

Phylosymbiosis with limited functional divergence in bee gut microbiota

Pierre Noiset

Ludwig-Maximilians-Universität München <https://orcid.org/0000-0003-0141-6051>

Andrea Pinos

University Hospital Carl Gustav Carus Dresden

Frederico Roncheti

Università degli Studi di Milano

Antonia Mayr

Bonn Institute of Organismic Biology

Fabienne Maihoff

Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg

<https://orcid.org/0000-0003-3246-3213>

Shao Chui

Technical University of Munich

Gemma Villagomez

Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg

Pedro Alonso-Alonso

University of Würzburg <https://orcid.org/0000-0001-8791-5227>

Gunnar Brehm

Friedrich-Schiller Universität, Jena

Alice Classen

University of Bremen <https://orcid.org/0000-0002-7813-8806>

Yenny Correa-Carmona

Friedrich-Schiller University Jena

Tim Heard

Sugarbag Bees

Kim Holzmann

University of Würzburg <https://orcid.org/0000-0002-2469-6212>

Sara Leonhardt

TU Munich <https://orcid.org/0000-0002-8154-9569>

Marcell Peters

University of Bremen

Carlo Polidori

Università degli Studi di Milano

Claus Rasmussen

Aarhus University <https://orcid.org/0000-0003-1529-6548>

Thomas Schmitt

Universität Würzburg <https://orcid.org/0000-0002-6719-8635>

Ingolf Steffan-Dewenter

University of Würzburg <https://orcid.org/0000-0003-1359-3944>

Helen Wallace

Queensland University of Technology,

Arne Weinhold

Ludwig-Maximilians-Universität München

Felipe Yon

Universidad Peruana Cayetano Heredia <https://orcid.org/0000-0002-5667-873X>

Alexander Keller

keller@bio.lmu.de

Ludwig-Maximilians-Universität München <https://orcid.org/0000-0001-5716-3634>

Article

Keywords: Host–microbe evolution, Anthophila, microbiome assembly, Environmental filtering, Evolutionary ecology

Posted Date: August 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10314404/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Abstract

Animal microbiomes often show phylosymbiosis, a pattern in which closely related hosts harbour more similar microbial communities than distant hosts. Yet, the processes generating this pattern remain unresolved, particularly whether phylosymbiosis requires sociality and vertical microbial transmission or can emerge through host filtering from environmental microbial pools. Bees (Anthophila) provide a powerful model to test this question because their major evolutionary radiation spans diverse life histories and contrasting microbiome transmission modes. Here, we analysed gut bacterial communities from 60 bee genera worldwide (1,989 samples), spanning all major bee families, and combined comparative phylogenetic models with host traits, bacterial phylogenies and predicted functional profiles to disentangle ecological and evolutionary drivers of phylosymbiosis. We found strong phylosymbiosis across and within bee families, with host phylogeny explaining substantial variation in gut microbiome composition despite weak effects of measured host traits. Geo-environmental context also explained considerable variation, indicating interactions between evolutionary and environmental filters. Despite extensive taxonomic and phylogenetic turnover, microbial functional divergence was weaker, suggesting convergence despite compositional differentiation. Together, these results show that microbiome composition can follow host phylogeny even under predominantly environmental acquisition and that phylosymbiosis can emerge through repeated assembly of functionally conserved communities rather than strict microbial inheritance.

Introduction

Animals host complex microbial communities (Ley et al. 2008; McFall-Ngai et al. 2013) that influence nutrition, immunity and behaviour (Zilber-Rosenberg and Rosenberg 2008; Lee and Hase 2014; Leitão-Gonçalves et al. 2017; Morais et al. 2021), yet the processes governing their assembly across evolutionary timescales remain unresolved (Groussin et al. 2020; Week et al. 2025). In many animal groups, closely related species harbour more similar gut microbiota than distant relatives, a pattern known as phylosymbiosis (Kohl 2020; Lim and Bordenstein 2020). However, the processes generating this pattern are still debated. Phylosymbiosis may arise in vertical transmission mode from adults to offspring or horizontal between nestmates in social bees and co-diversification of host-microbe lineages. Alternatively, it may also emerge from host filtering by phylogenetically conserved traits (e.g., diet or gut pH), stochastic assembly, or dispersal limitation (Mazel et al. 2018; Mallott and Amato 2021). It remains unclear whether host phylogeny structures microbiome composition independently of transmission mode, or whether observed patterns primarily reflect inheritance and social transmission. Disentangling the relative roles of host filtering, environmental acquisition and evolutionary history therefore remains a central challenge in understanding host-microbiome associations.

Bees (Anthophila) provide a powerful system to address these questions. They comprise more than 20,000 species worldwide and span a wide range of morphological, behavioural and physiological traits (Michener 2007; Roubik 2020). Their associated microbial communities play key roles in host nutrition and immunity by providing essential nutrients for larval development, detoxifying harmful compounds,

and protecting against pathogens (Engel et al. 2012; F. J. Lee et al. 2015; Kwong et al. 2017; Leonhardt et al. 2022; Motta and Moran 2024). Microbial communities also mediate plant–pollinator interactions, influencing bee foraging patterns and pollination dynamics (Keller et al. 2021; Steffan et al. 2024). Across bee lineages, modes of microbiome transmission vary along a continuum, from tightly socially mediated communities, often but not exclusively found in highly eusocial species such as *Apis mellifera* (Lombardo 2008; Zheng et al. 2018; Mazel et al. 2025), to predominantly horizontally acquired microbiota from the environment in solitary taxa (Keller et al. 2018; Kapheim et al. 2021; Peters et al. 2025; Hettiarachchi et al. 2025). Horizontal transmission of bacteria from the environment (hereafter environmental acquisition) includes transfer from flowers, plant materials, and soil (Voulgari-Kokota et al. 2019). Variation in the microbial pools in the environment due to abiotic filters (pH, temperature) or biotic filters (plant community composition, microbial competition) further defines the geo-environmental context for microbial acquisition, and can exhibit strong spatial turnover even across short geographic distances (Gaube et al. 2021; Du et al. 2023). Microbiome assembly may also be shaped by host-associated ecological factors, including body size, gut size and the corresponding volume of bacterial habitat, as well as foraging preferences, nesting habits and the use of antimicrobial materials such as resins (McFrederick et al. 2017; Keller et al. 2021; Chui et al. 2022; Vannette et al. 2025). This variation allows testing whether phylogenetic structure in microbiomes can emerge under differing degrees of host control and environmental filters over microbial transmission. At the same time, bees have gone through a major evolutionary radiation resulting in diverse ecologies, which provides an opportunity to evaluate how host traits (here, sociality, body size and resin use) and evolutionary history jointly structure microbial communities.

Although microbial communities have been characterised in both social and solitary bees, they have yet to be analysed within a unified framework spanning the full diversity of bee lineages. Here, we analyzed bee samples from all major (i.e. most speciose) bee families in ten countries, applying consistently the same methodological workflow within a comparative phylogenetic approach to test three predictions. First, if host evolution structures microbiome composition independently of transmission mode, gut communities should exhibit phylosymbiosis across bee phylogeny, including solitary taxa. Second, if phylosymbiosis results from host filtering, host-related factors should structure microbiome composition, such that host phylogeny and life history traits better explain microbiome variation relative to sampling location (i.e., geo-environmental context). Third, if microbiomes are constrained by host functional requirements, taxonomic and phylogenetic divergence across host lineages should be decoupled from functional divergence. This would reflect convergence in microbial functional capacity despite turnover in community composition: although phylosymbiosis predicts distinct communities in distantly related bees, conserved host needs may preserve similar microbial functions across hosts. Together, these predictions provide a framework to determine whether general principles of microbiome assembly emerge across a major evolutionary radiation despite variation in transmission mode and life history traits.

Results

Phylosymbiosis in bacterial community composition (α -diversity)

We first asked whether bee gut microbiomes show phylogenetic structure across the broad evolutionary radiation despite strong variation in sociality, life history and likely routes of microbial acquisition. To address this, we analysed 16S rRNA gene amplicon data from 1,989 bee gut samples representing 60 bee genera across all major bee families and ten countries (Fig. 1). This sampling design allowed us to test whether closely related hosts harbour more similar bacterial communities even when lineages differ in transmission mode and are embedded in different geo-environmental contexts.

We combined these microbiome data with a published bee phylogeny (Henríquez-Piskulich et al. 2024), public trait datasets (Michener 2007; Poulsen and Rasmussen 2020; Ferrari and Polidori 2022) and plot-level sampling location as a proxy for local geo-environmental context for microbial acquisition. To compare compositional, phylogenetic and functional dimensions of microbiome variation, we reconstructed bacterial phylogenetic relationships and inferred functional profiles (Keller et al. 2014; Douglas et al. 2020). We then quantified community dissimilarity (α -diversity) and analysed its major axes using phylogenetic mixed models.

We found a significant phylogenetic signal in gut bacterial communities, with host evolutionary history accounting for over a quarter (26%) of the variation in species composition. Closely related genera harboured more similar microbiota than more distantly related lineages (Figs. 1 & 2). This phylogenetic signal was consistent across the major axes of variation and remained evident within individual bee families (Supplementary Tables 3 & 4) and across multiple bacterial taxonomic levels (family, genus, and ASV; Supplementary Fig. 1), indicating that phylosymbiosis is pervasive across the bee phylogeny. These phylogenetically structured communities included lineage-specific associations with dominant bacterial groups, consistent with host-linked assembly processes (Fig. 2).

This signal holds true when measuring the specific identities of the bacteria (taxonomic diversity), phylogeny-based metric accounting for their evolutionary relationships (weighted UniFrac; 19.9%), or their predicted molecular functions (KEGG ortholog, 19.3%).

In contrast to host phylogeny, measured host traits including sociality, body size and resin use, did not significantly explain variation in microbiome taxonomic, phylogenetic or functional composition (Table 1). Instead, geo-environmental context, captured by plot-level location, accounted for a comparable or greater proportion of variance than host phylogeny (Fig. 1), highlighting a strong contribution of ecological filtering alongside evolutionary history. Together, these results support the first prediction that microbiome composition tracks host phylogeny, while indicating that phylosymbiosis emerges from the combined effects of host evolution and geo-environmental context rather than measured host traits.

