mellifera scutellata × sspp.). Microbiol Spectr 13:. https://doi.org/10.1128/spectrum.02475-24

Soares KO, Oliveira CJB de, Rodrigues AE, et al (2021) Tetracycline Exposure Alters Key Gut Microbiota in Africanized Honey Bees (Apis mellifera scutellata x spp.). Front Ecol Evol 9:1–11. https://doi.org/10.3389/fevo.2021.716660

Zheng D, Liwinski T, Elinav E (2020) Interaction between microbiota and immunity in health and disease. Cell Res 30:492–506. https://doi.org/10.1038/s41422-020-0332-7

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Sample collection, laboratory preparation, and DNA, Library preparation and sequencing, Bioinformatics and statistical analyses were performed by Kilmer Oliveira Soares, Priscylla CarvalhoVasconcelos, Núbia Michelle Vieira da Silva and Octavio Gomes da Cunha Filho. The first draft of the manuscript was written by Kilmer

Oliveira Soares, and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Data Availability

The 16S rRNA gene sequencing datasets generated and analyzed during this study are available in the NCBI Sequence Read Archive (SRA) repository under BioProject accession number PRJNA PRJNA732391. The dataset supporting the conclusions of this article will be included within the article and its additional files.

Ethics Approval

Sampling of *Melipona scutellaris* and Africanized *Apis mellifera* was conducted in accordance with institutional guidelines for non-cephalopod invertebrates. All experimental procedures were previously approved by the Biodiversity Authorization and Information System—SISBIO (Protocol #: 71750-1, approved on 09/19/2019).

Consent to Participate

Not applicable (this study did not involve human participants).

Consent to Publish

Not applicable (this study did not involve human participants).



Figure 1. Schematic representation of *Apis mellifera* and *Melipona scutellaris* with the shared intestinal bacterial community. Image generated with the assistance of the Gemini artificial intelligence (Google, 2025).

