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Cuban stingless bee livestock exhibit specialized floral resource use: a palynological study on honey samples from Matanzas and Mayabeque provinces

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Abstract

The knowledge of the different plant species that make up the feeding diet of animals is highly important to develop more efficient strategies. This research aimed to characterize the food potential available for the Cuban stingless bee livestock of the Matanzas and Mayabeque provinces. Palynological analysis was done using 60 g of pollen from sealed pots and 80 mL of honey from the ten randomly selected beehives (five in each province). The results showed that in the honey collected in Matanzas province, the most represented family was Amaranthaceae, followed by Myrtaceae and Fabaceae. Meanwhile, for Mayabeque, the most represented ones were the families Fabaceae and Myrtaceae. Regarding the stingless bee pollen of Matanzas provenance, the family Fabaceae prevailed, followed by Burseraceae and Myrtaceae. The pollen corresponding to Mayabeque coincided in showing Fabaceae as the most representative. In addition, pollen grains of small size (from 10 to 25 µm) were collected, with a marked representation of the pollen type of *Mimosa pudica* in the Mayabeque honey. It was concluded that the Cuban stingless bee livestock of the Matanzas and Mayabeque provinces had a specialist feeding behavior because a low number of plant taxa made up its diet.

Introduction

The Spanish word *ganad*o (livestock) is directly derived from the verb *ganar* (to gain) and has several definitions, available in the tercentenary edition (the most updated one) of the Spanish language dictionary. Among these definitions, livestock is: "a group of beasts that feed and move together", such as sheep, goats, and cattle. In its second definition, current since 1803, livestock is also defined as the group of bees in a beehive (RAE, 2017).

Taking the previous concept as a reference, Lóriga (2015) defines the Cuban stingless bee livestock as all the managed beehives of the bee species *Melipona beecheii* Bennett, 1831 (Apidae: Meliponini), included in a census or not.

According to Sáenz et al. (2000), during the foraging process, bees visit flowers in search of food, then pollen grains adhere to their body and later become part of honey. The palynology branch studying these grains to know the botanical origin of the different products elaborated or stored in the hives of diverse groups of bees is called melissopalynology.

With the use of this discipline, the study of the floral sources visited by bees is facilitated to a large extent (Sánchez, 2001).

The antecedents of using trophic resources by bees allow the evaluation of the plant taxa that contribute nectar and/or pollen to the bee diet. And consequently, from these data, the elaboration of floristic lists and phenological calendars can be done, contributing to better management of the beehives by farmers (Flores et al., 2015).

Due to the above-exposed facts, knowing the main plant species that make up the diet of these animals is a priority in developing efficient feeding strategies that guarantee higher production with an optimum health level. This research aimed to characterize the food potential available for the Cuban stingless bee livestock of the Matanzas and Mayabeque provinces and its possible application to other territories with beehives of this species.

Materials and Methods

Study sites

The study was conducted in two Cuban stingless bee apiaries in April 2018. The first one is located in the Matanzas municipality, of Matanzas province, at 23°02'25'' North latitude and 81°30'58'' West longitude, at an altitude of 14 m.a.s.l.; and the second one, in the San Nicolás municipality, belonging to the Mayabeque province, located at 22°47'13'' North latitude and 80°55'05'' West longitude, at an altitude of 34 m.a.s.l.

The characteristic climate of the studied area is classified as tropical, with marked seasonality of the rains in two periods corresponding to the dry season (DS), which extends from November to April, and the rainy season (RS), which lasts from May to October (Academia de Ciencias de Cuba, 1989).

During the sampling period, the Matanzas locality showed a warm climate that caused minimum temperature values and positive anomalies of 0.8ºC. The recorded cumulative rainfall was 22.8 mm, lower than the historical mean (52.6 mm). Regarding relative humidity, it was 74%, over the historical values. In the case of Mayabeque, it was also warm, conditioned by the influence of high oceanic pressure that weakly influenced the territory during most of the month. This situation is responsible for the cold air masses not reaching the territory, which provoked temperature values with significant positive anomalies of 1.7 ºC compared with the historical average. The minimum temperatures averaged 19.9ºC. Regarding rainfall, the cumulative exceeded the historical mean of 171.3 mm, and the mean air relative humidity was 75%, slightly higher than the historical average.

