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Abstract- Propolis is used by corbiculated bees to protect the bee hive; it is mostly used to seal cracks, to reduce or prevent microbial growth and to embalm invaders. Different factors have been reported to influence the chemical composition of propolis, including bee species and the flora surrounding the hive. Nevertheless, the majority of the studies are focused on propolis produced by *Apis mellifera*, while studies on the chemical composition of propolis produced by stingless bees are still limited. In this investigation, the chemical composition of 27 propolis samples collected in the Yucatan Peninsula from *A. mellifera* beehives, together with 18 propolis samples from six different species of stingless bees, were analyzed by GC-MS. Results showed that lupeol acetate and *β*-amyrin were the characteristic triterpenes in propolis samples from *A. mellifera*, while grandiflorenic acid and its methyl ester were the main metabolites present in samples from stingless bees. Multivariate analyses were used to explore the relationship between bee species and botanical sources on the chemical composition of the propolis samples. Differences in body size and, therefore, foraging abilities, as well as preferences for specific botanical sources among bee species, could explain the observed variation in propolis chemical composition. This is the first report on the composition of propolis samples from the stingless bees *Trigona nigra*, *Scaptotrigona pectoralis*, *Nannotrigona perilampoides*, *Plebeia frontalis* and *Partamona bilineata*.

Key Words - Chemometrics, Apini, Meliponini, Lupeol acetate, Grandiflorenic acid.

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INTRODUCTION

Resin-collecting bees, which include honey bees (Apini tribe) and stingless bees (Meliponini tribe), are known as corbiculated bees or bees with basket for pollen. These bees mix resins secreted or exuded from wounds, or buds, of different plants, with wax produced in specialized bee´s glands and, in some cases, with other type of materials, to produce propolis (Bankova 2005; Salatino et al. 2011; Martins et al. 2014). Propolis is the main material used by bees to prevent microbial infection of larvae and the honey comb. At the same time, bees use propolis to seal gaps and protect the hive against airflow, to reduce microbial growth, to embalm invaders, and to prevent external moisture (Simone-Finstrom and Spivak 2010; Kuropatnicki et al. 2013).

The use of propolis by humans is as old as the use of honey; there are reports that suggest propolis being used by ancient Egyptians to alleviate diverse ailments and for embalming corpses (Kuropatnicki et al. 2013). Presently, propolis is an important material with many applications for human use; it is known to have antimicrobial, astringent, anti-inflammatory, anaesthetic, antioxidant, antitumoural, immunomodulatory and hepatoprotective effects, among others (Kuropatnicki et al. 2013; Toreti et al. 2013; Bankova et al. 2014). These diverse biological activities depend on the chemical constituents of propolis and these constituents depend, in turn, on various factors related to the elaboration of propolis by the bee species (Pazin et al. 2017).

Although the composition of propolis can be rather variable, propolis is generally made of a mixture of 55% resins and/or balms, 30% of wax, 7% of essential oils, 5% of pollen and 3% of various materials (Huang et al. 2014). In terms of chemical composition, a recent review reported the existence of five main types of *A. mellifera* propolis (Salatino et al.

2011). However, to date, knowledge about the chemical composition of propolis produced by stingless bees in tropical regions is still limited, with the exception of Brazil where studies are concentrated mainly in the genus *Melipona* (Campos et al. 2014; Dutra et al. 2014; Araújo et al. 2016; Santos et al. 2017; Lavinas et al. 2018). Previous studies on the chemical composition of propolis from the Yucatan peninsula reported triterpenoids being the main components of the extract (Boisard et al. 2016), and the purification of extracts of Yucatecan propolis resulted in the identification of a number of non-polar, GC-volatile secondary metabolites such as terpenoids (Herrera-López et al., 2019) and phenolic lipids (Herrera-López et al., 2020),

