Ecological and evolutionary drivers of stingless bee honey variation at the global scale

Pierre Noiset[1], Madeleine Héger[1], Chloé Salmon[1], Peter Kwapong[2], Rofela Combey[2], Kumara Thevan[3], Natapot Warrit[4], Nathalie Cabirol[5], Marcelo Rojas-Oropeza[5], Carlos Zaragoza-Trello[6], Claus Rasmussen[7], Kiatoko Nkoba[8], and Nicolas Vereecken[1]

[1]Université Libre de Bruxelles
[2]University of Cape Coast
[3]Universiti Malaysia Kelantan
[4]Chulalongkorn University
[5]National Autonomous University of Mexico
[6]Universidad de La Laguna
[7]Aarhus Universitet
[8]International Centre of Insect Physiology and Ecology

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Abstract

Stingless bee honey (SBH) is a prime natural product consumed and used for diverse medicinal and traditional purposes by local communities across the (sub-)tropics. The drivers of its compositional variation within and among species remain poorly understood, although this could inform broader and less explored eco-evolutionary theories. In this study, we aimed to disentangle the roles of evolutionary and environmental drivers of SBH compositional variation using a sampling design that combines honey profiling by H1-NMR spectroscopy with the collection of honeys from honey bees and stingless bees. Our results show a clear differentiation between the chemical composition and functional diversity of honey bee and stingless bee honeys, without identifying a clear continental, phylogenetic or ecological pattern. We provide the first global and comprehensive characterization of SBH composition, a prerequisite for the establishment of standards for while highlighting the need for more interdisciplinary and trans-sectoral research.

Introduction

The concept of extended phenotype lies in the idea that a gene exerts effects beyond the organism itself, by creating physical structures, modifying the environment or manipulating other organisms to increase its reproductive success. Ever since Dawkins introduced the theoretical framework of "extended phenotype" (Dawkins, 1982), contemporary evolutionary biology has demonstrated its influence by providing numerous examples of complex relationships and adaptations in all taxonomic groups (Camargo & Pedro, 2003; Whitham et al., 2003; Cui et al., 2020; Fernandez et al., 2022; DeLong et al., 2022). As the introduction of this conceptual framework stimulated both heated debates and a great deal of research activity (Dawkins, 2004; Hunter, 2018), its incorporation into quantitative and empirical approaches in an attempt to bridge the gap between genotypes and environments towards an integrated theory of evolution remains a challenge. The environment, usually seen as an independent component of phenotypic variation in quantitative genetics, is instead viewed as a variable affected by the genotype (Bailey, 2012; Edelaar et al., 2023). The investigation into extended phenotypes, including behavioral manipulation or housing structure and associated microorganisms, has many potential applications in the fields of ecology and agriculture (Woods et al., 2021;

Favela et al., 2023). This is particularly relevant for keystones species ("whose impact on its community or ecosystem is large, and disproportionately large relative to its abundance" (Power et al., 1996)) such as social insects (Elizalde et al., 2020). In the case of social bees, their ecological and economical importance triggered research on their related extended phenotype, mainly focusing on nesting (Smith et al., 2015), foraging and communication (Hughes, 2008; Schaedelin & Taborsky, 2009) yet overlooking their most renowned product, honey. Honey production combines the features of the extended phenotype with a well-defined genetic basis, from foraging (Page et al., 2000) to nectar collection and processing by bee enzymes (Kunieda et al., 2006; Vannette et al., 2015) and symbiotic microorganisms (de Paula et al., 2021). Further, this contributes to the colony fitness not only as stored source of carbohydrates, but also for improving immunity and regulating temperature, development and adult longevity (Berenbaum & Calla, 2021).

Although the production of honey has been the hallmark of *Apis mellifera* in Europe, the well-known Western honey bee spread across the entire world, another tribe of social bees called stingless bees (Apidae, Subfamily Meliponinae) are found exclusively in the (sub-)tropical regions and produce honey used for its nutritional and medicinal properties (Chuttong et al., 2018; Pimentel et al., 2021; Noiset et al., 2022; Héger et al., 2023; Vit et al., 2024). Approximately 600 species of stingless bees belonging to 45 genera have been described to this date (Engel et al., 2023), making Meliponinae the largest group of corbiculate bees. Despite this great diversity, stingless bees and their products have been relatively less studied than honey bees and have mainly been investigated in a few Neotropical countries (Brazil & Mexico in the lead), neglecting other Neotropical countries, the Afrotropics and the Indomalayan, Papuasian, and Australian (IPA) region (Nordin et al., 2018). Stingless bee honey (SBH) has higher water content than honey from *A. mellifera* , which allows the persistence of hydrophilous symbiotic microorganisms involved in sugar fermentation (Menezes et al., 2013). This is a key process for the preservation of honey that results in a less sweet and more acidic product (Biluca et al., 2016) but also in the production of a range of unique by-products associated with health benefits (Fletcher et al., 2020). Additionally, honey microbiota and phytochemicals transferred from honey pots made of cerumen (a mix of wax and propolis) contributes to its medicinal properties by introducing antimicrobial compounds to reduce the proliferation of competing microorganism (Ngalimat et al., 2020; Chuttong et al., 2023). Therefore, SBH may be the extended phenotype of the hive but also the one of the microorganisms hosted by honey and bees. These microorganisms benefits as well from this aqueous solutions that has been proposed to be the natural habitat of some osmophilic yeasts (Matos et al., 2020).