Floristic inventory of each stingless bee apiary

Knowing the foraging area of bees is important to understand the use of floristic resources. Studies on species of the genus *Melipona* Illiger, 1806, found flight radios close to 2 km, with the studied species having body sizes larger than *M. beecheii* (Nunes Silva et al., 2020). However, a flight radius of 1 km was estimated for *M. beecheii*, similar to that of species of the genus *Trigona* Jurine, 1807 (Araújo et al., 2004), since they share similar biometric parameters (Fonte, 2007).

Considering that both stingless bee apiaries were located in urban areas, where gardens and house backyards prevail, the main existing plant species potentially contributing with pollen and nectar were determined: native, naturalized, and ornamental plants (Pérez-Piñeiro, 2017). In addition, to determine the representativeness of the species in the environment of stingless bee apiaries, four categories (abundant, frequent, little frequent, and rare or scarce) and their corresponding biological form (tree, shrub, and herbaceous) were established, adapting the methodology proposed by García-Abad (2015) to urban environments, which are less diverse from the botanical point of view (Wittig & Becker, 2010).

Construction of the palynotheque

From each inventoried species, floral buds were taken and preserved in a universal fixator preservative composed of a mixture of 95º ethylic alcohol, glacial acetic acid, 37-40% formaldehyde, and distilled water. Afterward, they were dissected with the aid of a surgical blade, extracting the anthers, which were placed in conical test tubes of 10 ml capacity, and the protocol used for the preparation of the samples for the palynological and melissopalynological qualitative analysis was followed.

The photographic shot of each grain from the previously identified botanical species of reference was done through a camera (LEICA DFC290) coupled to the optical microscope (NIKON OPTIPHOT-I) with a magnification of 400. The Leica Application Suite, version 3.3.1 for Windows XP, was used for this purpose.

The obtained photographs and bibliographic references (Aira et al., 2018, Pérez, 2016) served as a basis for determining pollen types found in the honey and pollen samples.

Sample collection

From the ten beehives under study in each province, five were randomly selected in each locality. In Mayabeque, beehives 14, 2, 48, 5, and 6 were chosen, while in Matanzas, beehives 1, 9, 11, 12 and 13 were selected. Sixty grams of stingless bee pollen from sealed pots and 80 mL of honey in each one of them were taken. Sampling was done in April 2018.

Melissopalynological qualitative analysis

The protocol used for sample preparation was that of honey analysis proposed by the Honey Work Group belonging to the Spanish Society of Palynology (APLE, for its initials in Spanish), recently constituted (Sánchez et al., 2018, González-Porto et al., 2018). The protocol is based, with modifications, on García et al. (2001), consisting of the previous treatment of the samples with 5% acidulated water with their respective washings and centrifugations to obtain sediment that is in turn deposited on the microscope slide and one drop of glycerin jelly with fuchsin is applied before taking it to the microscope for its later analysis.

Palynological qualitative analysis of stingless bee pollen

One gram of sample was weighed and was subject to the above-explained protocol, with modifications in the volume of 5% acidulated water that was added. This methodology was used to liken it to the one used in the processing of honey samples, also considering the collection conditions of stingless bee pollen, with different load sizes, variable from small masses of 5-6 mm to dust particles.

The reading of stingless bee honey and pollen samples was done under the optical microscope with a magnification of 400, identifying a minimum number of 300 to 500 pollen grains to guarantee correct botanical and geographical characterization. The samples were done in quadruple.

The observed pollen types were placed in the different class frequencies according to Loveaux et al. (1978), as shown below:

- Dominant pollen (**>45%**)
- Companion pollen (**16-45%**)
- Important isolated pollen (**4-15%**)
- Rare or sporadic pollen (**1-3%**)
- Present pollen (**<1%**)

The Shannon-Weaver diversity index was used to determine the browsing strategy deployed by *M. beecheii* from the pollen diversity found in the honey samples (Shannon & Weaver, 1949).

$$H' = -\sum_{n=1}^{S} pi \ln pi$$

Where:
H'= Shannon-Wiener Index.
p_i = Proportion of each pollen type found.
ln = Natural logarithm.