A number of factors have been reported to influence the chemical composition of propolis, these include altitude (Andelkovic et al. 2017), the season of collection (Maraschin et al. 2016), as well as the type of vegetation surrounding the beehive (Watson et al. 2006; Mot et al. 2010; Morlock et al. 2014; Chasset et al. 2016; Andelkovic et al. 2017). In recent years, multivariate analyses have been used as tools to find patterns in the variation observed on the chemical composition of propolis and the relationships of this variation with different explicative variables. In particular, multivariate analysis have shown differences between the composition of propolis collected by *A. mellifera* and that collected by stingless bee species (Sawaya et al. 2006). In this investigation, the chemical composition of propolis samples produced by different bee species, collected in various sites of the Yucatan peninsula, was evaluated and multivariate analyses were used to understand the relationship between bee species and botanical sources with the variations observed in the chemical composition of the different propolis samples.

METHODS AND MATERIALS

Propolis Samples. A total of 27 propolis samples from *A. mellifera* were provided by beekeepers from different apiaries (Fig. 1a; Table S1). In the studied area, meliponiculture is practiced by a small number of beekeepers, thus the collection of samples of this type of propolis was less extensive; 18 samples from stingless bees were collected in six locations (Fig. 1b; Table S2).

Preparation of Extracts. The collected samples of propolis were ground to a homogeneous powder using liquid nitrogen; 1 g of this powder was extracted three times (2 h) with ethanol (20 mL) at room temperature. The combined filtrates were kept overnight at −18°C and then filtered to separate precipitated waxes; finally the solvent was eliminated under reduced pressure to yield the corresponding propolis extracts (Boisard et al. 2016).

GC-MS Analyses of Propolis Extracts. Analyses were carried out in the Laboratory of Natural Substances and their Structural Analogues (SONAS) at the University of Angers, France. Taking into account the previous studies on the chemical composition of propolis from the Yucatan peninsula, in which the main components are non-polar, GC-volatile secondary metabolites, propolis extracts used for this investigation were analyzed without derivatization. The 45 samples were analyzed injecting 8 µL of extract, at a concentration of 1% (1mg/100 µL ethanol), in a gas chromatograph GCMS-QP2010 SE Shimadzu, with an ionization voltage of 70 eV. The separation was carried out using a Phenomenex Zebron ZB-5 (5% phenyl, 95% dimethylpolysiloxane) column (30 m × 0.25 mm Ø, thickness of 0.25 µm) and helium as a carrier gas (2 mL/min), with a temperature program of 110 °C (0.5 min), 110-280 °C (20 °C/min), 280 °C (20 min), 280-300 °C (5 °C/min), 300 °C (2 min).

Identification of Individual Metabolites. For a metabolite to be considered tentatively identified, its mass spectrum would have to match the corresponding mass spectra contained in the Shimadzu (FFNSC2) library of flavors and fragrances of natural and synthetic compounds and in the library of the National Institute of Standards and Technology (NIST 11) and coincide with its reported linear retention index (Adams 2007; Babushok et al. 2011; Zellner et al. 2008). Definitive identification of individual metabolites was carried out by comparing their mass spectra with those of available commercial standards or authentic samples previously purified and identified in our laboratories.

Multivariate Statistical Analysis. Multivariate analyses were performed to evaluate the effect of the different bee species on propolis composition, comparing two groups of samples, i.e. propolis samples from *A. mellifera vs* propolis samples from different species of stingless bees. A second analysis was performed to compare propolis composition among samples from the six different species of stingless bees, while a third analysis evaluated the effect of vegetation type (deciduous and semi-deciduous forests) on the composition of propolis produced by *A. mellifera.*