The understanding of the compositional variability of SBH is still limited due to the numerous sources of variation interacting with each other. Biochemical reactions in stingless bee honey vary according to the bee species producing enzymes, processing dynamics and the microbiota (Souza et al., 2021). Honey microbiota varies according to the foraging patterns, nectar microbiota, environment, hive and the bee species trough vertical and horizontal transmission, the relative importance of each has yet to be defined (Cerqueira et al., 2024). Stingless bee also visits a wide variety of (sub-)tropical plants (Bueno et al., 2023) whose nectar and microbiota composition vary, adding significant levels of phytochemicals and amino acids to honey (Shamsudin et al., 2019; Biluca et al., 2019). Nectar composition itself is influenced by ecological (water availability, temperature, soil factors, interactions with herbivores and nectar robbers) and evolutionary drivers such as plant phylogenetic constraints and pollination syndrome (Nicolson & Thornburg, 2007; Parachnowitsch et al., 2019). Foraging activity of stingless bees also include resin collection to build nests and honey pots which also influence honey composition and properties (Kegode et al., 2023; Villagomez et al., 2024; Nakamura et al., 2024). Beyond these complex factors, the lack of scientific research on the physico-chemical characteristics and bio-functional properties of SBH from the Afrotropics and the Indomalayan, Papuasian, and Australian regions hinder the exploration of the evolutionary ecology hypothesis regarding SBH.

To fill these gaps, our study aimed to work at the global level to i) characterize the differences between the composition of honeys from stingless bees and honey bees using advanced chemometric methods, ii) investigate patterns of compositional variation among tropical regions and finally iii) disentangle the roles of evolutionary history and environmental conditions on honey properties. Ultimately, we aim to provide the basis of a theoretical framework to understand the drivers of compositional variability of SBH.

Material and methods

Sample collection .

To investigate the compositional variation of SBH and *Apis* spp. honey, we collected honey samples from diverse geographic regions across the world within all five continents (Figure 1; Supplementary Table 1 & 2) between January 2019 and July 2022. We collected samples from different countries within each targeted region to ensure broad representation of the environmental and ecological diversity of stingless bee habitat. Collection sites within each country were selected based on the availability of honey samples produced by both stingless bees and honey bees. Honey samples were collected during similar periods across different locations whenever possible to minimize the impact of inter-seasonal variation in honey composition and properties (Aleixo et al., 2017).

Stingless bee honeys ($n = 150$) from 48 species distributed in 23 genera (Full list in Supplementary Table 1) in the subfamily Meliponinae were collected from managed colonies in the Afrotropics ($n = 50$), the Neotropics ($n = 50$) and the Indo-Malayan region ($n = 50$). Two extraction methods described by Mokaya et al., 2022 were employed to harvest honey directly from sealed pots within the nest. The stingless bee species identity was provided by the meliponiculturists (i.e. stingless bee beekeepers) during honey harvest and confirmed by identification of voucher specimens. *Apis mellifera* ($n = 69$), *Apis cerana* (n=12), *Apis florea* (n=2) and *Apis dorsata* (n=2) honeys were extracted from managed colonies by squeezing the honeycomb. A volume of 10 ml of honey was collected from each colony and placed in a sterilized container. After honey collection in the field, honey bee samples were stored in a fridge (< 5 °C) while SBH honeys were stored in a freezer to prevent fermentation due to their higher water content.