Meanwhile, the browsing uniformity of the bees was determined through Pielou's evenness Index (Pielou, 1984).

$$J' = \frac{H'}{H'\ max}$$

Where:
J'= Browsing uniformity of the bees.
H'= Shannon-Wiener index.
H'_{max} = Napierian logarithm of pollen richness.
The diversity (H') and evenness (J') indices were determined through the utilization of the software Diversity Species & Richness 3.02 (Henderson & Seaby, 2002).

Results

The results of the floristic inventory in both localities show that in the flight environment of stingless bees, there are 16 plant species in common. We identified 42 plants belonging to 27 families in Matanzas (Table 1). *Alternanthera sessilis* (L.) R. Br. ex DC, *Mangifera indica L.*, *Adonidia merrillii* (Becc.) Becc, *Bidens pilosa* L., *Bursera simaruba* (L.) Sarg, *Senna spectabilis* (DC.) H.S. Irwin & Barneby, *Persea americana* Mill., Musa *x* paradisiaca L., *Psidium guajava* L. and *Coccoloba uvifera* (L.) L. were the most abundant.

Table 1. Floristic inventory of the Matanzas locality, Cuba, 2018.

Matanzas meliponary			
Family	**Scientific name**	**Biological form**	**Appearance frequency**
Amaranthaceae	*Alternanthera sessilis* (L.) R. Br. ex DC.	Herbaceous	Abundant
Anacardiaceae	*Mangifera indica* L.	Tree	Abundant
Anacardiaceae	*Anacardium occidentale* L.	Tree	Rare or Scarce
Annonaceae	*Annona muricata* L.	Tree	Frequent
Annonaceae	*Annona squamosa* L.	Tree	Little Frequent
Arecaceae	*Cocos nucifera* L.	Tree	Frequent
Arecaceae	*Roystonea regia* (Kunth) O.F. Cook	Tree	Little Frequent
Arecaceae	*Adonidia merrillii* (Becc.) Becc.	Tree	Abundant
Asteraceae	*Bidens pilosa* L.	Shrub	Abundant
Asteraceae	*Viguiera dentata* Spreng.	Shrub	Little Frequent
Bignoniaceae	*Crescentia cujete* L.	Tree	Little Frequent
Boraginaceae	*Cordia collococca* L.	Tree	Rare or Scarce
Boraginaceae	*Bourreria virgata* (Sw.) G. Don	Shrub	Frequent
Boraginaceae	*Cordia alliodora* (Ruiz & Pav.) Oken	Tree	Frequent
Burseraceae	*Bursera simaruba* (L.) Sarg	Tree	Abundant
Cactaceae	*Nopalea auberi* (Pfeiff.) Salm-Dyck	Tree	Rare or Scarce

Table 1. Floristic inventory of the Matanzas locality, Cuba, 2018. (Continuation)