Data matrices were built using, as variables, the relative abundance of each metabolite (% of the total area in the chromatogram), placed in columns according to their retention times, and the propolis samples, placed in rows. Only metabolites which had a relative abundance $\geq$ 1% in at least one sample were included. The Plymouth Routines in Multivariate Ecological Research (PRIMER) version 7, were used for multivariate analyses (Clarke et al. 2014). A Log(x+1) transformation was used for relative abundance values (% chromatogram area) to perform the multivariate analysis. Similarities were evaluated using the Bray-Curtis similarity index (S), obtained from the transformed matrix. The ordination

method used was non-Metric Multidimensional scaling (nMDS), were the similarity of samples decreases with the distance in the nMDS plot (Clarke et al. 2014; Dittmar et al. 2007). The stress value indicates the distortion between the similarity ranks and the corresponding distance ranks in the ordination; ordinations with low stress values in the range 0.2-0.05 correspond to an adequate representation of data similarities (Clarke et al. 2014).

The analysis of similarities test (ANOSIM) was used to determine if the observed differences, according to each factor (bee species and vegetation type), were statistically significant under the null hypothesis, considering there are no differences in the propolis composition according to the bee species or the vegetation type. The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups, $R = 0$ indicates that no differences are present between sample groups, while $R = 1$ indicates results when groups differ completely. A set of 999 permutations was randomly calculated to build the null distribution of R (Clarke et al. 2014). The Similarity Percentages (SIMPER) analysis was used to evaluate the contribution of different metabolites in order to explain differences among the propolis samples (Clarke et al. 2014).

RESULTS

Metabolites in Propolis Extracts. A total of 93 metabolites were detected in the propolis samples representing, on average, 94% of the total chromatogram area. Triterpenes, diterpenes, phenolic lipids, fatty acids, alkanes, as well as sterols, sesquiterpenes, long chain alcohols, alkenes, flavonoids and lipids were recorded (Table S3). Lupenone, lupeol and α-amyrin, showed concentration values higher than 10% in both types of propolis,

representing, in average, 49.5% of the chromatogram area in samples from *A. mellifera* and 57.6% in stingless bees propolis (Table 1, Fig. 2). Certain metabolites were exclusive to propolis samples from each particular bee species (Table S3), i.e. 17 metabolites were present only in propolis samples from *A. mellifera* (*e.g*. kaura-5,16-dien-18-ol, octacosanol, 5,23-ergostadienol acetate), while 15 metabolites were found only in propolis samples from stingless bee species (*e.g*. sandaracopimarinal, totarol, lignoceric alcohol).

Bee Species and Chemical Composition of Propolis. Triterpenes were the only type of metabolites present in all propolis samples. *A. mellifera* samples showed a slightly higher content (6.3%), with an important variation between 0.3 and up to 22% of the chromatogram area. In comparison, in propolis samples from stingless bees, triterpenes presented an average relative abundance of 5.6%, and values varied between 0.4 and 27.4%. The other types of metabolites were found in different relative abundances according to the bee species (Table 1). It is important to mention that, of all the chromatographic profiles of propolis extracts, only three (one from *A. mellifera* and two from stingless bees) showed the presence of phenolic metabolites as minor components, when visualized using DPPH (data not shown).

The nMDS ordination plot of propolis samples from *A. mellifera* and stingless bees presented a stress value of 0.2, indicating that the plot corresponds to a reliable representation of the data; most of the propolis samples from *A. mellifera* or stingless bees were grouped closer to their corresponding bee-type (Fig. 3). As expected, this grouping suggests important differences in the composition of propolis samples from *A. mellifera* and Meliponini species.

Results obtained from the ANOSIM test (global $R = 0.21$, $P < 0.001$) indicated a significant difference between these two groups, confirming that the composition of propolis samples depends on the type of bee that collects and elaborates the propolis.