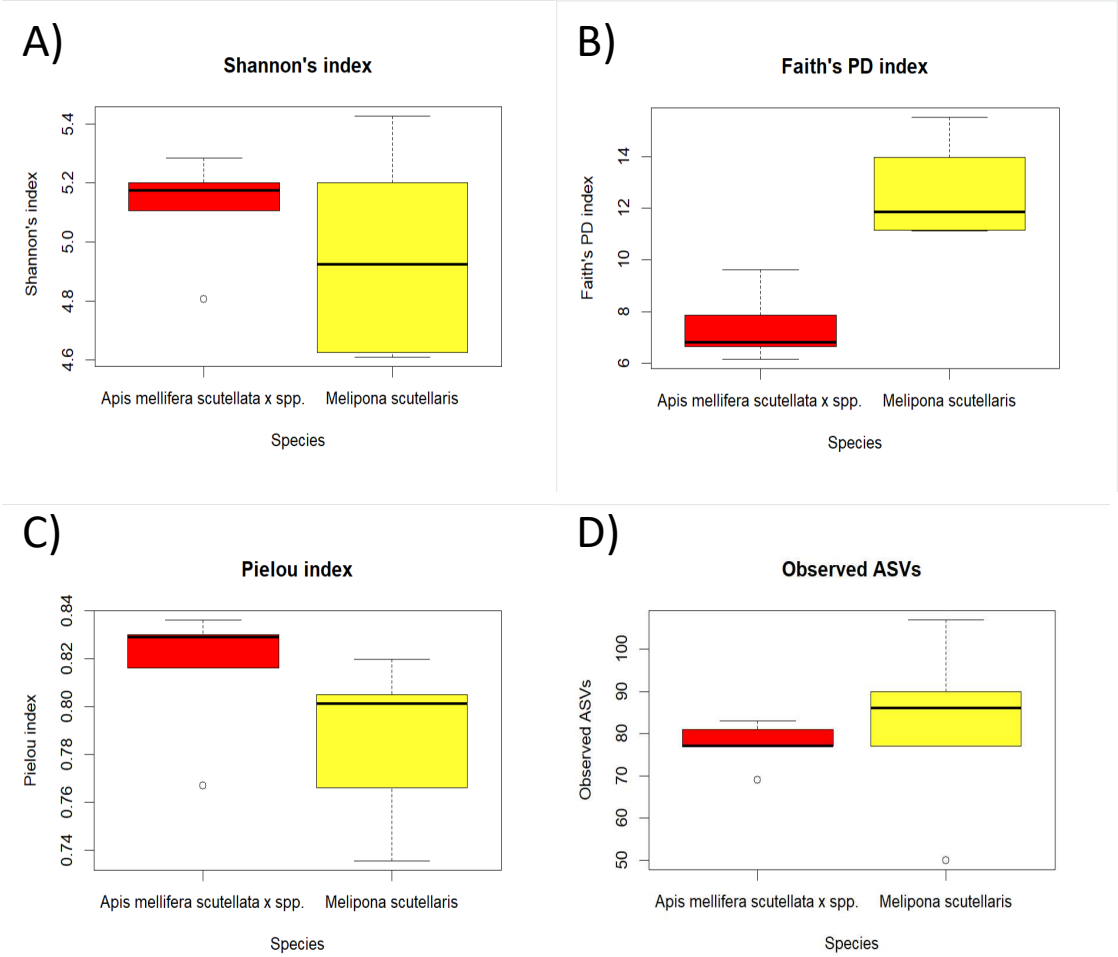


Figure 2. Gut microbial diversity *of Melipona scutellaris* and *Apis mellifera scutellata* x spp. (A) Shannon index, (B) Faith's PD, (C) Pielou's evenness, and (D) observed features. Box plots show outliers, first and third quartiles (lower and upper edges), and highest, lowest, and median values (horizontal black dash). (Kruskal-Wallis: Shannon index $P = 0.601$; Faith's PD $P = 0.009$; Pielou's evenness $P = 0.075$; observed features $P = 0.341$). Significant differences ($P \leq 0.05$ Tukey's test) are denoted with different letters over each box plot.