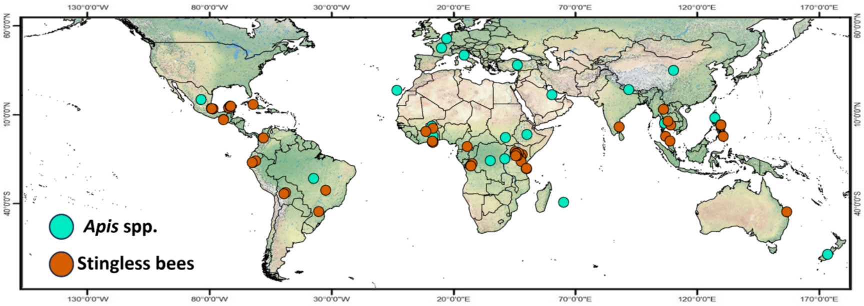


Figure 1 : Geographical distribution of honey samples harvested from honey bees (n=90) and stingless bees (n=150) colonies. We collected SBH from twenty-three genera in the subfamily Meliponinae from managed colonies (meliponaries, i.e., stingless bee colony nurseries) in the Afrotropics ($n = 50$), the Neotropics ($n = 50$) and the Indo-Malayan region ($n = 50$). See Methods and Results sections for more details.

Honey profiling by 1H-NMR spectroscopy

To characterize the compounds and properties of our honey samples, we used H^1 Nuclear Magnetic Resonance spectroscopy (hereafter H1-NMR spectroscopy), a state-of-the-art analytical technique increasingly used alongside chemometrics statistical approaches for the qualitative and quantitative control of honeys, as well as to assess the botanical origin of honeys and to quantify their major constituting compounds (Schievano et al., 2012; Ohmenhaeuser et al., 2013). H1-NMR spectroscopy was carried out on all 240 samples described above at the laboratories of Quality Services International GmbH (QSI, Bremen, Germany) following the method described in Noiset et al, 2022. We quantified 36 compounds grouped in five categories : sugars (n=10), organic acids (n=3), amino acids (n=8), fermentation markers (i.e., all the compounds involved in sugar transformation trough alcoholic, acetic and lactic fermentation; n=10) and anti-microbial compounds (n=5) (Supplementary Table 3).

Another set of physicochemical data of honey samples of *Apis mellifera* ($n = 10$) from Mexico analysed according to the protocol described previously at the QSI laboratories were pooled with our dataset of 90 A*pis* spp. samples; this provided a better balance between the number of honey bee and stingless bee samples analysed in this study.

Statistical analyses

All the statistical analyses presented here were performed in RStudio (RStudio Team, 2020) for R (R Core Team, 2021). Our raw data were first transformed using the Hellinger transformation (square root transformation of the relative concentration in each sample) to limit the influence of higher values and compute Euclidean distance matrix between each sample. We performed a Principal Component Analysis (PCA) on this distance matrix to identify variables significantly explaining the variation in physico-chemical characteristics in our dataset using the *vegan* package (version 2.6-4) (Oksanen et al., 2022) to obtain a set of 13 variables in our final dataset : fructose, glucose, turanose, maltose, mannose, glutamine, leucine, proline, valine, acetic acid, lactic acid, formic acid, pyruvic acid.

The raw data of the selected compounds were standardized using the double Wisconsin transformation. Using this approach, each value in the "species x compounds" matrix was divided by its column maximum, and then divided by the row total, producing values between 0 and 1 that equalize emphasis among sample units and among species. Bray–Curtis dissimilarities were computed on the transformed data to conduct multivariate analyses using the *vegan* package. We then performed Permutational Multivariate Analysis of Variance (PERMANOVA) to evaluate significant differences among honey samples from stingless bees and honey bees. These differences between groups of honey samples were illustrated in Non-Metric Multidimensional Scaling (NMDS) plots using the *ggplot2* package (version 3.3.3) (Wickham, 2016) and a dendrogram (hierarchical clustering) using the *ggtree* package (version 3.4.1) (Yu, 2020). Indicator compounds analysis was performed to identify the compounds associated with each taxa using the*indicspecies* package version (1.7.14) (Cáceres & Legendre, 2009). We also validated the classification of our honey samples using model-based approach. Our dataset was split in two equal parts to (1) train and (2) test a conditional random forest model that assigns honey bee or stingless bee label to each sample using the*extendedForest* package (version 1.6.1) (Liaw & Wiener, 2002). We also compared honeys from stingless bees and honeys bees in terms of chemical diversity and dissimilarity by comparing diverse types of indexes : Hill diversity, Functional Hill diversity, Shannon's diversity, Simpson diversity, Rao's Q, Pielou's evenness and Hill evenness. Diversity indexes assessments were based on the chemical structure of the 36 compounds quantified using the *chemodiv* package version (0.3.0) (Petrén et al., 2023).