The results obtained from the SIMPER analysis indicated that both groups had a total average dissimilarity of 53.6%. The triterpene, lupeol acetate, with an average abundance of 11.6% in the propolis samples from *A. mellifera* and 3.4% in those from stingless bees, was the metabolite that contributed most (5.1% of the total dissimilarity) to the differentiation between the two groups of propolis samples, being present in 88% and 66% of *A. mellifera* and stingless bees propolis samples, respectively. A similar pattern was observed for *β*-amyrin acetate, with an average abundance of 9.6% in *A. mellifera* and 1.5% in stingless bees (Table 2). On the contrary, grandiflorenic acid, a metabolite that contributes with 3.9% of total dissimilarity, was present in 72% of stingless bees samples and only in 22% of *A. mellifera*, presenting a higher abundance (5.5%) in propolis samples from stingless bees, when compared to those in *A. mellifera* (2.7%). A similar pattern was observed for α-amyrin, grandiflorenic acid methyl ester and lupenone, with higher values in propolis samples from stingless bee (Table 2).

Chemical Composition of Propolis among Stingless Bee Species. The nMDS ordination plot for stingless bees propolis samples, with a stress factor of 0.14, grouped together samples corresponding to the same stingless bee species (Fig. 4). This pattern could be observed more clearly in those species with more than two propolis samples, i.e. *M. beecheii* and *T. nigra.* Samples from *T. nigra* were placed closer together, while *M. beecheii* showed a higher intraspecific variation. Samples could be grouped in three clusters according to their similarities-dissimilarities in propolis chemical composition:

group 1) *P. bilineata*, group 2) *T. nigra* and group 3) *S. pectoralis*, *N. perilampoides*, *M. beecheii* and *P. frontalis* (Fig. 4).

Consistently, the results of the ANOSIM test (global R = 0.626, P = 0.001) indicated a significant difference in the chemical composition of the propolis extracts according to the stingless bee species. In order to determine between-group differences, the value of R was examined for each pairwise comparison (Table S4). The *Partamona bilineata* sample showed a clear separation from the other five species. *T. nigra* was different from the four remaining species *S. pectoralis*, *N. perilampoides*, *M. beecheii* and *P. frontalis*, species that showed non-significant differences in propolis chemical composition.

The results obtained showed that the metabolites that contributed more to the dissimilarities among propolis samples from stingless bee were the triterpenes, lupeol, cycloartenol acetate and β-amyrin, together with the diterpenes grandiflorenic acid and grandiflorenic acid methyl ester, and the fatty acids, palmitic acid, oleic acid and stearic acid ethyl ester (Table S5). Palmitic and oleic acids were the metabolites that explained the separation of the propolis produced by *P. bilineata* from the other samples (Fig. 3, Table S5), while grandiflorenic acid was present in higher abundance in propolis samples from *T. nigra* compared to samples of *S. pectoralis*, *N. perilampoides* and *M. beecheii* (Fig. 3, Table S5).

Botanical Sources and Propolis Composition. The nMDS ordination plot (Fig. 5) did not show a clear separation of propolis samples collected from sites located in the two different vegetation types (global R = 0.081, P = 0.06). A qualitative comparison of the propolis samples collected from both *A. mellifera* and stingless bees in those sites where both types of bees were present and therefore, shared botanical sources (Fig. 1, Tables S1, S2), showed that, while the chemical composition of the propolis sample from *A. mellifera* (Am22) and that of the sample from the medium size stingless bee *S. pectoralis* (Sp2) were

quite similar, the smaller stingless bee *N. perilampoides* (Np2), presented a different chemical composition (Fig. 2). Similarly, propolis collected from five species of stingless bees at the Xmatkuil site (Table S2) showed that the composition of the propolis produced by the larger *M. beecheii* (Mb1) was considerably distinct, especially in the relative abundance of lupenone, grandiflorenic acid and its methyl ester, when compared to the composition of the other four species, the two medium-sized *T. nigra* (Tn1, Tn2) and *S. pectoralis* (Sp1), and the two smaller *N. perilampoides* (Np1) and *P. frontalis* (Pf1) (Fig. 2).