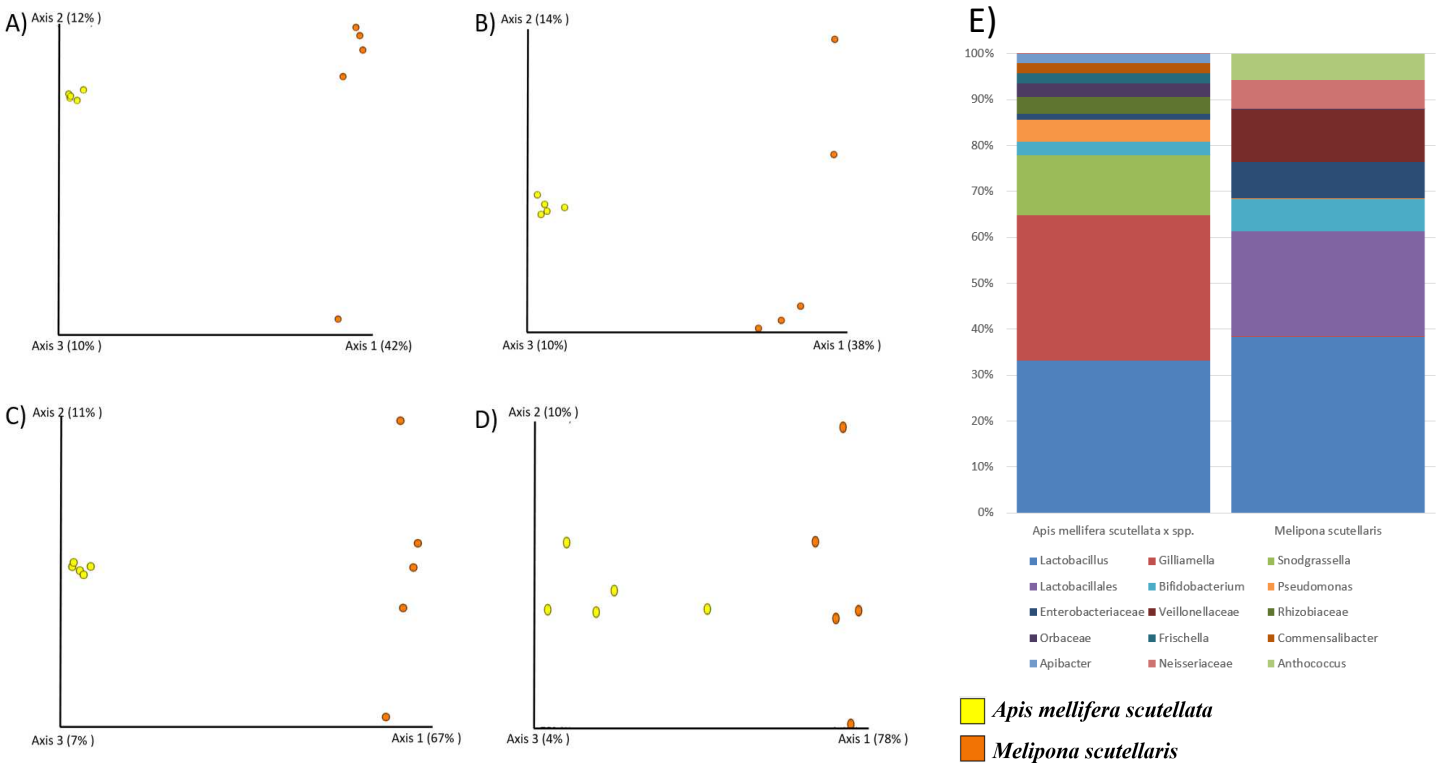


Figure 3. Gut microbial composition of *Apis mellifera scutellata* x spp. and *Melipona scutellaris*. Overall honey bee gut microbial composition demonstrated marked microbial composition differences between species. Qualitative metrics A) Jaccard coefficient (PERMANOVA: $p = 0.006$) and B) unweighted UniFrac ($p = 0.008$) indicated significant variations in taxa presence/absence. Quantitative metrics C) Bray-Curtis coefficient ($p = 0.003$) and D) weighted UniFrac ($p = 0.011$) highlighted differences in relative abundance.