We then investigated patterns of variation in SBH composition by testing the differences in the composition according to the tropical regions. Further analyses performed to estimate the importance of environmental and phylogenetic constraints on honey composition, using the full dataset at the global scale but also datasets at the continental scale including honey samples from each of the tropical region. We computed phylogenetic dissimilarities based on the patristic phylogenetic distances between genera using the phylogeny established by Rasmussen & Cameron, 2010 (Supplementary Fig. 1) using the *adephylo* package (version 1.1-16) (Jombart & Dray, 2008). Environmental dissimilarities between locations were computed based on 8 variables impacting nectar production (soil moisture, precipitation, solar radiation) and composition (Tree cover, moisture, temperature, forest density, plant biodiversity). Solar irradiance (The World Bank Group, 2023) and water availability (Topographic wetness; Title & Bemmels, 2018 & Annual Mean precipitation; Fick & Hijmans, 2017) are the main limiting factors in nectar production, influencing its concentration in sugar. Humidity (Climatic Moisture Index; Title & Bemmels, 2018) and temperature (Annual Temperature; Fick & Hijmans, 2017) impact evaporation rate leading to differences nectar viscosity and concentration (Nicolson & Thornburg, 2007). Honey produced in forest differed in its composition (Lowore et al., 2018; Cannizzaro et al., 2022) due to the influence of tree cover and density (Forest density; Riitters et al., 2000) on microclimatic factors (Parachnowitsch et al., 2019). Finally, we included plant biodiversity (native plant diversity; Ellis et al., 2012) which impact resource availability and pollen profile of honeys (mono-/polyfloral; de Sousa et al., 2016; Ávila et al., 2019). Data were extracted from Raster files using annual or monthly mean value when available.

We estimated the part of variation of chemical dissimilarity explained by the phylogenetic and environmental dissimilarity matrix using the*varpart* function in *vegan* . Finally, we tested correlations and linear relationships between the Bray-Curtis dissimilarity matrix of chemical composition and the phylogenetic/environmental dissimilarities using partial mantel tests and distance-based RDA. Variation partitioning and correlation analysis were performed at the global but also at continental scale to disentangle ecological and evolutionary drivers since phylogeny follows a geographical split.

Results

The PERMANOVA showed significant differences between honey samples from stingless bees and honey bees (p-value = 0.001, df = 1, R^2 = 0.230). Our NMDS plot (Fig. 2) illustrated these differences, showing a clear general distinction between the two discrete clusters of samples. The composition of a few samples did not follow this pattern and had opposite characteristics, resulting in a small number of errors (4 out of 75 samples) for stingless bees in the classifications of the conditional random forest models. Major fermentation markers (acetic and lactic acid) and sugars (fructose and glucose) were recorded in all honeys, and they were the most relevant compounds to discriminate the two groups of honeys. Stingless bee honeys had lower sugars levels and higher fermentation marker levels than honey bees samples. The amino acid levels also varied significantly among our samples independently of the bee taxa. Finally, our indicator compounds analysis showed no significant association between bee taxa and a specific compound.

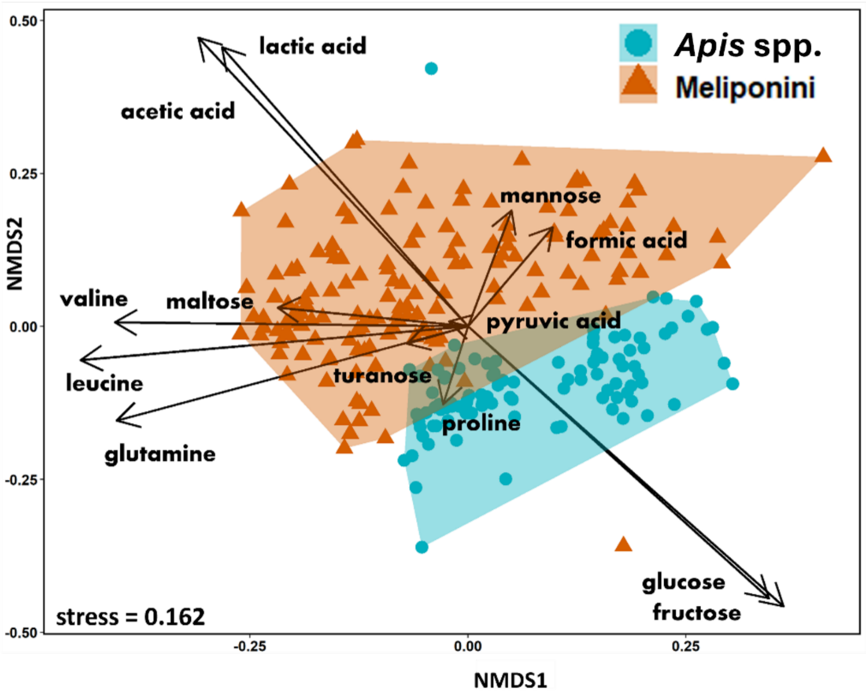


Figure 2. Non-linear Multidimensional Scaling (NMDS) ordination plots showing the differentiation of honey samples grouped into two convex hulls to the bee tribe. The results show that SBH were divided in two discrete groups with little overlap due to one sample of each group that differed from the compositional profile of its tribe.