Matanzas meliponary			
Family	**Scientific name**	**Biological form**	**Appearance frequency**
Calophyllaceae	*Calophyllum antillanum* Britton	Tree	Little Frequent
Combretaceae	*Terminalia catappa* L.	Tree	Frequent
Commelinaceae	*Tradescantia pallida* (Rose) D.R. Hunt	Herbaceous	Frequent
Convolvulaceae	*Ipomoea triloba* L.	Herbaceous	Frequent
Convolvulaceae	*Turbina corymbosa* (L.) Raf.	Herbaceous	Frequent
Cucurbitaceae	*Cucurbita pepo* L.	Herbaceous	Little Frequent
Fabaceae	*Mimosa pudica* L.	Herbaceous	Little Frequent
Fabaceae	*Vachellia farnesiana* (L.) Wight & Arn.	Tree	Little Frequent
Fabaceae	*Senna spectabilis* (DC.) H.S. Irwin & Barneby	Tree	Abundant
Fabaceae	*Tamarindus indica* L.	Tree	Rare or Scarce
Fabaceae	*Trifolium incarnatum* L.	Herbaceous	Frequent
Fabaceae	*Dichrostachys cinerea* (L.) Wight & Arn.	Tree	Frequent
Lamiaceae	*Tectona grandis* L. f.	Tree	Rare or Scarce
Lauraceae	*Persea americana* Mill.	Tree	Abundant
Malvaceae	*Hibiscus rosa-sinensis* L.	Shrub	Frequent
Malvaceae	*Hibiscus elatus* Sw.	Tree	Rare or Scarce
Meliaceae	*Swietenia mahagoni* (L.) Jacq.	Tree	Rare or Scarce
Moraceae	*Morus alba* L.	Tree	Rare or Scarce
Moringaceae	*Moringa oleifera* Lam.	Tree	Frequent
Musaceae	*Musa x paradisiaca* L.	Herbaceous	Abundant
Myrtaceae	*Psidium guajava* L.	Tree	Abundant
Passifloraceae	*Passiflora edulis* Sims	Herbaceous	Little Frequent
Polygonaceae	*Coccoloba uvifera* (L.) L.	Tree	Abundant
Rutaceae	*Citrus x aurantium* L.	Tree	Frequent
Rutaceae	*Citrus x limon* (L.) Osbeck	Tree	Frequent
Sapotaceae	*Manilkara zapota* (L.) P. Royen	Tree	Frequent

In Mayabeque (Table 2), a lower number of plant species (33) was identified, grouped in 22 families. In the category of abundant are *Mangifera indica* L., *Bursera simaruba* (L.) Sarg, *Ipomoea triloba* L., *Vachellia farnesiana* (L.) Wight & Arn., *Gliricidia sepium* (Jacq.) Kunth, *Persea americana* Mill., *Psidium guajava* L., *Gouania polygama* (Jacq.) Urb. In general, in the two localities, the biological form that prevailed was the tree, followed by shrubs and herbaceous.

In the honey samples from Matanzas (Table 3) higher richness of pollen forms is observed, with a mean of 11.2 and a maximum of 12 (beehives 9 and 11). In the analyze honey from this locality, the predominant pollen percentage was that of *Alternanthera* Forssk., with a mean of 38.26%, with the dominant pollen (>45%) in three of the five samples, followed by *Psidium guajava* L. (17.50%) and the genus *Trifolium* L. (16.07%). The most represented family was Amaranthaceae, followed by Myrtaceae, and finally Fabaceae because only one sample (beehive 9) showed pollen of *Mimosa pudica* L., justifying its inclusion in the companion category, being lower than in the other beehives.

Meanwhile, in the samples of the Mayabeque provenance (Table 4), the quantity of pollen forms or types is considerably lower, with a mean value of 7 and a maximum of 13 for the case of the honey belonging to beehive 6. It should be highlighted that the pollen from *Mimosa pudica* prevailed in all the examined honey samples, with a mean percentage of 74.05%, besides being the dominant pollen (>45%) in the five studied beehives. Fabaceae and Myrtaceae, represented by the species *Psidium guajava,* stand out as companion pollen (16-45%) of the honey obtained from beehives 2 and 48, with a mean of 23.21%.

When analyzing the foraging strategy of *M. beecheii* (Fig 1), we found that in the Matanzas honey, the mean value of the diversity index (H´) was 1.34, with a maximum value in beehive 11 (1.88). On the contrary, Mayabeque showed a lower mean (0,84). These results are in correspondence with the ones exposed in Table 4. Regarding the evenness index (Fig 1), in Matanzas, the mean value was 0.45, and again in beehive 11, the highest value was recorded (0.63). For Mayabeque, this indicator had a mean value of 0.28.

Table 2. Floristic inventory of the Mayabeque locality, Cuba, 2018.