Host phylogenetic signal across bacterial diversity dimensions

To assess how host phylogeny structures different dimensions of microbial diversity, we analysed taxonomic, phylogenetic and functional α -diversity using phylogenetic mixed models. Taxonomic diversity was quantified using the Shannon diversity index (Hill number, $q = 1$; e^H), phylogenetic diversity using Faith's index, and functional diversity using the Shannon diversity of predicted KEGG ortholog (KO) profiles. Patterns differed markedly among these dimensions (Fig. 3).

Phylogenetic diversity (Faith's index) exhibited a clear signal of host evolutionary history, with host phylogeny explaining a significant proportion of variance (approximately 8%), indicating that closely related hosts harbour more phylogenetically similar bacterial communities. Taxonomic diversity (e^H), in contrast, was influenced by both host phylogeny and geo-environmental context, suggesting that ecological variation contributes to community assembly. Strikingly, functional diversity (Shannon diversity of KO profiles) showed minimal variation across bee lineages, with no significant effects of host phylogeny, location or traits. This indicates that microbial communities show similar functional diversity across hosts despite pronounced taxonomic and phylogenetic divergence.

Phylogenetic structure within bacterial communities

Many bee genera harboured phylogenetically clustered microbiota. Nearly half of the genera ($n = 34$) showed significant clustering (NRI/NTI), indicating that co-occurring taxa are more closely related than expected by chance (Fig. 4). In contrast, phylogenetic overdispersion was rare and largely restricted to specific lineages, such as stingless bees.

Phylogenetically clustered communities were established by positive interactions within major bacterial phyla. Interactions among *Proteobacteria* accounted for approximately 25% of all inferred associations, while *Firmicutes-Firmicutes* interactions contributed about 19%. Cross-phyla interactions were less frequent, indicating that co-occurrence patterns are structured predominantly within major taxonomic groups. This suggests that co-occurring taxa may share similar ecological niches or engage in complementary metabolic interactions. The prevalence of within-phyla associations indicates that community assembly may favour functionally compatible or mutually facilitating taxa, contributing to the observed phylogenetic clustering of microbiota within hosts.

Co-phylogenetic structure of host-microbe associations

Co-phylogenetic analyses revealed significant congruence between bee and bacterial phylogenies (PACO, $p = 0.001$ & DTL, $p = 0.045$). Together, these complementary analyses demonstrate significant congruence between host and microbial phylogenies, indicating a consistent evolutionary association between bee lineages and their gut microbiota.

Table 1

Wald test results for fixed effects in phylogenetic generalised linear mixed models assessing gut microbial community structure and diversity in bees. We tested the effects of sociality (df = 3), body size (df = 5), and resin use (df = 1) on multivariate community composition (taxonomic, phylogenetic and functional dissimilarity) and univariate diversity metrics (taxonomic, phylogenetic, and functional diversity), while controlling for bee phylogeny, location and sample ID. Reported values are χ^2 statistics. Statistical significance is indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

Type of model	Type of Response	Sociality	Body Size	Resin use
Multivariate	Taxonomic β -diversity	2.372	1.583	0.010
	Phylogenetic β -diversity	0.179	0.497	0.0005
	Functional β -diversity	0.058	0.207	0.0001
Univariate	Taxonomic α -diversity	8.067*	3.010***	6.527**
	Phylogenetic α -diversity	3.504	2.445***	0.752
	Functional α -diversity	1.268	1.994	0.226

Discussion

Our results show that gut microbiota of bees are structured by host phylogeny across a global scale, revealing pervasive phylosymbiosis across a major and predominantly non-social insect radiation (Sanders et al. 2014; Dietrich et al. 2014; Novakova et al. 2017; Leigh et al. 2018; Qin et al. 2023). At the same time, microbiome composition is jointly shaped by geo-environmental context, with location contributing as strongly as host phylogeny, while measured host traits (like sociality, body size or resin use) explain little variation. Strikingly, this pronounced taxonomic and phylogenetic structuring is not mirrored at the functional level, with functionally stable assemblages across host lineages (Engel et al. 2012; Bonilla-Rosso and Engel 2018; Wu et al. 2021). Together, these findings indicate that microbiome assembly is overarchingly governed by functionally mediated filtering, whereby host evolution and geo-environmental context shape community composition, while functional boundaries constrain the range of viable microbial assemblages.

Phylosymbiosis is often interpreted as a consequence of co-evolution due to social or vertical transmission mode of endosymbionts allowing for long-term co-diversification between hosts and their

symbionts (Moran et al. 2008; Kaltenpoth et al. 2014). Here this mainly applies to social bees with generational overlap and nursing behaviour (Su et al. 2022; Kowallik and Mikheyev 2021; Prasad et al. 2025). However, our results show that this pattern persists across bee lineages that are largely solitary and rely predominantly on environmental acquisition of microbes. Holometabolous development involves gut remodelling that can disrupt microbial continuity (Powell et al. 2014; Hammer and Moran 2019; Danforth et al. 2019), making the persistence of phylogenetic structure particularly striking. This indicates that strict vertical transmission is not necessarily required for microbiome composition to track host phylogeny over evolutionary timescales. Instead, repeated acquisition from environmental pools may still generate phylogenetically structured communities if hosts consistently select from available microbes. In bees, microbial transmission via pollen provisions or nest environments contribute to this process (McFrederick and Rehan 2019; Voulgari-Kokota et al. 2019; Keller et al. 2021; Christensen et al. 2024), but such pathways are likely supplemented by strong host-associated filtering that stabilizes microbiome composition despite recurrent environmental exposure. Bees span a wide continuum of life histories, from solitary ground-nesting species to highly eusocial colonies, with corresponding differences in life history traits and microbial exposure, providing diverse ecological contexts for microbiome assembly. The joint effects of host phylogeny and geo-environmental context indicate that microbiome assembly in bees is shaped by multiple, interacting filters operating across scales. Host phylogeny explained a substantial proportion of variation in community composition, consistent with filtering mediated by phylogenetically conserved traits such as physiology, immune function or gut environment. At the same time, geo-environmental context, captured by location, contributed equally or more strongly, indicating that the pool of available microbes varies across geographic and ecological settings (Gaube et al. 2021; Quevedo-Caraballo et al. 2025). This includes variation in floral resources and nesting substrates that structure microbial exposure across bee lineages. Together, these results suggest that microbiome assembly reflects a two-step process in which environmental filtering determines the set of potential colonists, while host-associated filtering selects among these taxa. This process is further reinforced by bidirectional microbial exchanges, whereby bees both acquire and deposit microbes on flowers, feeding back into the pool of available colonists (Keller et al. 2021). In addition to these deterministic filters, ecological drift may further reinforce divergence among communities through the stochastic loss of low-abundance taxa (Zeng et al. 2017; Kohl 2020). The weak explanatory power of measured host traits further implies that key dimensions of host filtering were either unavailable (e.g. gut pH) in our study or operate through complex, phylogenetically structured mechanisms that are difficult to measure (e.g. food choice or flight behavior) and cannot be captured by coarse trait categories (Ostwald et al. 2024).

Despite strong taxonomic and phylogenetic structuring of microbiomes across bee lineages, functional diversity remained remarkably stable, indicating that community composition and functional capacity are decoupled. This pattern suggests that bee-associated microbiomes are constrained by functional requirements (Engel et al. 2012; Bonilla-Rosso and Engel 2018; Wu et al. 2021), such that multiple, phylogenetically distinct microbial taxa can fulfil similar ecological roles. As a result, host filtering may act primarily on functional traits rather than on specific microbial lineages, allowing taxonomic turnover

while maintaining consistent functional diversity. This functional convergence provides a mechanism by which phylosymbiosis can emerge even under predominantly environmental acquisition, as hosts repeatedly select for taxonomically divergent but functionally redundant taxa from variable microbial pools (Brooks et al. 2016; Haag et al. 2026). More broadly, these findings highlight the importance of functional redundancy in stabilizing host-microbiome interactions across evolutionary timescales, and underscore that understanding these assembly processes is essential for predicting how environmental stressors affect bee health and fitness and for developing more effective conservation strategies (Fernandez De Landa et al. 2023; Crowley and Schaeffer 2024; Peters et al. 2025). Protecting host-associated symbionts through reduced pesticide and antibiotic exposure (Hotchkiss et al. 2022; Allen et al. 2025), along with habitat preservation (Peters et al. 2025), can maintain their community-driven adaptive capacity to environmental stressors, a response that occurs far more rapidly than genetic adaptation in the host itself.

Patterns of phylogenetic clustering and co-abundance further support the role of ecological filtering in structuring microbial communities within hosts. In bees, such structuring may reflect the consistent association of microbes with pollen, nectar and nest materials, which act as recurring ecological reservoirs (Keller et al. 2021). Many bee genera harboured microbiota composed of more closely related taxa than expected by chance, indicating that co-occurring bacteria are not randomly assembled but constrained by shared ecological or physiological requirements. Consistent with this, co-abundance networks were dominated by positive associations within major bacterial phyla, particularly among Proteobacteria and Firmicutes, suggesting that taxa with similar evolutionary histories or metabolic capabilities tend to co-occur (Kwong et al. 2017; Bonilla-Rosso and Engel 2018). At the same time, competitive exclusion mediated by host immunity or inhibitory compounds may limit the persistence of other lineages (Emery et al. 2017; Guo et al. 2023; Corral López et al. 2026). Such patterns are consistent with niche-based assembly processes, which may promote the persistence of functionally coherent microbial consortia. Together, these findings indicate that interactions among microbes, alongside host and environmental filters, contribute to the assembly of structured and stable microbiomes. Behaviours such as resin collection or social interactions may further modulate these communities by altering microbial exposure and persistence (Saelao et al. 2020; Dalenberg et al. 2020; Shanahan and Spivak 2021; Chui et al. 2022).

Overall, we provide here a broader perspective on how host-associated microbiomes assemble across evolutionary timescales. The combination of phylogenetic structure in community composition, strong environmental effects, and functional convergence suggests that similar principles may govern microbiome assembly across diverse insect systems. Rather than requiring strict inheritance, host-associated microbiomes may emerge from re-assembly processes within every adult generation in which hosts select for functionally suitable microbes from environmentally available pools. This framework reconciles the widespread occurrence of phylosymbiosis with variable transmission modes and highlights how evolutionary history, ecological context and functional constraints jointly shape microbial communities. Extending this perspective beyond bees may help explain why phylogenetic

patterns in microbiomes are observed across taxa with very different life histories and transmission strategies (Lim and Bordenstein 2020; Bordenstein and The Holobiont Biology Network 2024).