DISCUSSION

Even though polyphenols have been frequently reported as major components in propolis samples from *A. mellifera* (Salatino et al. 2011; Huang et al. 2014) the results of this investigation showed that propolis from both *A. mellifera* and the six species of stingless bees, contain triterpenes, both, pentacyclic and cycloartane type, as their major components. These findings coincide with those reported previously for Yucatecan propolis (Herrera-López et al., 2020, 2019; Boisard et al., 2016), as well as with the increasing number of reports of triterpenes as major components in samples of tropical propolis from both *A. mellifera* (Bracho et al. 2009; Kardar et al. 2014; Boisard et al. 2016; Nina et al. 2016; Carol et al. 2017; Omar et al. 2017) and stingless bees (Santos Pereira et al. 2003; Sanpa et al. 2015; Torres-González et al. 2016; Zhao et al. 2017). However, comparing the composition of different samples of propolis is often difficult because of the lack of standardized protocols, since it has been shown that the chemical composition of propolis depends on the extraction method, on the solvent type, and on the method used to identify the metabolites (Huang et al. 2014).

Body size in *A. mellifera* and stingless bees has been related to their ability to collect pollen and nectar (Araújo et al. 2004; Abou-Shaara 2014; Rodrigues and Ribeiro 2014). Presently, there is no information about bee flight ranges and their ability of resin foraging; assuming that a bigger body size could mean a higher ability to reach a larger diversity of resin sources, the differences in body size among the studied species could partially explain the differences found in the chemical composition of propolis. *A. mellifera*, is by far, the largest species, with the widest flight range. In the case of stingless bees *M. beecheii* has the largest body size, although its reported flight range is half of that of *A. mellifera* (Table 3). The medium size (5-6 mm) stingless bee species *T. nigra*, *P. bilineata* and *S. pectoralis*, have shorter flight ranges, about half than that of *M. beecheii;* while small size (3-4 mm) stingless bees such as *N. perilampoides* and *P. frontalis*, have the shortest flight ranges (Ayala 1999). Based on body size and flight ranges, *A. mellifera* could reach a larger diversity of resins sources than stingless bees. Similarly, among stingless bees, the diversity of resin sources available to *M. beechii* would be larger than that of other species with smaller body size and flight range.

M. beecheii is considered of great importance for the Mayan people since ancient times (Quezada-Euán 2005; Vit et al. 2013; Ortiz et al. 2016). It has been reported a strong preference for *M. beecheii* over other stingless bees because of its higher capacity for honey production, which could be associated to their larger foraging area (Ortiz et al. 2016). This species has also received a greater attention in terms of the chemical composition of its propolis; Pino et al. (2006) compared the chemical composition of the volatile contituents of propolis samples from *A. mellifera* and *M. beecheii* collected in Yucatan, Mexico, reporting 99 and 92 constituents respectively. The propolis chemical

composition in our study shows important differences with this previous report, with spathulenol, caryophyllene oxide, palmitic acid and manool being the only metabolites shared in both studies. These differences could be due not only to differences in collection sites, but also to in the type of extraction method used.

Alternatively, the differences found in the chemical composition of propolis samples among the stingless bee species of this study could be due to factors such as body size, as previously mentioned, as well as different preferences for botanical sources. The importance of botanical sources to explain the observed differences in the chemical composition of propolis has been well documented, with results showing that, in the presence of a large number of resiniferous species on a given site, different bee species have preferences for specific resin sources (Sawaya et al. 2006; Leonhardt and Blüthgen 2009; Salatino et al. 2011; Leonhardt 2017). Unfortunately, since meliponiculture is not a common practice in the Yucatan peninsula, it is difficult to have experimental designs in which the chemical composition of the propolis produced by different bee species can be compared in sites where they share botanical sources.