Our chemodiversity analysis showed that SBH had a significantly higher richness, evenness, disparity and functional diversity than honey bees according to all the indexes assessed investigated (Supplementary Fig. 2). Non-sugar compounds represented 7% of the composition of samples from honey bees while these represented 19% in SBH composition, mainly fermentation markers. Among the three main fermentation markers, lactic ($X^2 = 136.87$, df = 1, p-value $< 2.2e\text{-}16$) and acetic acid ($X^2 = 155.64$, df = 1, p-value $< 2.2e\text{-}16$) levels were significantly higher in SBH while ethanol was present in equal concentration.

Differentiation in stingless bee honey composition

The pairwise comparison of SBH between each pair of tropical regions using PERMANOVA showed significant differences (p=0.003) in the composition of honeys across regions. However, the proportion of the variation explained by the geographic group was low (<20%, R^2 ranging from 0.05 to 0.183) , with the lowest value recorded being between the Neotropics and the Afrotropics (5%).

Random forests models and hierarchical clustering (Table 1 & Supplementary Fig. 3) illustrated that the distinction between groups of honey samples was ambiguous, particularly among samples from the Neotropics and the Afrotropics. None of the clusters included samples from a single tropical region; instead they were composed of a mix of samples from widely different geographical origins. Amino acids, fermentation makers and sugars were significantly varying among each tropical regions (Fig. 3), especially those involved in lactic acid fermentation (sugars including fructose glucose, raffinose and maltose, pyruvic acid and lactic acid) which were the most important compounds in the random forest classification. Indicator compound analysis showed that honeys from the Afrotropics were associated to kynurenic acid (stat = 0.424, p-value = 0.005) and acetoin (stat = 0.511, p-value = 0.01) and honeys from the Neotropics to methylglyoxal (stat = 0.532, p-value = 0.005) while we found no significant association between a specific compound and honeys from the Indo-Malayan region.

Table 1 : Prediction results of the conditional random forest models to predict the geographical origin of SBH samples.

Predicted Origin True Origin	Afrotropics	Indo-Malayan region	Neotropics	Prediction accuracy
Afrotropics	18	2	5	72%
Indomalayan region	3	22	0	88%
Neotropics	3	5	17	68%

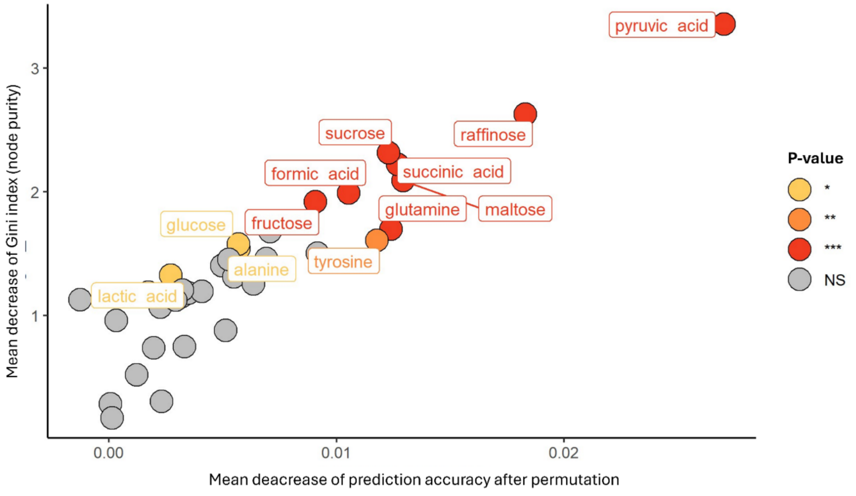


Figure 3 : Multi-way importance plot illustrating importance measures of each variable in the random forest decision trees. Significant variables (that are used significantly more often for splitting the decision trees than if the selection was random) are coloured according to the p-value (*** = <0.001, ** = prediction accuracy are two measures correlated to the reduction of misclassification when the variable is selected in the decision tree of the random forest model.