Several limitations should be considered when interpreting these results. Functional profiles were inferred from 16S rRNA data, which may underestimate variation due to incomplete reference genomes and limited resolution for specialised taxa (Matchado et al. 2024). However, the consistency of functional patterns across analyses suggests that the observed convergence reflects robust large-scale constraints rather than artefacts of inference. More comprehensive genomic resources will nevertheless be essential to resolve finer-scale functional pathways and to identify the metabolic interactions underlying community assembly (Giannari et al. 2021). In addition, the host traits included here represent only a subset of potentially relevant ecological and physiological factors, and finer-scale trait data may help disentangle the mechanisms linking host biology to microbiome composition. Future studies integrating genomic, ecological and experimental approaches will be critical to test these mechanisms and to determine how host-microbiome interactions respond to environmental change.

Concluding, microbiome composition can track host phylogeny even under predominantly environmental transmission, indicating that phyllosymbiosis emerges from repeated, functionally constrained assembly rather than strict inheritance. By decoupling taxonomic turnover from functional stability, host-associated microbiomes behave as dynamic yet constrained systems shaped by both evolutionary history and ecological context. This framework provides a general explanation for how phylogenetic structure in microbiomes can emerge across hosts with contrasting transmission modes and ecological contexts.

Methods

Sample collection

This study integrates datasets generated across research projects conducted in 10 countries using consistently the same methodological workflow (Fig. 1). Overall 1,929 adult bees from 60 genera were collected in the field either by hand using sterile containers or by using entomological nets. Specimens were preserved immediately after collection using cold-chain approaches, including storage on dry ice or below 0°C followed by transfer to – 20°C freezers. In the laboratory, whole guts or abdominal tissues were dissected under sterile conditions. Dissected tissues were preserved in Zymo DNA/RNA shield lysis solution (Zymo Research, Irvine, CA, USA) to preserve nucleic acid or processed directly for downstream analyses. Sample collection was performed with permissions granted through respective local authorities, namely Germany (ROB–55.1–8646.NAT_02-8-81-5; Weinhold et al. 2024; ROB-55.1-8646.NAT_02-8-121-20; Maihoff et al. 2025), Italy (9/06/2020 from Parco Regionale della Maremma and Terre Regionali Toscana; Ronchetti et al. 2022), Ecuador (MAE-DNB-CM-2015-0068 & 144-2019-EXP-CM-FAU-DNB/MA; Villagómez et al. 2024), Peru (AUT-IFS2022-102; Pinos et al. 2025), Tanzania (COSTECH 2015-177-NA-96-44; Mayr et al. 2021).

Bee taxonomy, traits and phylogeny

Taxonomic identification was primarily based on morphological characteristics and, when necessary, confirmed using cytochrome oxidase I (COI) DNA barcoding from leg tissue. All nomenclature was standardized by aligning identifications with accepted names and known synonyms listed in the Catalogue of Life (Bánki et al. 2026). Given the inherent challenges of species-level identification in diverse bee assemblages, species-level identification was not possible for all samples and characterization of phylogenetic relationships and life-history traits was conducted at the genus or subgenus level.

We characterized each subgenus based on three functional traits (Supplementary Table 1) previously hypothesized to influence gut microbiota composition: body size (Minute < 5 mm; Small = 5–10 mm; Moderate = 10–17 mm; Large > 17 mm), sociality (kleptoparasitic, solitary & eusocial), and resin use (Resin as a nest material or not). We initially considered a broader set of ecological traits, including nesting and foraging type. However, trait assignment had to remain relatively coarse because analyses were conducted at the genus/subgenus level rather than the species level. Preliminary exploration revealed substantial collinearity between nesting and foraging behaviour and other ecological predictors, resulting in several unique trait combinations represented by only a small number of taxa. This prevented robust statistical comparison among factor levels and reduced model interpretability. We therefore excluded nesting and foraging type from the final analyses. Using the recently published review on bee trait ecology (Ostwald et al. 2024), we identified two large-scale studies covering most of the taxa investigated in this study (Poulsen and Rasmussen 2020; Ferrari and Polidori 2022) to ensure consistency in the definition of trait categories. When traits varied within one genus and species identification were not available, we assigned a “mixed” category. Missing information for a few bee subgenera were extracted from Michener (2007).

We used the most recently published and time-calibrated bee ‘tree-of-life’ supermatrix phylogeny at the genus level (Henríquez-Piskulich et al. 2024). We pruned this phylogenetic tree, which includes 427 bee and five wasp genera, to remove unsampled genera and associated internal branches using the *ape* package (version 5.8-1) (Paradis and Schliep 2019).

Microbiome profiling by DNA metabarcoding

The genomic DNA from the guts was isolated using the ZymoBIOMICS 96 DNA kit for microbiome, according to manufacturer instructions (Zymo Research). Each plate contained 88 samples and was accompanied by at least two negative extraction controls (water) and four positive extraction controls (ZymoBiomics Mock community). We constructed 16S rRNA gene libraries using a dual-indexing approach as described in Kozich et al. (2013) to amplify the V4 variable region of the 16S rRNA gene. We used 16 forward primers with index sequences SA501–SB508 and 24 reverse primers with indices SA701–SB712, allowing a total of 384 unique combinations for sample indexing. PCR was performed in triplicates following the 96-well PCR sample design two as described in Sickel et al. (2015). To avoid

bias, triplicate reactions of each sample were combined after PCR and further processed as described in Kozich et al. (2013). To pool each 96-well plate sample, the normalization was performed using the SequalPrep Normalization Plate Kit (Invitrogen) and concentrated using AMPure XP beads. Pools were quality controlled using a Bioanalyzer High Sensitivity DNA Chip (Agilent Technologies, Santa Clara, CA, USA), quantified with the dsDNA High Sensitivity Assay using a Qubit 4.0 Fluorometer (Invitrogen). Then, pools were combined into a single pool and afterward diluted to have an equimolar concentration of 1.8 nM. Eight pM of the libraries combined with 5% Phix Control (Illumina Inc., San Diego, CA, USA) was sequenced with the Illumina MiSeq platform either at The Genomics Service Unit (LMU Munich) or at the Department of Animal Ecology and Tropical Biology (University of Würzburg), using 2×250 cycles reagent kit v2 according to the manufacturer's instructions (Illumina Inc.).

BeeBiome data

To improve the taxonomic coverage of this study, we further integrated selectively 16S rRNA V4 sequencing data from bee guts deposited in the NCBI BioProject database (Supplementary Table 2) using the BeeBiome portal while ensuring data compatibility (Laval et al. 2025). In total, we collected 60 samples from 8 genera, including *Anthophora abrupta* and *Centris atripes* (Kwong et al. 2017), *Habropoda laboriosa*, *Svastra petulca* and *Svastra obliqua* (Holley et al. 2022), *Nomia melanderi* (Kapheim et al. 2021), *Osmia cornuta* and *Osmia bicornis* (Manthey et al. 2023), *Sphecodes* spp. (Rubin et al. 2018) and *Tetragonisca angustula* (Cerqueira et al. 2021).

Bioinformatic analysis and data availability

Raw sequence reads were obtained directly from the Illumina MiSeq output, which includes sample reads already demultiplexed by MID barcode with the Illumina device's software. Bioinformatic analysis followed the pipeline established by Leonhardt et al. (2022), available at https://github.com/chiras/metabarcoding_pipeline. Low-quality (E_{max} = 1, no ambiguous base pairs) and short (< 170 bp) reads were removed. Cleaning of reads, i.e., removal of bad quality reads and filtering of chimeric artifacts, denoising, and dereplication to amplicon sequence variants (ASVs) was performed with VSEARCH global alignments and MetAnoDe (Rognes et al. 2016, Keller 2025). The taxonomy assignment was made using direct global alignment classifications against the databases RDP_16s (v18; Edgar 2016), GreenGenes 16s (v13.5; DeSantis et al. 2006), and SILVA 16S (v123; Quast et al. 2013) using VSEARCH and a threshold of 97% identity. Remaining unclassified reads were hierarchically classified using the SINTAX implementation of VSEARCH against SILVA 16S (v123) using a threshold cutoff of 0.8 (Edgar 2016).

We predicted abundances of KEGG ortholog (KO) abundances based on clean and classified reads using Picrust2 (version 2.6.2) (Douglas et al. 2020). Additionally, we used IQ-TREE (version 3.0.1) to reconstruct a bacterial phylogenetic tree (Wong et al. 2025) based on 16S rRNA sequences aligned with muscle5 (version 5.3) (Edgar 2022). The best substitution model was defined with the ModelFinder feature, which identified the General Time Reversible framework with empirical base frequencies and rate heterogeneity across sites using the FreeRate approach with ten rate categories (GTR + F+R10) as

the optimal solution (Kalyaanamoorthy et al. 2017). Pre-analysed data is publicly deposited at Zenodo 10.5281/zenodo.21289939.

Data analyses

Microbiome data was imported into R (version 4.5.1) (R Core Team, 2021) and managed with the *phyloseq* package (version 1.54.0) (McMurdie and Holmes 2013). Any chloroplast or mitochondrial ASVs were excluded. Additionally, all bacterial genera with a relative abundance below 1% within a given sample were excluded from downstream analysis. The threshold was estimated based on estimation of cross-contamination levels in the positive control samples.

Sampling effort was uneven across bee genera (Supplementary Table 1). Roughly two-thirds were represented by fewer than ten samples, whereas others had hundreds (e.g., *Bombus* spp., $n = 355$). To balance the number of samples per genera, we first detected and removed multivariate outliers based on robust Mahalanobis distances using the package *mvoutlier* (version 2.1.1) (Filzmoser et al. 2005). We then performed a leave-one-out (jackknife) analysis to compare the original and reduced ordination by procrustes analysis using the *vegan* package (version 2.7-2) (Oksanen et al. 2022). We retained ten samples whose removal most impacted the ordination (i.e., produced the lowest Procrustes correlations) for each genus, as these samples contributed most strongly to the overall multivariate structure of the dataset. A Procrustes analysis comparing the original and subsampled datasets after this procedure confirmed that the two datasets remained highly similar (Procrustes correlation = 0.9996, $p = 0.001$).