This is the first report about the presence of grandiflorenic acid and its methyl ester as propolis components; while both metabolites have been reported in the leaves of *Wedelia trilobata*, a species found in the Yucatan Peninsula (Balekar et al. 2014), further studies are needed in order to confirm the botanical source of these metabolites in the propolis samples. Lupeol acetate, β-amyrin acetate, α-amyrin, lupenone, and lupeol are all pentacyclic triterpenes and have been reported as resin components in the large tropical families Burseraceae, Dipterocarpaceae, and Anacardiaceae (Bracho et al. 2009). Recently, *Bursera simaruba* was proposed as the botanical source of several of these pentacyclic

triterpenes in propolis samples from *A. mellifera* (Boisard et al., 2016, Herrera-López et al. 2019), while the botanical origin of a number of resorcinolic lipids, was determined to be the resin of *Mangifera indica* (Herrera-López et al. 2020).

Although the collection sites of *A. mellifera* propolis were classified as having two different vegetation types, i.e. deciduous and semi-deciduous forest, it is well known that the forests throughout the Yucatan Peninsula have suffered important natural and anthropogenic disturbances, resulting in significant changes to the primary vegetation (Caamal-Maldonado and Armendariz-Yañez 2002; Zamora et al. 2008). Additionally, beehives are generally located near human settlements, where the original vegetation has been transformed, mainly through deforestation and the introduction of agricultural crops or ornamental plant species. Therefore, it is likely that most of the studied sites share resiniferous species characteristic of secondary vegetation, as well as introduced and cultivated species; this would explain, in turn, why the botanical source of the previously mentioned bioactive resorcinol lipids in a propolis sample of *A. mellifera* was the resin of *Mangifera indica* (Herrera-López et al. 2020), an introduced species to the Yucatan Peninsula.

In this study, vegetation type was used as a proxy to analyze the role of botanical sources in the chemical composition of propolis. This was possible only for the *A. mellifera* samples because the beehives are widely distributed throughout the studied territory. However, in the case of stingless bees, given the low number of hives, this was not possible.

According to Islebe et al. 2015 tropical dry forest in the Yucatan Peninsula is highly disturbed due to a long history of human intervention. Disturbed dry tropical forest is the largest category with more than 3.9 million ha, spatially distributed in an intricate mosaic landscape. The deciduous forest is a low stature forest, with canopy heights less than 15 m,

nearly 100% of the tree species drop their leaves during the dry season. *Lysiloma bahamensis, Baeucarnea pliabilis, Gymnopodium floribundum, Cassia alata, Acacia milleriana, Mimosa bahamensis, Diospyros anisandra, Pseudophoenix sargentii and Piscidia piscipula* are characteristic species of this forest type. On the other hand, the semi-deciduous forest presents canopy heights from 20 up to 35 m. Fifty to 75 % of all tree species drop their leaves during the dry season. *Brosimum alicastrum, Vitex gaumeri, Byrsonima* spp. and *Lysiloma latisiliquum*, are some of the characteristic species in this type of forest. Huechacona et al. 2020 described the spatial distribution of deciduousness in these two different types of dry forests in Yucatan, deciduousness decreased from the northwest to the southeast, with mean values of 86.6% and 75.0%, for deciduous and semi-deciduous forests, respectively.

In the interviews conducted with the beekeepers who provided the propolis samples for this study, 33 species were mentioned as sources of resins for bees. However, beekeepers did not specify which sources were used by *A. mellifera* and which by stingless bees. Of all the species, seven are unique to the deciduous forest and eight to the semi-deciduous forest. The remaining species are shared between the two vegetation types (Table S6).

Recently da Silva Mendonça et al. 2021, reported an important seasonal variation in propolis composition in the Brazilian caatinga, finding that monoterpenes were more abundant in the dry season, while aliphatic hydrocarbons were more abundant during the rainy season. However, no significant seasonal variation was observed in the case of sesquiterpenes, the predominant volatile component in green propolis. In forests with a strong seasonality, as the ones described in this study, it is important to take into account the phenological changes in botanical sources in propolis sampling design. This study lays

the foundation for understanding the role of different botanical sources in the chemical composition of *A. mellifera* and stingless bees propolis.