Mayabeque meliponary			
Family	**Scientific name**	**Biological form**	**Appearance frequency**
Anacardiaceae	*Mangifera indica* L.	Tree	Abundant
Arecaceae	*Cocos nucifera* L.	Tree	Frequent
Arecaceae	*Roystonea regia* (Kunth) O. F. Cook	Tree	Frequent
Arecaceae	*Adonidia merrillii* (Becc.) Becc.	Tree	Frequent
Asteraceae	*Bidens pilosa* L.	Shrub	Frequent
Boraginaceae	*Cordia gerascanthus* L.	Tree	Little Frequent
Burseraceae	*Bursera simaruba* (L.) Sarg	Tree	Abundant
Cactaceae	*Dendrocereus nudiflorus* (Engelm. ex Sauvalle) Britton & Rose	Tree	Little Frequent
Convolvulaceae	*Ipomoea triloba* L.	Herbaceous	Abundant
Convolvulaceae	*Turbina corymbosa* (L.) Raf.	Herbaceous	Frequent
Ericaceae	*Lyonia myrtilloides* Griseb.	Tree	Little Frequent
Fabaceae	*Mimosa pudica* L.	Herbaceous	Frequent
Fabaceae	*Vachellia farnesiana* (L.) Wight & Arn.	Tree	Abundant
Fabaceae	*Gliricidia sepium* (Jacq.) Kunth	Tree	Abundant
Lauraceae	*Persea americana* Mill.	Tree	Abundant
Malvaceae	*Ceiba pentandra* (L.) Gaertn.	Tree	Little Frequent
Meliaceae	*Trichilia hirta* L.	Tree	Little Frequent
Moringaceae	*Moringa oleifera* Lam.	Tree	Little Frequent
Myrtaceae	*Psidium guajava* L.	Tree	Abundant
Nyctaginaceae	*Pisonia aculeata* L.	Shrub	Frequent
Oxalidaceae	*Averrhoa carambola* L.	Tree	Rare or Scarce
Polygonaceae	*Antigonon leptopus* Hook. & Arn.	Herbaceous	Frequent
Rhamnaceae	*Gouania polygama* (Jacq.) Urb.	Shrub	Abundant
Rosaceae	*Prunus domestica* L.	Tree	Frequent
Rosaceae	*Pyrus communis* L.	Tree	Little Frequent
Rubiaceae	*Coffea arabica* L.	Shrub	Little Frequent
Rutaceae	*Citrus x aurantium* L.	Tree	Frequent
Rutaceae	*Citrus x limon* (L.) Osbeck	Tree	Frequent
Sapindaceae	*Melicoccus bijugatus* Jacq.	Tree	Frequent
Sapindaceae	*Nephelium lappaceum* L.	Tree	Rare or Scarce
Sapindaceae	*Serjania diversifolia* (Jacq.) Radlk.	Herbaceous	Little Frequent
Sapotaceae	*Manilkara zapota* (L.) P. Royen	Tree	Frequent
Sapotaceae	*Pouteria dictyoneura* (Griseb.) Radlk.	Tree	Little Frequent

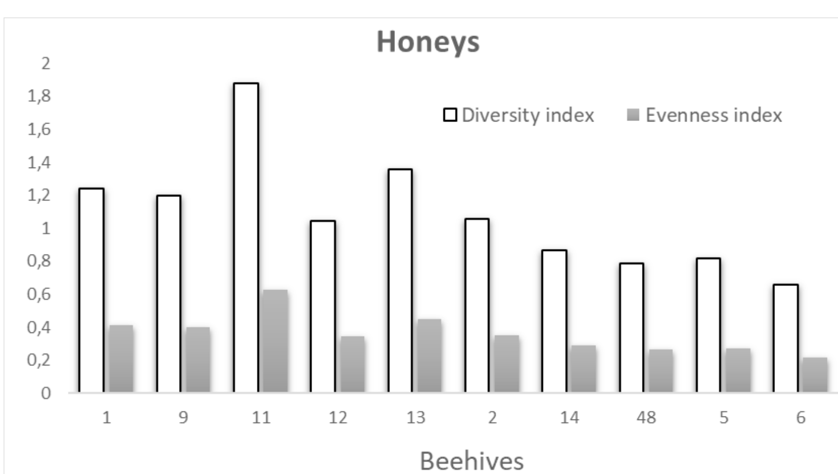

Fig 1. Values obtained in the diversity (H´) and evenness (J´) indices of the Matanzas (1, 9, 11, 12, 13) and Mayabeque honeys (2, 14, 48, 5, 6).