Phylosymbiosis in community composition (α -diversity)

We fitted Multivariate Generalised Mixed Models (GLMMs) using Monte Carlo Sampler accounting for correlated random effects derived from phylogenies using the *MCMCglmm* package (version 2.36) (Hadfield 2010). We transformed the ASV table of the subsampled dataset into a Bray-Curtis dissimilarity matrix using *phyloseq*. We then performed a Principal Coordinate Analysis (PCoA) and selected the two first axes as a joint response following a Gaussian distribution in the model. We specified inverse-Wishart priors for the variance–covariance matrices with expected covariance matrix $V = \text{diag}(k)$ and degrees of belief parameter $\nu = k + 1$, where k is the number of response variables (Halliwell et al. 2025). Bee traits were included as fixed effects, while phylogeny, location, and sample ID were modeled as random effects. Sample ID was included as a random factor to prevent different species or populations within the same genus from being treated as evolutionary identical. Convergence diagnostics were conducted by calculating potential scale reduction factors ($\hat{R}$) across multiple MCMC chains using the *Rhat* function from the *rstan* package (version 2.32.7), and all models presented here showed adequate convergence with $\hat{R}$ values consistently below 1.01 (Vehtari et al. 2021).

We illustrated evolutionary patterns in the gut bacterial communities by a Principal Coordinates of Phylogenetic Structure (PCPS) using the *PCPS* package (version 1.0.8) (Debastiani 2025). Bray-curtis dissimilarity in gut bacterial communities among samples were weighted by phylogenetic distance between bee genera.

Phylogenetic signal across diversity dimensions

We estimated bacterial species diversity by computing Shannon diversity index ($q = 1$) using the *iNEXT* package (version 3.0.2) (Chao et al. 2014). This approach applies interpolation and extrapolation to standardize sampling effort and coverage. We further computed Faith's phylogenetic diversity by integrating bacterial phylogeny using *picante* (version 1.8.2) (Kembel et al. 2010). Functional diversity was assessed using the same approach as for bacterial diversity applied to KO abundances predicted with Picrust2.

To test the effects of bee phylogeny on the species, phylogenetic and functional diversity, we fitted Multivariate Generalised Mixed Models using the same approach as described in the α -diversity section. Log-transformed diversity values were used as response variables following a Gaussian distribution. Priors were specified as $V = 1$ and $v = 0.002$ (Halliwell et al. 2025).

Phylogenetic structure within bacterial communities

To further investigate patterns of phylogenetic diversity, we used net relatedness index (NRI) and Nearest Taxon Index (NTI) to measure the degree of phylogenetic clustering or overdispersion within each bee sample using *picante*. We first calculated the standardized effect size of mean pairwise distances and mean nearest taxon distances in bacterial communities, which are equal to $-1 \times \text{NRI}$ and $-1 \times \text{NTI}$ respectively. We additionally predicted ecological interactions between bacteria based on co-abundance using *FlashWeave* (version 0.19.2) (Tackmann et al. 2019). It allows statistical detection of direct associations, while controlling for compositionality and bystander effects, and biases in shared-niche and sequencing.

Co-phylogenetic structure of host-microbe associations

We conducted co-phylogenetic analyses with bee and bacterial phylogeny described above using two different types of methods. We first used a distance-based method, the Procrustean Approach to Cophylogeny (PACo), using the *paco* package (version 0.4.2) (Hutchinson et al. 2017). This approach superimposes two phylogenies to test their congruence based on their interactions. Secondly, we reconciled the two phylogenies under the Duplication-Transfer-Loss (DTL) model using the default settings in *eMPRESS* (version 1.2.1) (Santichaivekin et al. 2021).

Declarations

Competing interest statement

The authors declare no competing interests.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) under projects 460916175, 444827997, 279735434, and 325176232 to AK and the “Bayerische Forschungsallianz GmbH” to SDL, and the Bavarian State Ministry of Science and the Arts in the context of the Bavarian Climate Research Network (bayklif) to FM and AC.

Acknowledgments

We appreciate the laboratory support of Gudrun Grimmer and Beate Krischke at the University of Würzburg as well as Anika Schopf and Uschi Schkölziger at LMU Munich. We gratefully acknowledge the support of the relevant authorities and organizations that granted permission for access to the sampling areas and facilitated sample collection.

References

1. Allen NO, Duan C, Copeland BM, Mott R, Erickson, Anderson KE (2025) Antibiotic Treatment of Honey Bee Colonies Alters Early Gut Microbiome Assembly and Induces Persistent Dysbiosis in Newly Emerged Workers. *Sci Rep* 15(1):29031. <https://doi.org/10.1038/s41598-025-12823-9>
2. Bánki O, Roskov Y, Döring M et al (2026) Catalogue of Life. Versions 2026-02-13 XR. <https://doi.org/https://doi.org/10.48580/dgwnl>
3. Bonilla-Rosso Germán (2018) and Philipp Engel. Functional Roles and Metabolic Niches in the Honey Bee Gut Microbiota. *Current Opinion in Microbiology, Environmental Microbiology * The New Microscopy*, vol. 43 (June): 69–76. <https://doi.org/10.1016/j.mib.2017.12.009>
4. Bordenstein SR, The Holobiont Biology Network (2024) The Disciplinary Matrix of Holobiont Biology. *Science* 386(6723):731–732. <https://doi.org/10.1126/science.ado2152>
5. Brooks AW, Kevin D, Kohl RM, Brucker, Edward J, van Opstal, Bordenstein SR (2016) Phyllosymbiosis: Relationships and Functional Effects of Microbial Communities across Host Evolutionary History. *PLoS Biol* 14(11):e2000225. <https://doi.org/10.1371/journal.pbio.2000225>
6. Cerqueira AES, Hammer TJ, Moran NA, Santana WC, Kasuya MCM, da Silva CC (2021) Extinction of Anciently Associated Gut Bacterial Symbionts in a Clade of Stingless Bees. *ISME J* 15(9):2813–2816. <https://doi.org/10.1038/s41396-021-01000-1>. Scopus
7. Chao A, Nicholas J, Gotelli TC, Hsieh et al (2014) Rarefaction and Extrapolation with Hill Numbers: A Framework for Sampling and Estimation in Species Diversity Studies. *Ecol Monogr* 84(1):45–67. <https://doi.org/10.1890/13-0133.1>
8. Christensen SM, Sriram N, Srinivas QS, McFrederick, Bryan N, Danforth SL, Buchmann, Vannette RL (2024) Symbiotic Bacteria and Fungi Proliferate in Diapause and May Enhance Overwintering Survival in a Solitary Bee. *ISME J* 18(1):wrae089. <https://doi.org/10.1093/ismejo/wrae089>
9. Chui S, Xiong A, Keller, Leonhardt SD (2022) Functional Resin Use in Solitary Bees. *Ecol Entomol* 47(2):115–136. <https://doi.org/10.1111/een.13103>

10. Corral López, Roberto JA, Bonachela MG, Dominguez-Bello et al (2026) Imbalance in Gut Microbial Interactions as a Marker of Health and Disease. *Science* 391(6788):890–895.
<https://doi.org/10.1126/science.ady1729>
11. Crowley BL, Robert NS (2024) Bee Microbiomes in a Changing Climate: Investigating the Effects of Temperature on Solitary Bee Life History and Health. *Environ Microbiol* 26(11):e70002.
<https://doi.org/10.1111/1462-2920.70002>
12. Dalenberg H, Maes P, Mott B, Anderson KE, and Marla Spivak (2020) Propolis Envelope Promotes Beneficial Bacteria in the Honey Bee (*Apis mellifera*) Mouthpart Microbiome. *Insects* 11(7):453.
<https://doi.org/10.3390/insects11070453>
13. Danforth BN, Minckley RL, Neff JL, and Frances Fawcett (2019) *The Solitary Bees: Biology, Evolution, Conservation*. Princeton University Press. <https://doi.org/10.2307/j.ctvd1c929>
14. Debastiani VJ (2025) *PCPS: Principal Coordinates of Phylogenetic Structure*. V. 1.0.8. Released. R package. <https://doi.org/10.32614/CRAN.package.PCPS>
15. DeSantis TZ, Hugenholtz P, Larsen N et al (2006) Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Appl Environ Microbiol* 72(7):5069–5072.
<https://doi.org/10.1128/AEM.03006-05>
16. Dietrich C, Köhler T, and Andreas Brune (2014) The Cockroach Origin of the Termite Gut Microbiota: Patterns in Bacterial Community Structure Reflect Major Evolutionary Events. *Appl Environ Microbiol* 80(7):2261–2269. <https://doi.org/10.1128/AEM.04206-13>
17. Douglas GM, Vincent J, Maffei JR, Zaneveld et al (2020) PICRUST2 for Prediction of Metagenome Functions. *Nat Biotechnol* 38(6):685–688. <https://doi.org/10.1038/s41587-020-0548-6>
18. Du X, Gu S, Zhang Z et al (2023) Spatial Distribution Patterns across Multiple Microbial Taxonomic Groups. *Environ Res* 223:115470. <https://doi.org/10.1016/j.envres.2023.115470>
19. Edgar RC (2016) SINTAX: A Simple Non-Bayesian Taxonomy Classifier for 16S and ITS Sequences. Preprint, bioRxiv. 9. <https://doi.org/10.1101/074161>
20. Edgar RC (2022) Muscle5: High-Accuracy Alignment Ensembles Enable Unbiased Assessments of Sequence Homology and Phylogeny. *Nat Commun* 13(1):6968. <https://doi.org/10.1038/s41467-022-34630-w>
21. Emery O, Schmidt K, and Philipp Engel (2017) Immune System Stimulation by the Gut Symbiont *Frischella Perrara* in the Honey Bee (*Apis mellifera*). *Mol Ecol* 26(9):2576–2590.
<https://doi.org/10.1111/mec.14058>
22. Engel P, Martinson VG, Moran NA (2012) Functional Diversity within the Simple Gut Microbiota of the Honey Bee. *Proceedings of the National Academy of Sciences* 109 (27): 11002–7.
<https://doi.org/10.1073/pnas.1202970109>
23. De Landa F, Gregorio D, Alberoni L, Baffoni et al (2023) The Gut Microbiome of Solitary Bees Is Mainly Affected by Pathogen Assemblage and Partially by Land Use. *Environ Microbiome* 18(1):38.
<https://doi.org/10.1186/s40793-023-00494-w>