In order to elucidate the relationship between resin botanical sources and propolis composition, future studies that incorporate a detailed description of the botanical sources surrounding the beehives as well as the phenological changes of botanical sources during the dry and wet seasons are needed. Additionally, these studies will allow us to verify if any of the species mentioned by beekeepers as botanical sources (e.g. *Gymnopodium floribundum, Bursera simaruba, Piscidia piscipula, Lysiloma latisiliquum, Sabal yapa* , *Bourreria pulchra,* and *Vitex gaumeri*) are actually visited by bees to collect resinous materials, taking into account that *B. simaruba, L. latisiliquum* and *P. piscipula* are well known for their production of resin and exudates (Flores and Vermont 1996; Langenheim 2003).

The intraspecific variation observed in the composition of two propolis samples, collected from two different bee hives of *T. nigra* at the Xmatkuil site, could be explained by genetic factors as mentioned by Salatino et al. (2011). Specific experiments, with sufficient samples, are needed in order to confirm these hypotheses.

The results obtained in this investigation suggest that bee species is the main factor responsible for the observed differences in the composition of propolis samples collected in the Yucatan Peninsula. Different foraging abilities among species, as well as preferences for specific botanical sources, could explain the observed variation. While triterpenes were present in all of the analyzed propolis samples, these natural products were the major components of *A. mellifera* propolis, while propolis from stingless bees were dominated by diterpenes and fatty acids. To the best of our knowledge, this is the first report on the

chemical composition of propolis samples from *T. nigra*, *S. pectoralis*, *N. perilampoides*, *P. frontalis* and *P. bilineata*.

Declarations

Competing interests

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

Authors' contributions

-The authors declare that they have no potential conflict of interest in relation to the study in this paper.

-M.G.H., L.M.C and L.M.P., contributed to the study conception and design. Material preparation, data collection and analysis were performed by M.G.H. The first draft of the manuscript was written by M.G.H. and L.M.C, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

- Data used in this study are available from the corresponding author upon request

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Table 1 Average relative abundance (% chromatogram area) of the metabolite types present in the ethanol extracts of propolis samples from *A. mellifera* and six species of stingless bees. Maximum and minimum values (excluding zero values) are shown in parenthesis. Only metabolites with a relative abundance > 1%, in at least one sample, were considered

Type of metabolite	*A. mellifera*	Stingless bees
Triterpene	6.29 (21.6-0.3)	5.60 (27.4-0.4)
Diterpene	1.67 (3.3-0.7)	3.10 (7.2-0.9)
Phenolic lipid	2.80 (5.3-0.9)	1.89 (3.5-0.9)

Fatty acid	1.03 (1.9-0.3)	1.54 (1.9-1.1)
Alkane	0.85 (1.0-0.7)	1.20 (1.5-1.0)
Sterol	1.24 (1.4-1.0)	2.28 (3.4-1.6)
Sesquiterpene	0.90 (1.1-0.7)	1.17 (0.8-1.6)
Alkene	0.66 (0.8-0.5)	1.83 (3.8-0.6)
Flavonoid	0.93	1.49
Lipid	3.65 (5.2-0.8)	3.05 (3.5-0.9)
Lignane	3.43 (4.8-2.0)	0.42

Table 2 Average abundance (%) of the metabolites that contribute to 50% of the dissimilarity in propolis samples collected from *A. mellifera* and stingless bees