Table 3. Pollen types identified in the honeys from the beehives of Matanzas, Cuba, 2018.

Beehives	Dominant pollen (>45%)	Companion pollen (16-45%)	Important isolated pollen (4-15%)	Sporadic or rare pollen (1-3%)	Present pollen (<1%)
1	*Psidium guajava* (**65,96%**)	-	*Mimosa pudica* (**13,98%**) *Citrus* sp. (**6,07%**)	*Alternanthera* (2,9%) *Moringa oleífera* (2,37%) *Mimosa pigra* (1,58%) Cariofilaceae (1,85%)	*Acasia farnesiana* (0,26%) Trifolium (0,26%) *Bidens pilosa* (0,53%) Unknown (0,53%)
9	*Alternanthera* (**54,11%**)	*Mimosa pudica* (**25,44%**)	*Trifolium* (**7,23%**) *Psidium guajava* (**5,49%**)	*Citrus* sp. (2,49%) *Moringa oleífera* (1,5%)	*Acasia farnesiana* (0,75%) *Bursera simaruba* (0,99%) *Bidens pilosa* (0,75%) Unknown (0,25%) *Mimosa pigra* (0,75%) Cariofilaceae (0,25%)
11	*Trifolium* (**55,97%**)	-	*Mimosa pudica* (**14,68%**) Alternanthera (**10,95%**) *Acasia farnesiana* (**4,48%**) *Bursera simaruba* (**4,73%**)	*Psidium guajava* (2,49%) *Citrus sp* (1,24%) *Moringa oleífera* (1,74%) *Bidens pilosa* (1,74%) Cariofilaceae (1,49%)	*Albizia lebbeck* (0,25%) Unknown (0,25%)
12	*Alternanthera* (**64,79%**)	**-**	*Mimosa pudica* (**9,26%**) *Psidium guajava* (**10,66%**) *Trifolium* (**8,25%**)	*Acasia farnesiana* (2,21%) *Bursera simaruba* (1,41%) *Moringa oleífera* (1,21%) *Bidens pilosa* (1,81%)	*Albizia lebbeck* (0,2%) *Mimosa pigra* (0,2%)
13	*Alternanthera* (**58,53%**)	-	*Mimosa pudica* (**9,71%**) *Acasia farnesiana* (**6,82%**) *Trifolium* (**8,66%**) *Bursera simaruba* (**4,2%**)	*Psidium guajava* (2,89%) *Moringa oleífera* (1,05%) *Bidens pilosa* (1,81%) *Albizia lebbeck* (1,84%) *Citrus* sp. (2,36%) *Coccoloba uvifera* (1,31%)	-

According to Terrab et al. (2004), pollen grain size is one of the factors influencing higher or lower representation of plant species pollen in honey. Pollen size can be defined by the length of its polar axis and equatorial diameter, and considering the major axis, they are differentiated into several types (Erdtman, 1952). The results indicate that the Cuban

Table 4. Pollen types identified in the honeys from Mayabeque beehives.