24. Ferrari A, and Carlo Polidori (2022) How City Traits Affect Taxonomic and Functional Diversity of Urban Wild Bee Communities: Insights from a Worldwide Analysis. *Apidologie* 53(4):46.
<https://doi.org/10.1007/s13592-022-00950-5>
25. Filzmoser P, Garrett RG, and Clemens Reimann (2005) Multivariate Outlier Detection in Exploration Geochemistry. *Comput Geosci* 31(5):579–587. <https://doi.org/10.1016/j.cageo.2004.11.013>
26. Gaube P, Junker RR, and Alexander Keller (2021) Changes amid Constancy: Flower and Leaf Microbiomes along Land Use Gradients and between Bioregions. *Basic Appl Ecol* 50(February):1–15. <https://doi.org/10.1016/j.baae.2020.10.003>
27. Giannari D, Ho CH, Mahadevan R (2021) A Gap-Filling Algorithm for Prediction of Metabolic Interactions in Microbial Communities. *PLoS Comput Biol* 17(11):e1009060.
<https://doi.org/10.1371/journal.pcbi.1009060>
28. Groussin M, Mazel F, and Eric J. Alm (2020) Co-Evolution and Co-Speciation of Host-Gut Bacteria Systems. *Cell Host Microbe* 28(1):12–22. <https://doi.org/10.1016/j.chom.2020.06.013>
29. Guo L, Tang J, Tang M, Luo S, Xin, Zhou (2023) Reactive Oxygen Species Are Regulated by Immune Deficiency and Toll Pathways in Determining the Host Specificity of Honeybee Gut Bacteria. *Proceedings of the National Academy of Sciences* 120 (33): e2219634120.
<https://doi.org/10.1073/pnas.2219634120>
30. Haag K, Luisa LQ, Stein (2026) Carlos Gustavo Nunes da Silva, Florent Mazel, Aiswarya Prasad, and Philipp Engel. Conserved Gut Microbiomes with Cross-Species Spillover between Sympatric Neotropical Stingless Bees and Honey Bees. *Applied and Environmental Microbiology* 0 (0): e02483-25. <https://doi.org/10.1128/aem.02483-25>
31. Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *J Stat Softw* 33(February):1–22. <https://doi.org/10.18637/jss.v033.i02>
32. Halliwell B, Holland BR, Yates LA (2025) Multi-Response Phylogenetic Mixed Models: Concepts and Application. *Biol Rev* 100(3):1294–1316. <https://doi.org/10.1111/brv.70001>
33. Hammer TJ, Moran NA (2019) Links between Metamorphosis and Symbiosis in Holometabolous Insects. *Philosophical Trans Royal Soc B: Biol Sci* 374(1783):20190068.
<https://doi.org/10.1098/rstb.2019.0068>
34. Henríquez-Piskulich, Patricia AF, Hugall, Devi Stuart-Fox (2024) A Supermatrix Phylogeny of the World's Bees (Hymenoptera: Anthophila). *Mol Phylogenet Evol* 190(January):107963.
<https://doi.org/10.1016/j.ympev.2023.107963>
35. Hettiarachchi A, Tuerlings T, Weekers T et al (2025) The Gut Microbial Community of Solitary Bees Is Acquired through Host and Location Filtering. *Microb Ecol* 88. <https://doi.org/10.1007/s00248-025-02617-x>
36. Holley Jo-anneC, Mary N, Jackson AT, Pham SC, Hatcher, Moran NA (2022) Carpenter Bees (Xylocopa) Harbor a Distinctive Gut Microbiome Related to That of Honey Bees and Bumble Bees. *Appl Environ Microbiol* 88(13):e00203–e00222. <https://doi.org/10.1128/aem.00203-22>

37. Hotchkiss MZ, Alexandre J, Poulain, Jessica RK, Forrest (2022) Pesticide-Induced Disturbances of Bee Gut Microbiotas. *FEMS Microbiol Rev* 46(2):fuab056. <https://doi.org/10.1093/femsre/fuab056>
38. Hutchinson MC, Fernando Cagua E, Balbuena JA, Stouffer DB, and Timothée Poisot (2017) Paco: Implementing Procrustean Approach to Cophylogeny in R. *Methods Ecol Evol* 8(8):932–940. <https://doi.org/10.1111/2041-210X.12736>
39. Kaltenpoth M, Roeser-Mueller K, Koehler S et al (2014) Partner Choice and Fidelity Stabilize Coevolution in a Cretaceous-Age Defensive Symbiosis. *Proceedings of the National Academy of Sciences* 111 (17): 6359–64. <https://doi.org/10.1073/pnas.1400457111>
40. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nat Methods* 14(6):587–589. <https://doi.org/10.1038/nmeth.4285>
41. Kapheim K, Johnson M, and Maggi Jolley (2021) Composition and Acquisition of the Microbiome in Solitary, Ground-Nesting Alkali Bees. *Sci Rep* 11. <https://doi.org/10.1038/s41598-021-82573-x>
42. Keller A (2025) Anomaly Detection in Metabarcoding Amplicon Reads Using an LSTM-CNN Deep Neural Network Ensemble (MetAnoDe). March 20. <https://ecoevorxiv.org/repository/view/8792/>
43. Keller A, Brandel A, Becker MC et al (2018) Wild Bees and Their Nests Host *Paenibacillus* Bacteria with Functional Potential of Avail. *Microbiome* 6(1):229. <https://doi.org/10.1186/s40168-018-0614-1>
44. Keller A, Horn H, Förster F, and Jörg Schultz (2014) Computational Integration of Genomic Traits into 16S rDNA Microbiota Sequencing Studies. *Gene* 549(1):186–191. <https://doi.org/10.1016/j.gene.2014.07.066>
45. Keller A, McFrederick QS, Dharampal P, Steffan S, Danforth BN, Leonhardt SD (2021) (More than) Hitchhikers through the Network: The Shared Microbiome of Bees and Flowers. *Curr Opin Insect Sci* 44(April):8–15. *Ecology * Parasites/Parasitoids/Biological control*<https://doi.org/10.1016/j.cois.2020.09.007>
46. Kembel SW, Peter D, Cowan MR, Helmus et al (2010) Picante: R Tools for Integrating Phylogenies and Ecology. *Bioinformatics* 26(11):1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
47. Kohl KD (2020) Ecological and Evolutionary Mechanisms Underlying Patterns of Phyllosymbiosis in Host-Associated Microbial Communities. *Philosophical Trans Royal Soc B: Biol Sci* 375(1798):20190251. <https://doi.org/10.1098/rstb.2019.0251>
48. Kowallik V, Mikheyev AS (2021) Honey Bee Larval and Adult Microbiome Life Stages Are Effectively Decoupled with Vertical Transmission Overcoming Early Life Perturbations. *mBio* 12(6):e02966–e02921. <https://doi.org/10.1128/mBio.02966-21>
49. Kozich JJ, Westcott SL, Nielson T, Baxter SK, Highlander, and Patrick D. Schloss (2013) *Appl Environ Microbiol* 79(17):5112–5120. <https://doi.org/10.1128/AEM.01043-13>. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform
50. Kwong WK, Luis A, Medina H, Koch et al (2017) Dynamic Microbiome Evolution in Social Bees. *Sci Adv* 3(3):e1600513. <https://doi.org/10.1126/sciadv.1600513>

51. Laval V, Dainat B, Engel P, and Marc Robinson-Rechavi (2025) The BeeBiome Data Portal Provides Easy Access to Bee Microbiome Information. BMC Bioinformatics 26.
<https://doi.org/10.1186/s12859-025-06229-7>
52. Lee FJ, Douglas B, Rusch FJ, Stewart HR, Mattila, Newton ILG (2015) Saccharide Breakdown and Fermentation by the Honey Bee Gut Microbiome. Environ Microbiol 17(3):796–815.
<https://doi.org/10.1111/1462-2920.12526>
53. Lee W-J, and Koji Hase (2014) Gut Microbiota–Generated Metabolites in Animal Health and Disease. Nat Chem Biol 10(6):416–424. <https://doi.org/10.1038/nchembio.1535>
54. Leigh BA, Sarah R, Bordenstein AW, Brooks AM, Bordenstein SR (2018) Finer-Scale Phyllosymbiosis: Insights from Insect Viromes. mSystems 3(6). <https://doi.org/10.1128/msystems.00131-18>.
10.1128/msystems.00131 – 18
55. Leitão-Gonçalves R, Carvalho-Santos Z, Patrícia A Francisco, et al (2017) Commensal Bacteria and Essential Amino Acids Control Food Choice Behavior and Reproduction. PLoS Biol 15(4):e2000862.
<https://doi.org/10.1371/journal.pbio.2000862>
56. Leonhardt S, Diana B, Peters, and Alexander Keller (2022) Do Amino and Fatty Acid Profiles of Pollen Provisions Correlate with Bacterial Microbiomes in the Mason Bee *Osmia Bicornis*? Philosophical Trans Royal Soc B: Biol Sci 377(1853):20210171. <https://doi.org/10.1098/rstb.2021.0171>
57. Ley RE, Catherine A, Lozupone M, Hamady R, Knight, and Jeffrey I. Gordon (2008) Worlds within Worlds: Evolution of the Vertebrate Gut Microbiota. Nat Rev Microbiol 6(10):776–788.
<https://doi.org/10.1038/nrmicro1978>
58. Lim SJ (2020) and Seth R. Bordenstein. An Introduction to Phyllosymbiosis. *Proceedings of the Royal Society B: Biological Sciences* 287 (1922): 20192900. <https://doi.org/10.1098/rspb.2019.2900>
59. Lombardo MP (2008) Access to Mutualistic Endosymbiotic Microbes: An Underappreciated Benefit of Group Living. Behav Ecol Sociobiol 62(4):479–497. <https://doi.org/10.1007/s00265-007-0428-9>
60. Maihoff F, Bofinger L, Brenzinger K, Keller A, and Alice Classen (2025) Exploring Climate-Related Gut Microbiome Variation in Bumble Bees: An Experimental and Observational Perspective. Ecology 106(3):e70066. <https://doi.org/10.1002/ecy.70066>
61. Mallott EK, and Katherine R. Amato (2021) Host Specificity of the Gut Microbiome. Nat Rev Microbiol 19(10):639–653. <https://doi.org/10.1038/s41579-021-00562-3>
62. Manthey C, Johnston PR, Nakagawa S, and Jens Rolff (2023) Complete Metamorphosis and Microbiota Turnover in Insects. Mol Ecol 32(23):6543–6551. <https://doi.org/10.1111/mec.16673>
63. Matchado M, Steffi M, Rühlemann S, Reitmeier et al (2024) On the Limits of 16S rRNA Gene-Based Metagenome Prediction and Functional Profiling. Microb Genomics 10(2):001203.
<https://doi.org/10.1099/mgen.0.001203>
64. Mayr AV, Keller A, Peters MK et al (2021) Cryptic Species and Hidden Ecological Interactions of Halictine Bees along an Elevational Gradient. Ecol Evol 11(12):7700–7712.
<https://doi.org/10.1002/ece3.7605>