Metabolite[a]	Average abundance in *Apis mellifera*	Average abundance in stingless bees	Contribution to dissimilarity (%)	Acumulated dissimilarity (%)
Lupeol acetate	11.66	3.43	5.17	5.17
β-Amyrin acetate	9.63	1.46	5.04	10.21
Grandiflorenic acid	2.68	5.50	3.90	14.11
α-Amyrin	12.63	14.31	3.82	17.93
Grandiflorenic acid methyl ester	3.30	5.95	3.73	21.66
Lupenone	21.56	27.44	2.94	24.60
Lupeol	15.37	15.85	2.83	27.43
Cycloeucalenol acetate	2.79	2.73	2.74	30.17
Olean-12-en-3-one	10.02	8.38	2.54	32.71
Simiarenol	1.67	2.05	2.41	35.12
Cycloartenol acetate	2.16	2.79	2.28	37.40
Palmitic acid	1.52	1.61	2.25	39.64
Oleic Acid	1.86	1.29	2.23	41.88
9,19-Cyclolanostan-3-ol, 24-methylene	3.85	1.56	2.23	44.10
Betulic acid	6.50	7.37	2.16	46.27
Cycloartenol	1.72	1.45	2.14	48.40
β-Amyrin	7.58	6.48	1.99	50.39

[a]Metabolites are presented in decreasing contribution to the Bray-Curtis index.

Table 3 Characteristics of body size and flight ranges of the studied bee species

Species	Total body length (mm)[1]	Maximum flight range (m)
Apis mellifera	13	5000[2]
Mellipona beecheii	9.7-10.7	2,400[2]
Trigona nigra	5.7	1159[a3]
Partamona bilineata	5.6	800[2]
Scaptotrigona pectoralis	5.2-5.4	1,200[a3]
Nannotrigona perilampoides	4.1-4.2	600[3]
Plebeia frontalis	3.5-4.4	No data

[a]Indicates the flight range of the genus, not the species. [1]Quezada-Euán 2018, [2]Quezada-Euán 2005, [3]Enriquez et al. 2006.

Fig. 1 Location of the propolis samples for a) *A. mellifera* and b) stingless bees, in different vegetation types. Am = *Apis mellifera*; Mb = *Melipona beecheii*; Tn = *Trigona nigra*; Sp = *Scaptotrigona pectoralis*; Np = *Nannotrigona perilampoides*; Pb = *Plebeia frontalis*; Pb = *Partamona bilineata*

Fig. 2 Heatmap for the most important (% contribution to dissimilarity) metabolites in the propolis samples according to bee type. Am = *A. mellifera* Sb = Stingless bee species. Mb = *Melipona beecheii*; Tn = *Triogna nigra*; Sp = *Scaptotrigona pectoralis*; Np = *Nannotrigona perilampoides*; Pb = *Plebeia frontalis*; Pb = *Partamona bilineata.* Symbols * and + are used to designate samples collected in the same site

Fig. 3 Non-metric multidimensional scaling (nMDS) ordination showing differences in propolis samples between *A. mellifera* (▲) and stingless bee species (▲) based on Bray-Curtis similarities calculated from Log(x+1) transformed relative abundances (% chromatogram area) of metabolites. Am = *A. mellifera* samples; Sb = Stingless bee samples: Mb = *Melipona beecheii*; Tn = *Trigona nigra*; Sp = *Scaptotrigona pectoralis*; Np = *Nannotrigona perilampoides*; Pb = *Plebeia frontalis*; Pb = *Partamona bilineata*

Fig. 4 Non-metric multidimensional scaling (nMDS) ordination of propolis samples according to the stingless bee species: ▼*Melipona beecheii*, ■*Trigona nigra*, ♦*Scaptotrigona pectoralis*, ●*Nannotrigona perilampiodes*, +*Plebeia frontalis* and X *Partamona bilineata*. Analysis is based on Bray-Curtis similarities of the log(x+1) transformed relative abundances of metabolites (% chromatogram area)

Fig. 5 Non-metric multidimensional scaling (nMDS) ordination showing differences between *A. mellifera* propolis samples collected in deciduous (▲) and semi-deciduous (▲) forest. Stress value (0.18) indicates that the plot corresponds to a reliable representation of

the data. Analysis based on Bray-Curtis similarities calculated from log(x+1) transformed relative abundances