Ecology and evolution of stingless bee honey

Beyond the geographical origin, our analysis of variation partition in SBH composition showed that ecological and evolutionary drivers and their joint effects were all explaining at least in part, the differences in honey characteristics (Fig. 4). The importance of each type of drivers varied according to the spatial scale, with a greater role of the joint effect of ecology and bee phylogeny at the global and Afrotropical level whereas it was non-significant in IPA region and in the Neotropics. Overall, 40 to 60% of the variation remained unexplained.

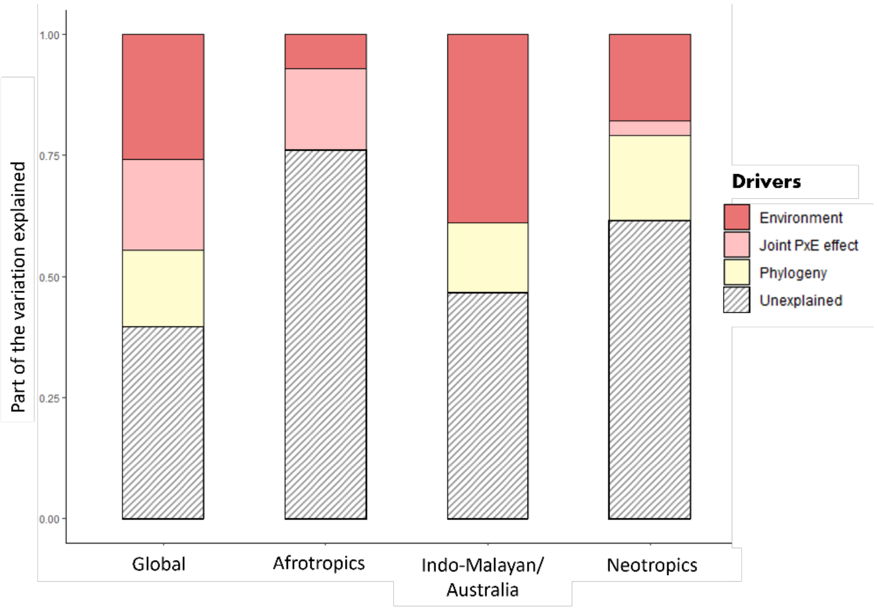
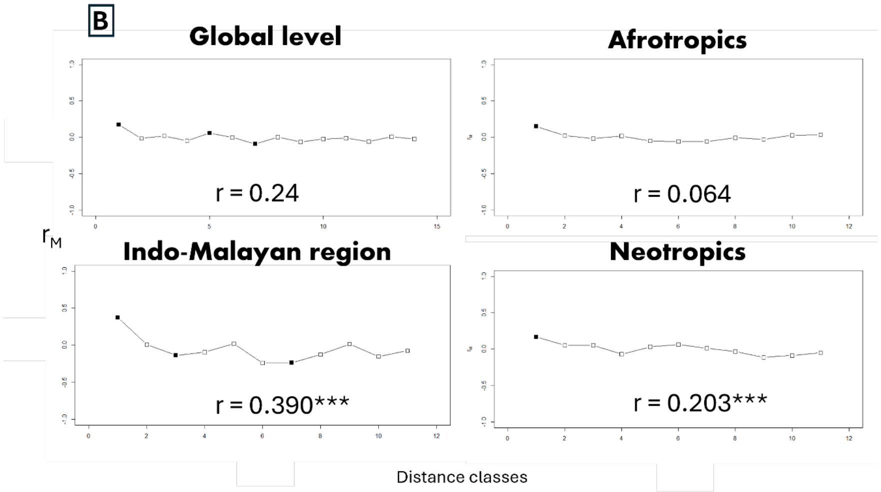


Figure 4 : Stacked bar plot showing the part of variation in SBH composition explained by each class of drivers at the global and regional scale.



The analyses of variation partitioning were consistent with the partial Mantel tests and distance-based RDA that showed significant correlation and linear relationships between honey composition and phylogenetic and ecological dissimilarities respectively at both global and continental scale (Supplementary Table 4). The only exception was the absence of significant correlation between honey composition and ecological dissimilarities at the global and Afrotropical levels. Mantel correlogram (Fig. 5) showed that the correlations with ecological drivers were significant for samples collected in similar environment while correlation with phylogeny was significant for a larger range of distances.