65. Mazel F, Davis KM, Loudon A, Kwong WK, Groussin M, and Laura Wegener Parfrey (2018) Is Host Filtering the Main Driver of Phyllosymbiosis across the Tree of Life? *mSystems* 3(5). 10.1128. /msystems.00097 – 18
66. Mazel F, Prasad A, and Philipp Engel (2025) Host Specificity of Gut Microbiota Associated with Social Bees: Patterns and Processes. *Microbiol Mol Biol Rev* 0(0):e00080–e00023. <https://doi.org/10.1128/mmbr.00080-23>
67. McFall-Ngai M, Hadfield MG, Bosch TCG et al (2013) Animals in a Bacterial World, a New Imperative for the Life Sciences. *Proceedings of the National Academy of Sciences* 110 (9): 3229–36. <https://doi.org/10.1073/pnas.1218525110>
68. McFrederick QS, and Sandra M. Rehan (2019) Wild Bee Pollen Usage and Microbial Communities Co-Vary Across Landscapes. *Microb Ecol* 77(2):513–522. <https://doi.org/10.1007/s00248-018-1232-y>
69. McFrederick QS, Jason M, Thomas JL, Neff et al (2017) Flowers and Wild Megachilid Bees Share Microbes. *Microb Ecol* 73(1):188–200. <https://doi.org/10.1007/s00248-016-0838-1>
70. McMurdie PJ, and Susan Holmes (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 8(4):e61217. <https://doi.org/10.1371/journal.pone.0061217>
71. Michener CD (2007) *The Bees of the World*, 2nd edn. Johns Hopkins University
72. Morais LH, Schreiber HL, Mazmanian SK (2021) The Gut Microbiota–Brain Axis in Behaviour and Brain Disorders. *Nat Rev Microbiol* 19(4):241–255. <https://doi.org/10.1038/s41579-020-00460-0>
73. Moran NA, McCutcheon JP (2008) and Atsushi Nakabachi. Genomics and Evolution of Heritable Bacterial Symbionts. *Annual Review of Genetics* 42 (Volume 42, 2008): 165–90. <https://doi.org/10.1146/annurev.genet.41.110306.130119>
74. Motta EVS, Moran NA (2024) The Honeybee Microbiota and Its Impact on Health and Disease. *Nat Rev Microbiol* 22(3):122–137. <https://doi.org/10.1038/s41579-023-00990-3>
75. Novakova E, Woodhams DC, Sonia M, Rodríguez-Ruano et al (2017) Mosquito Microbiome Dynamics, a Background for Prevalence and Seasonality of West Nile Virus. *Front Microbiol* 8. <https://doi.org/10.3389/fmicb.2017.00526>
76. Oksanen J, Simpson GL, Guillaume Blanchet F et al (2022) *Vegan: Community Ecology Package*, 2.7-2. Vienna (Austria): R Foundation for Statistical Computing
77. Ostwald MM, Victor H, Gonzalez C, Chang N, Vitale M, Lucia, Seltsmann KC (2024) Toward a Functional Trait Approach to Bee Ecology. *Ecol Evol* 14(10):e70465. <https://doi.org/10.1002/ece3.70465>
78. Paradis E, and Klaus Schliep (2019) Ape 5.0: An Environment for Modern Phylogenetics and Evolutionary Analyses. *Bioinformatics* 35(3):526–528. <https://doi.org/10.1093/bioinformatics/bty633>
79. Peters B, Leonhardt S, Schlöter M, and Alexander Keller (2025) Direct and Indirect Effects of Land Use on Microbiomes of Trap-Nesting Solitary Bee Larvae and Nests. *Front Microbiol* 15.

<https://doi.org/10.3389/fmicb.2024.1513096>

80. Piños A, Correa-Carmona PA-AY et al (2025) Host Identity, More than Elevation, Shapes Bee Microbiomes along a Tropical Elevation Gradient. *Front Microbiol* 16.
<https://doi.org/10.3389/fmicb.2025.1671348>
81. Poulsen NR, and Claus Rasmussen (2020) Island Bees: Do Wood Nesting Bees Have Better Island Dispersal Abilities? *Apidologie* 51(6):1006–1017. <https://doi.org/10.1007/s13592-020-00778-x>
82. Powell J, Elijah VG, Martinson KU-M, Moran NA (2014) Routes of Acquisition of the Gut Microbiota of the Honey Bee *Apis mellifera*. *Appl Environ Microbiol* 80(23):7378–7387.
<https://doi.org/10.1128/AEM.01861-14>
83. Prasad A, Pallujam AD, Siddaganga R et al (2025) Evolution of Gut Microbiota across Honeybee Species Revealed by Comparative Metagenomics. *Nat Commun* 16.
<https://doi.org/10.1038/s41467-025-64115-5>
84. Qin M, Jiang L, Qiao G, Chen J (2023) Phyllosymbiosis: The Eco-Evolutionary Pattern of Insect–Symbiont Interactions. *Int J Mol Sci* 24(21):15836. <https://doi.org/10.3390/ijms242115836>
85. Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res* 41(D1):D590–D596.
<https://doi.org/10.1093/nar/gks1219>
86. Quevedo-Caraballo S, Vega Cde, Lievens B, Fukami T, Sergio Álvarez-Pérez (2025) Tiny but Mighty? Overview of a Decade of Research on Nectar Bacteria. *New Phytol* 245(5):1897–1910.
<https://doi.org/10.1111/nph.20369>
87. Rognes Torbjørn, Flouri Tomáš, Nichols B, Quince C, Frédéric Mahé (2016) VSEARCH: A Versatile Open Source Tool for Metagenomics. *PeerJ* 4(October):e2584. <https://doi.org/10.7717/peerj.2584>
88. Ronchetti F, Polidori C, Schmitt T, Steffan-Dewenter I, and Alexander Keller (2022) Bacterial Gut Microbiomes of Aculeate Brood Parasites Overlap with Their Aculeate Hosts', but Have Higher Diversity and Specialization. *FEMS Microbiol Ecol* 98(November).
<https://doi.org/10.1093/femsec/fiac137>
89. Roubik DW (2020) Bees, Ecological Roles Bees: Ecological Roles. In *Encyclopedia of Social Insects*, edited by Christopher K. Starr. Springer International Publishing. https://doi.org/10.1007/978-3-319-90306-4_158-1
90. Rubin BER, Jon G, Sanders KM, Turner NE, Pierce, Kocher SD (2018) Social Behaviour in Bees Influences the Abundance of Sodalis (Enterobacteriaceae) Symbionts. *Royal Soc Open Sci* 5(7):180369. <https://doi.org/10.1098/rsos.180369>
91. Saelao P, Borba RS, Ricigliano V, Spivak M, Simone-Finstrom M (2020) Honeybee Microbiome Is Stabilized in the Presence of Propolis. *Biol Lett* 16(5):20200003.
<https://doi.org/10.1098/rsbl.2020.0003>
92. Sanders JG, Powell S, Kronauer DJC, Vasconcelos HL, Frederickson ME, Pierce NE (2014) Stability and Phylogenetic Correlation in Gut Microbiota: Lessons from Ants and Apes. *Mol Ecol* 23(6):1268–1283. <https://doi.org/10.1111/mec.12611>

93. Santichaivekin S, Yang Q, Liu J et al (2021) eMPress: A Systematic Cophylogeny Reconciliation Tool. *Bioinformatics* (Oxford England) 37(16):2481–2482.
<https://doi.org/10.1093/bioinformatics/btaa978>
94. Shanahan M, and Marla Spivak (2021) Resin Use by Stingless Bees: A Review. *Insects* 12.
<https://doi.org/10.3390/insects12080719>
95. Sickel W, Ankenbrand MJ, Grimmer G et al (2015) Increased Efficiency in Identifying Mixed Pollen Samples by Meta-Barcoding with a Dual-Indexing Approach. *BMC Ecol* 15(1):20.
<https://doi.org/10.1186/s12898-015-0051-y>
96. Steffan SA, Prarthana S, Dharampal JG, Kueneman et al (2024) Microbes, the ‘Silent Third Partners’ of Bee–Angiosperm Mutualisms. *Trends Ecol Evol* 39(1):65–77.
<https://doi.org/10.1016/j.tree.2023.09.001>
97. Su Q, Tang M et al (2022) Jiahui Hu,. *Diet Outweighs Genetics in Shaping Gut Microbiomes in Asian Honeybee*. <https://doi.org/10.1101/2022.01.23.477436>
98. Tackmann J, João, Rodrigues FM (2019) and Christian von Mering. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data. *Cell Systems* 9 (3): 286–296.e8. <https://doi.org/10.1016/j.cels.2019.08.002>
99. Vannette R, Williams N, Stephen Peterson, and Alexia Martin (2025) Pollen Diet, More than Geographic Distance, Shapes Provision Microbiome Composition in Two Species of Cavity-Nesting Bees. *FEMS Microbiol Ecol* 101. <https://doi.org/10.1093/femsec/fiaf067>
100. Vehtari A, Gelman A, Simpson D, Carpenter B, Paul-Christian Bürkner (2021) Rank-Normalization, Folding, and Localization: An Improved $R^{\hat{}}$ for Assessing Convergence of MCMC (with Discussion). *Bayesian Anal* 16(2):667–718. <https://doi.org/10.1214/20-BA1221>
101. Villagómez G, Spaethe J, and Sara Leonhardt (2024) The Stingless Bee *Trigona fulviventr*is Prefers Sweet and Salty over Savory Nectar. *Apidologie* 55. <https://doi.org/10.1007/s13592-024-01081-9>
102. Voulgari-Kokota A, McFrederick QS, Ingolf Steffan-Dewenter, and Alexander Keller (2019) Drivers, Diversity, and Functions of the Solitary-Bee Microbiota. *Trends Microbiol* 27(12):1034–1044.
<https://doi.org/10.1016/j.tim.2019.07.011>
103. Week B, Russell SL, Schulenburg H, Brendan JM, Bohannan, and Marjolein Bruijning (2025) Applying Evolutionary Theory to Understand Host–Microbiome Evolution. *Nat Ecol Evol* 9(10):1769–1780.
<https://doi.org/10.1038/s41559-025-02846-w>
104. Weinhold A, Grüner E, and Alexander Keller (2024) Bumble Bee Microbiota Shows Temporal Succession and Increase of Lactic Acid Bacteria When Exposed to Outdoor Environments. *Front Cell Infect Microbiol* 14. <https://doi.org/10.3389/fcimb.2024.1342781>
105. Wong TKF, Ly-Trong N, Ren H et al (2025) *IQ-TREE 3: Phylogenomic Inference Software Using Complex Evolutionary Models*. April 7. <https://ecoevorxiv.org/repository/view/8916/>
106. Wu Y, Zheng Y, Wang S et al (2021) Genetic Divergence and Functional Convergence of Gut Bacteria between the Eastern Honey Bee *Apis Cerana* and the Western Honey Bee *Apis mellifera*. *J Adv Res* 37. <https://doi.org/10.1016/j.jare.2021.08.002>