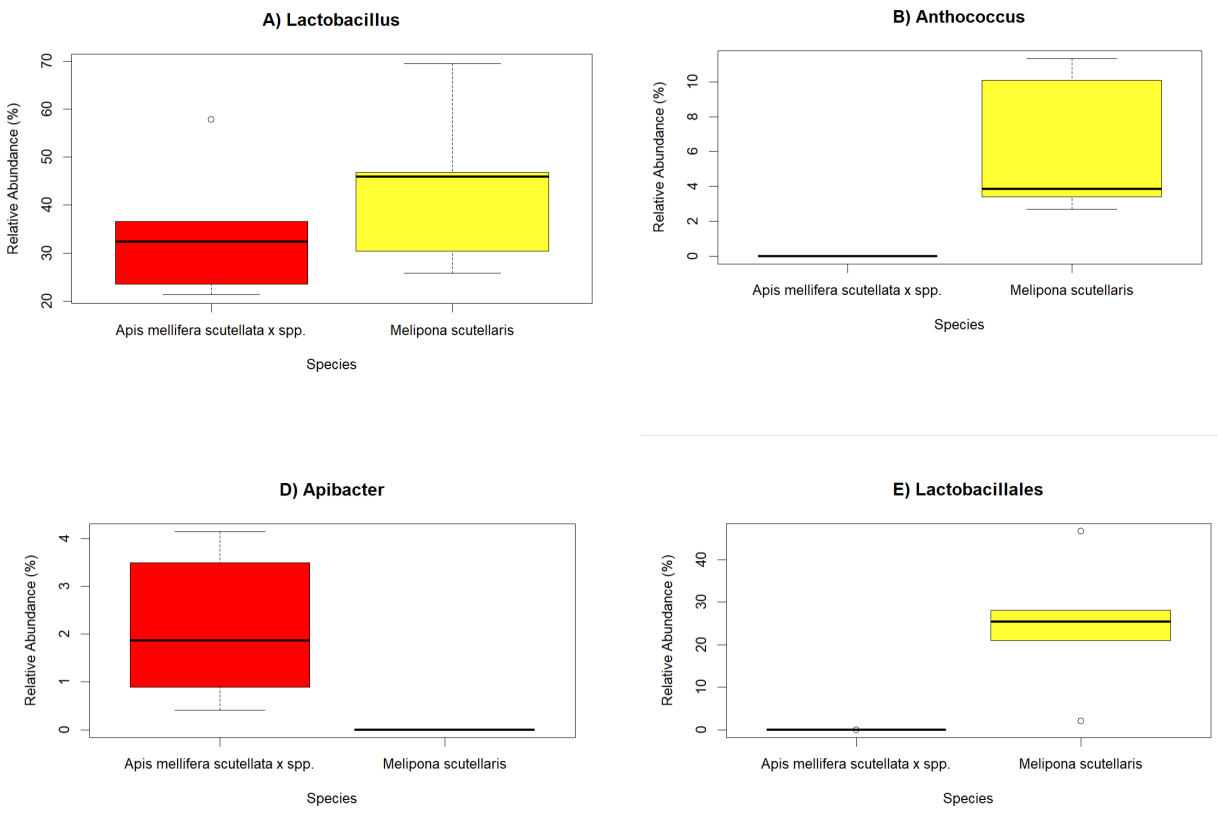


Figure 4. Relative abundance of dominant bacterial taxa in the gut microbiota of *Melipona scutellaris* and *Apis mellifera scutellata* × *spp.* (A) *Lactobacillus*, (B) *Anthococcus*, (C) *Bifidobacterium*, (D) *Apibacter*, (E) *Lactobacillales*, (F) *Commensalibacter*, (G) *Rhizobiaceae*, (H) *Neisseriaceae*, (I) *Enterobacteriaceae*, (J) *Snodgrassella*, and (K) *Gilliamella*. Box plots display outliers, first and third quartiles (lower and upper edges), and minimum, maximum, and median values (horizontal black line). Statistical analyses were performed using ANOVA followed by Tukey's test or Kruskal–Wallis followed by Dunn's post hoc test, as appropriate. Significant differences ($P \leq 0.05$) are indicated by different letters above the boxes. (Lactobacillus P = 0.38; Anthococcus P = 0.0091; Bifidobacterium P = 0.0157; Apibacter P = 0.005/0.0027; Lactobacillales P = 0.007/0.0035; Commensalibacter P = 0.007/0.0035; Rhizobiaceae P = 0.008/0.0041; Neisseriaceae P = 0.008/0.0041; Enterobacteriaceae P = 0.009/0.0045; Snodgrassella P = 0.009/0.0045; Gilliamella P = 0.0088/0.0044). Taxa with significant differences were more abundant in *M. scutellaris*.