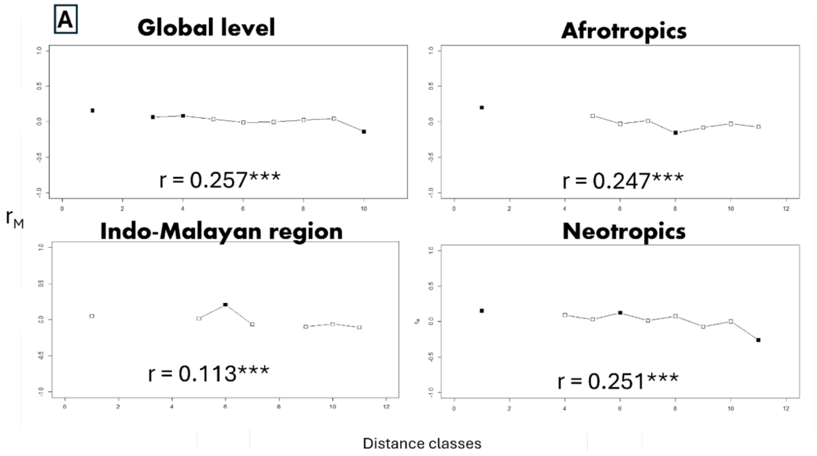


Figure 5 : Mantel correlograms showing correlation between each class of distances of honey composition and phylogenetic (A) and environmental (B) dissimilarities. Pearson's r and the significance of each association (*** = <0.001, ** = <0.01, * = were tested using the environmental or the phylogenetic distance matrix as a control matrix respectively.

Discussion

In this study, we provide evidence for differentiation in compositional profile of honeys produced by stingless bees and honey bees at an unprecedented spatial scale using advanced analytical and chemometric methods. Our results highlight that fermentation markers and sugars are the main compounds explaining the pattern observed between stingless bee and honey bee honeys, which supports recent studies (Muhammad & Sarbon, 2021; Gadge et al., 2024). The transformation of sugars into acids, ethanol and a variety of secondary metabolites during the fermentation process that occurs in SBH is a major driver of increased chemical composition diversity. These key differences lies in the transformation of sugars into acetic acid and lactic acid performed by bacteria, including lactic acid bacteria (LAB) and *Bacillus* spp. found in SBH (Menezes et al., 2013; Ngalimat et al., 2020). Our analysis of chemodiversity show that compared to honey bee honey, SBH had not only a higher compound richness but also a higher structural diversity of compounds (i.e functional chemodiversity). The simultaneous presence of a greater number of compounds with different biochemical properties fosters synergetic actions among them (Whitehead et al., 2021; Petrén et al., 2023), presumably conferring significant reported therapeutic benefits of SBH for both the bee colony and human health (Rao et al., 2016; Berenbaum & Calla, 2021; Héger et al., 2023). For instance, organic acids produced during fermentation result in increased antimicrobial properties of honey (e.g. formic and lactic acid; Raftari et al., 2012) while coupling with the effects of compounds naturally present in nectar, including amino acids (e. g. phenylalanine which has known neuroprotective effects (Zamri et al., 2023)) and organic acids with metal-chelating antioxidant activity (Viuda-Martos et al., 2008). Some notable examples include kynurenic acid, a metabolite known for its anti-inflammatory and antioxidative activity (Turska et al., 2022), that was only identified in SBH from the Afrotropics (25-198 mg/kg) in proportions approaching levels of chestnut honey (305 mg/kg in the chestnut honey sample from Turkey in our dataset), one of the main food enriched in kynurenic acid (Turski et al., 2016). Neotropical SBH were associated to methylglyoxal (0-30 mg/kg), a key compound of the medical grade Manuka (*Leptospermum scoparium*) honey bee honey (400+ mg/kg) known worldwide for its exceptionally high levels of antibacterial activity (Adams et al., 2009). These results show how the shared use of SBH by bees and microorganisms may increase both their fitness by protecting the colony against microbial pathogens and toxins while providing propitious and controlled environment for symbiotic microorganisms (Menezes et al., 2013; Fernandes et al., 2022), following theoretical research on extended fitness hypothesis (de Araújo et al., 2021).