107. Zeng Q, Wu S, Sukumaran J, and Allen Rodrigo (2017) Models of Microbiome Evolution Incorporating Host and Microbial Selection. *Microbiome* 5(1):127. <https://doi.org/10.1186/s40168-017-0343-x>
108. Zheng H, Steele MI, Leonard SP, Motta EVS, Moran NA (2018) Honey Bees as Models for Gut Microbiota Research. *Lab Anim* 47(11):317–325. <https://doi.org/10.1038/s41684-018-0173-x>
109. Zilber-Rosenberg I, and Eugene Rosenberg (2008) Role of Microorganisms in the Evolution of Animals and Plants: The Hologenome Theory of Evolution. *FEMS Microbiol Rev* 32(5):723–735. <https://doi.org/10.1111/j.1574-6976.2008.00123>

Figures

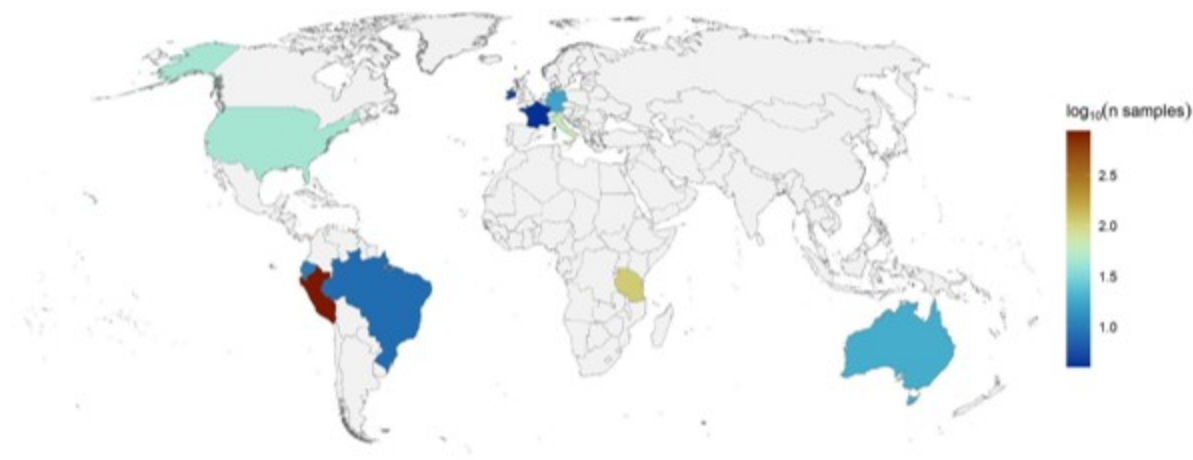


Figure 1

Global distribution of sampling effort. Countries are coloured according to the number of samples available, with colour intensity representing $\log_{10}$ -transformed sample counts. Grey areas indicate countries for which no samples were available. The map highlights spatial variation in sampling coverage across the globe.

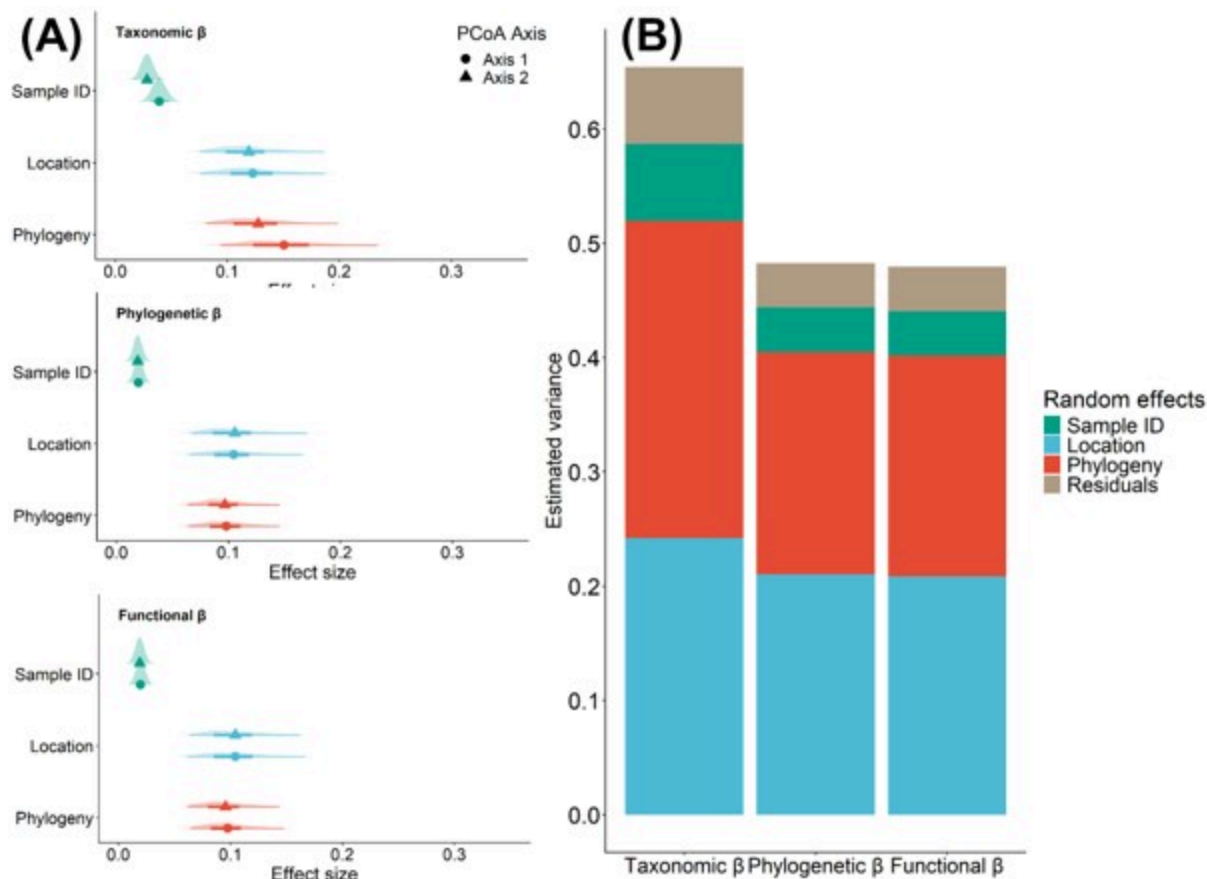


Figure 2

Results from a multi-response phylogenetic generalised mixed models (pGLMMs) quantifying drivers of taxonomic (Bray-Curtis), phylogenetic (weighted UniFrac) and functional (dissimilarity among predicted KEGG ortholog profiles) β -diversity across 60 genera of bees. **(A)** Estimated posterior distributions of random effects. Points represent the posterior mean for each estimate, with 50% and 95% confidence intervals (CIs) represented by heavy and light wicks respectively. **(B)** Variance decomposition of random effects, showing the proportional contribution of host phylogeny, geo-environmental context (plot location) and sample identity to variation in β -diversity. Models were fitted using Markov chain Monte Carlo techniques, with correlated random effects based on the bee genus-level phylogeny. Community dissimilarity was ordinated via principal coordinates analysis (PCoA), and the first two axes were used jointly as Gaussian response variables. Bee traits were included as fixed effects, while phylogeny, location, and sample ID were modeled as random effects, with sample ID accounting for evolutionary divergence among samples within the same genus.

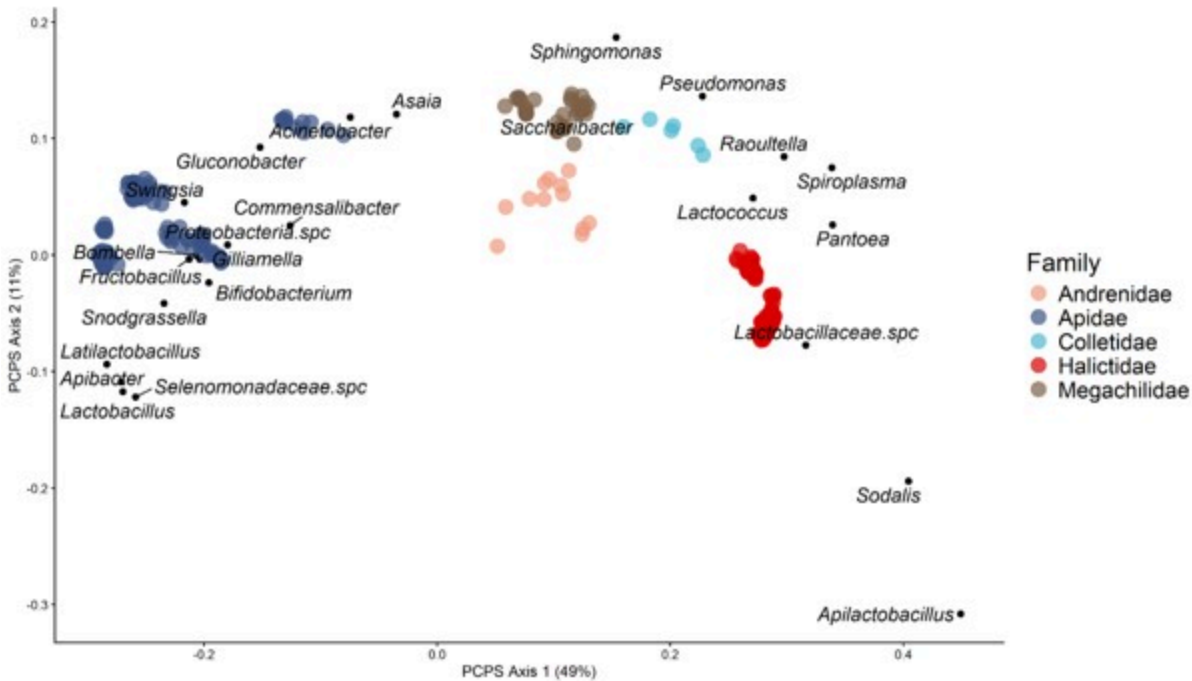


Figure 3

Principal Coordinates of Phylogenetic Structure (PCPS) illustrating dissimilarities among bacterial community profiles, weighted by the phylogenetic distances among bee genera. Only bacterial taxa with a mean relative abundance >0.01% and present in at least 10% of samples are shown. Bray–Curtis dissimilarities among samples were weighted by the phylogenetic relationships among bee genera to account for host evolutionary structure.