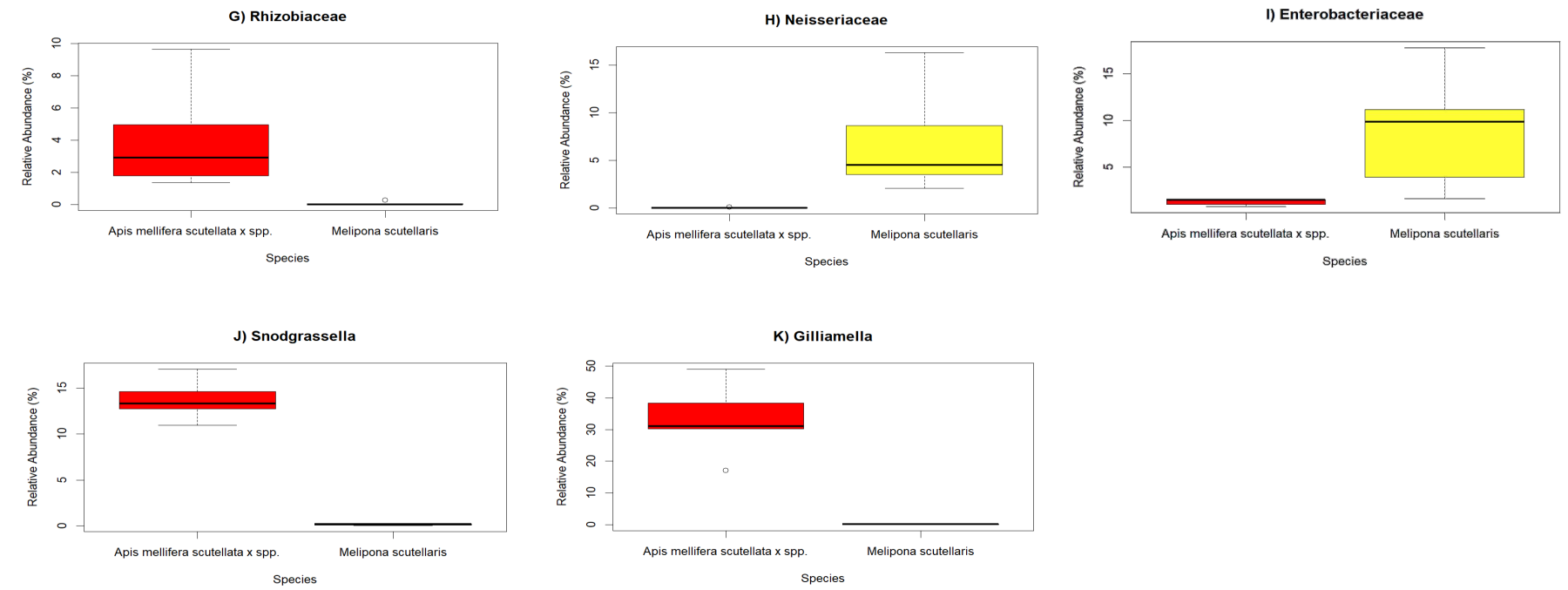


Figure 5. Relative abundance of dominant bacterial taxa in the gut microbiota of *Melipona scutellaris* and *Apis mellifera scutellata* × *spp.* (A) *Lactobacillus*, (B) *Anthococcus*, (C) *Bifidobacterium*, (D) *Apibacter*, (E) *Lactobacillales*, (F) *Commensalibacter*, (G) *Rhizobiaceae*, (H) *Neisseriaceae*, (I) *Enterobacteriaceae*, (J) *Snodgrassella*, and (K) *Gilliamella*. Box plots display outliers, first and third quartiles (lower and upper edges), and minimum, maximum, and median values (horizontal black line). Statistical analyses were performed using ANOVA followed by Tukey's test or Kruskal–Wallis followed by Dunn's post hoc test, as appropriate. Significant differences ($P \leq 0.05$) are indicated by different letters above the boxes.(Lactobacillus P = 0.38; Anthococcus P = 0.0091; Bifidobacterium P = 0.0157; Apibacter P = 0.005/0.0027; Lactobacillales P = 0.007/0.0035; Commensalibacter P = 0.007/0.0035; Rhizobiaceae P = 0.008/0.0041; Neisseriaceae P = 0.008/0.0041; Enterobacteriaceae P = 0.009/0.0045; Snodgrassella P = 0.009/0.0045; Gilliamella P = 0.0088/0.0044). Taxa with significant differences were more abundant in *M. scutellaris*.

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