Our study illustrates a significant magnitude of variation in the compositional profiles of SBH, driven by a range of compounds including sugars, amino acids and fermentation markers. Unexpectedly, our analyses did not allow to pinpoint a clear ecological, evolutionary or geographical driver explaining these differences in chemical properties among stingless bee honeys. SBH from the Afrotropics tend to be more similar to honeys from Central and South America than honeys from the other tropical regions of the Old world, even though global phylogeny suggests a division of the *Meliponinae* subfamily into Old and New World groups (Rasmussen & Cameron, 2010). The non-systematic composition of SBH highlights its important plasticity but also our lack of knowledge regarding ecology and evolution of the tribe. Environmental and evolutionary drivers investigated in this study alone do not explain all the variations in chemical composition. The environmental variables were mainly selected according to the available literature on nectar chemistry (Petanidou, 2007; Parachnowitsch et al., 2019; Nicolson, 2022). The impact of ecological conditions on nectar processing by stingless bees remains poorly understood, even though some species perform little or no thermoregulation within the hive (Roubik, 2006; Ayton et al., 2016) potentially exposing stored nectar to certain levels of alterations. Available flora and the proportional use of host plants play a key role in honey composition (Silva et al., 2009; Machado et al., 2020; Shamsudin et al., 2022) but cannot be included in formal analysis since the foraging ecology and seasonality of stingless bees in the Afrotropics and Indo-Malayan region remains largely undocumented (Aleixo et al., 2017; Bueno et al., 2023). The phylogeny of stingless bees is also only partially resolved and remains at the genus level, masking the variations in honey composition that are likely to occur within the same genus (de Sousa et al., 2016; Ismail et al., 2021). In addition of microbiota whose role is depicted above, pollen profile (monofloral *vs.* polyfloral) (Sousa et al., 2016; Chuah et al., 2023), maturation stage of the fermentation process acids (Silva et al., 2022), propolis composition and the management type that varies from rearing wild colonies to advanced beekeeping methods across the tropics (Delgado et al., 2020) have demonstrated impacts on honey properties and should be investigated throughout the tribe. This is of particular importance as the observed complex interplay of biotic and abiotic factors shaping honey composition leads to an important phenotypic variation in properties of SBH that may provide fitness benefits for the colony as shown for other extended phenotypes (Blamires et al., 2018).

The study of SBH is not only of fundamental interest, but also has applied purposes. Beekeeping with stingless bees (i.e. meliponiculture) is promoted as a tool for rural development and sustainable income generation activity, out of the Maya region where it's an age-old tradition and first emerged to meet the medicinal and nutritional needs of an exploding population (Cortopassi-Laurino et al., 2006; Bahri et al., 2016; Mustafa et al., 2018; Chemurot et al., 2021; Rasmussen et al., 2024). The development of meliponiculture is also supported to prevent destructive honey-hunting practices of stingless bee species, otherwise severely threatened by agrochemicals (Lima et al., 2016), pathogens (Fleites-Ayil et al., 2023), climate change (Giannini et al., 2017; Dalsgaard, 2020) and drastic land use change (Gibbs et al., 2010; Curtis et al., 2018). One of the main challenges in achieving economically sustainable meliponiculture is the absence of reliable market access due to the lack of international standards for SBH. Although a number of initiatives have been undertaken at national and regional level (Vit et al., 2004; Malaysian Standards, 2017; de Camargo et al., 2017), SBH has not been formally acknowledged by the Codex Alimentarius (Codex Alimentarius Commission, 2019), preventing wider market access for meliponiculturists and multiplying counterfeits on sale. In order to establish standards, several studies have investigated into the species- and genus-specific composition of SBH (Chuttong et al., 2016; Mokaya et al., 2022; Vit et al., 2022) and the impact of particular climatic and environmental conditions on its properties (Sousa et al., 2016; Dezmirean et al., 2017; Lavinas et al., 2023; Noiset et al., 2024) but relatively few at global scale and on the joint effect of evolutionary and ecological constraints. Yet, according to our results, if closely related species or species from the same region tend to produce similar honeys, this rationale does not extant to more distant species that can produce comparable or very contrasting honeys depending on the studied regions. Compositional profile of stingless bee can also be similar to those produced by honey bees, a pattern observed in this study and at intercontinental scale (Noiset et al., 2022). Therefore, precise and unique standards for a species or an environmentally heterogeneous region (i.e. a country) can ultimately turn out to be ineffective. By contrast, quality labels associated with specific local production conditions correspond more effectively to variations in the chemical composition of SBH. Additionally, compounds not quantified in this study such as trehalulose are promising markers to differentiate SBH (Fletcher et al., 2020; Zawawi et al., 2022). International standards for SBH should remain very broad to include all the possible compositional profiles, especially as some of the key compounds that differentiate them from *A. mellifera* (sugars and fermentation products) are among those that vary the most within stingless bee honeys.

Our study confirms the differences in composition between *Apis* honeys and SBH, while showing the significant variations in SBH composition across the Tropics due to the many ecological, evolutionary and human management factors involved. Phenotypic variation in SBH makes it an ideal model for the study of ecological and evolutionary theory but it requires more fundamental research on this under-studied tribe to fully understand the role of honey in bee colony and human health. Comprehensive characterization of SBH properties in Afrotropics and the Indo-Malayan region/Australia that takes into account its specificity and dynamism is a key step for the establishment of scientifically informed standards to promote sustainable livelihood through nature-based solutions and the exploitation of non-timber forest products.

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