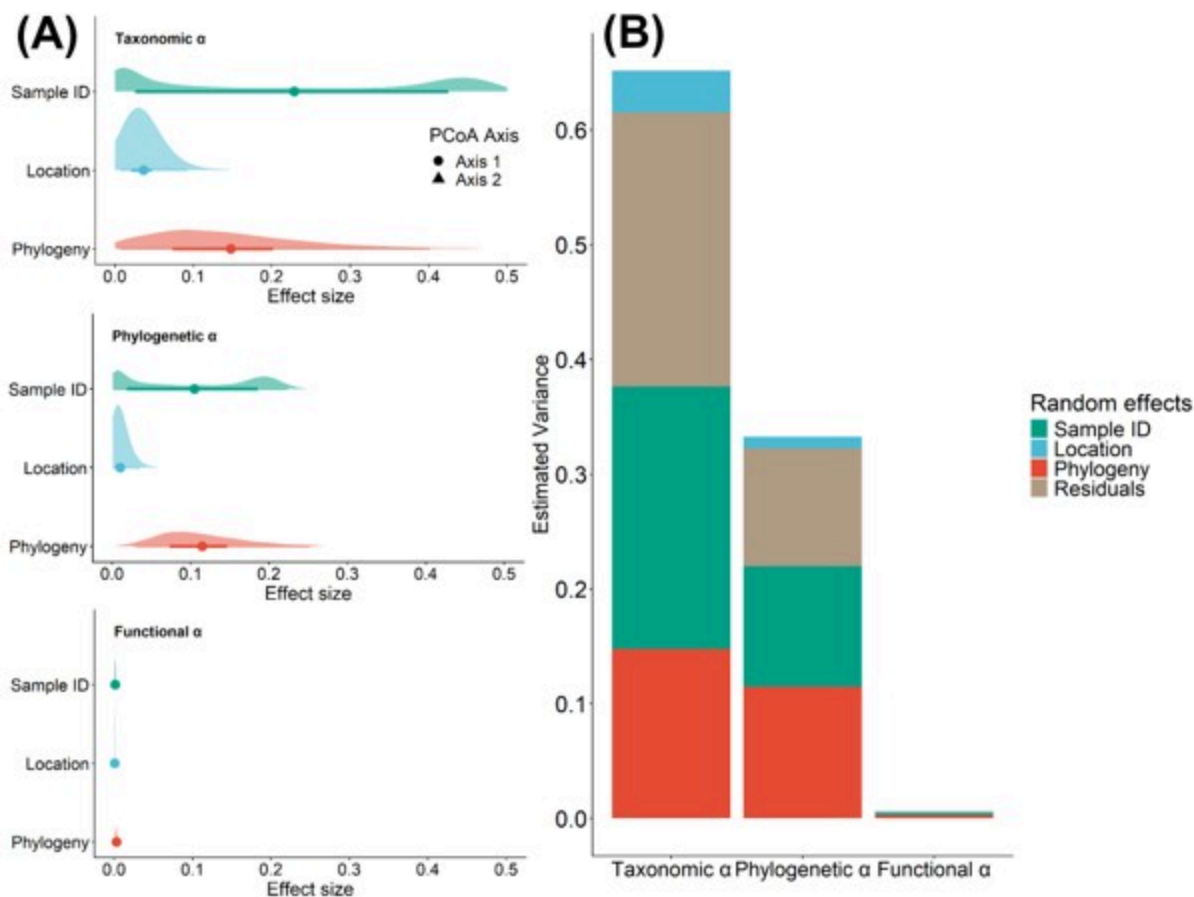


Figure 4

Results from single response phylogenetic generalised mixed models (pGLMMs) of bacterial taxonomic (e^H), phylogenetic (PD) and functional (FD) α -diversity across 60 genera of bees. **(A)** Estimated posterior distribution of random effects. Points represent the posterior mean for each estimate, with 50% and 95% confidence intervals (CIs) represented by heavy and light wicks respectively. **(B)** Variance decomposition of random effects, showing the proportional contribution of host phylogeny, geo-environmental context (plot location) and sample identity to variation in α -diversity. Species diversity was estimated using the Shannon index, with sampling effort standardized through interpolation and extrapolation. Phylogenetic diversity was calculated using Faith's index, and functional diversity was derived from predicted functional profiles. Log-transformed diversity metrics were modeled as Gaussian response variables in pGLMMs to assess the influence of bee phylogeny. Bee traits were included as fixed effects, while phylogeny, location, and sample ID were modeled as random effects, with sample ID accounting for evolutionary divergence among samples within the same genus.

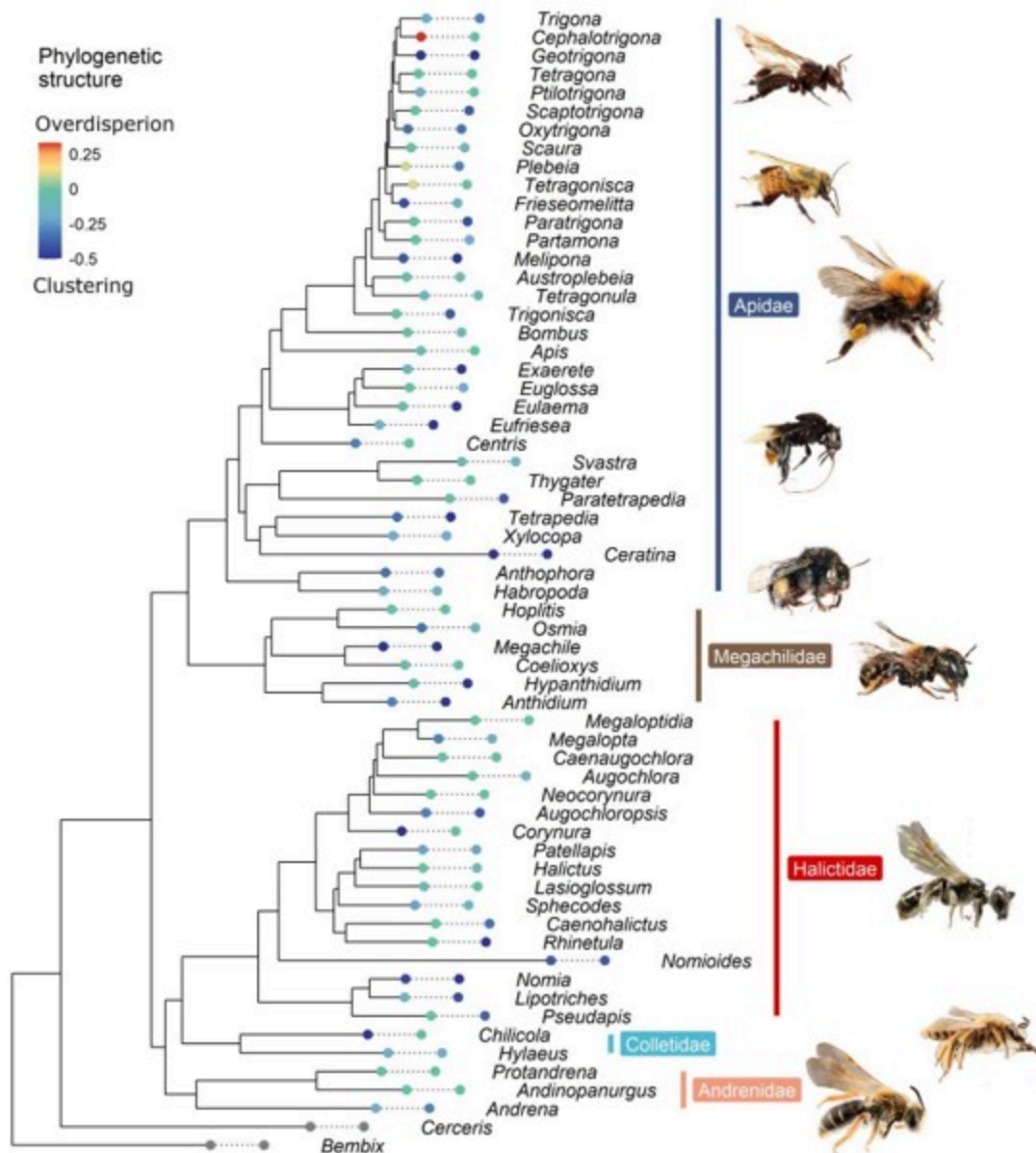


Figure 5

Bee phylogenetic tree at the genus level illustrating the phylogenetic structure of their associated gut microbiota. Tip point colors are based on the Net Relatedness Index (NRI, inner point) and the Nearest Taxon Index (NTI, outer point): blue points represent genera with more samples with phylogenetic clustering (closely related taxa co-occur more than expected), grey points indicate no detectable phylogenetic pattern, and red points show phylogenetic overdispersion (distantly related taxa co-occur more than expected). NRI and NTI values are respectively derived from the standardized effect size of mean pairwise distances and mean nearest taxon distances in bacterial communities. Overall, a large proportion of bee genera hosted phylogenetically clustered gut microbiota, with nearly half ($n=34$) exhibiting significant clustering (based on NRI and/or NTI), indicating that co-occurring bacterial taxa are more closely related than expected by chance. Bee pictures shown from top to bottom: *Trigona cilipes*, *Melipona eburnea* (photos by Clauss Rasmussen), *Bombus hypnorum*, *Eulaema peruvia*, *Anthophora*

plumipes, *Osmia aurulenta*, *Lasioglossum pauxillum*, *Dasypoda suripes*, and *Andrena chrysos* (photos by Pedro Alonso-Alonso). All specimens are from Peru.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SuppMatBeePhyloSymbio.docx](#)