Beehives	Dominant pollen (>45%)	Companion pollen (16-45%)	Important isolated pollen (4-15%)	Sporadic or rare pollen (1-3%)	Present pollen (<1%)
14	*Mimosa pudica* (**71,29%**)	-	*Psidium guajava* (**11,88%**) *Mimosa pigra* (**4,95%**) Cariofilaceae (**4,95%**)	*Acasia farnesiana* (3,96%) *Moringa oleífera* (2,97%)	-
2	*Mimosa pudica* (**64,11%**)	*Psidium guajava* (**22,6%**)	*Moringa oleífera* (**5,52%**)	*Acasia farnesiana* (1,54%) *Mimosa pigra* (2,45%) *Bursera simaruba* (3,68%)	-
48	*Mimosa pudica* (**70,24%**)	*Psidium guajava* (**23,81%**)	*Mimosa pigra* (**4,76%**)	*Acasia farnesiana* (1,19%)	-
5	*Mimosa pudica* (**78,03%**)	-	*Mimosa pigra* (**9,85%**) *Psidium guajava* (**6,44%**)	*Acasia farnesiana* (1,14%) *Moringa oleífera* (3,41%) Cariofilaceae (1,14%)	-
6	*Mimosa pudica* (**86,6%**)	-	*Mimosa pigra* (**2,9%**) *Psidium guajava* (**6,5%**)	-	*Acasia farnesiana* (0,4%) *Moringa oleífera* (0,4%) *Coccoloba uvifera* (0,9%) Arecaceae *Tipo Adonidia* (0,2%) *Nopalia auberi* (0,3%) *Pristimera coriacea* (0,2%) *Mangifera indica* (0,3%) *Myrtaceae* *Tipo Syzygium* (0,6%) Unknown (0,3%) Unknown (0,2%)

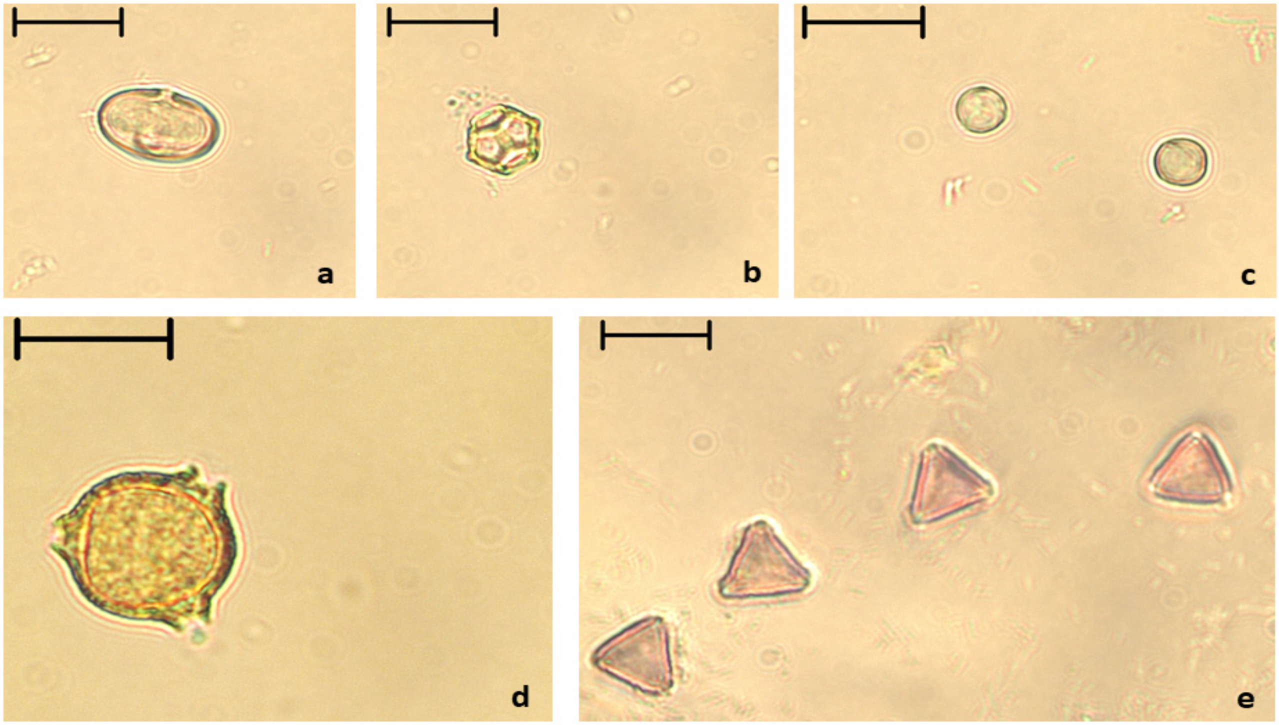

Fig 2. Pollen grains of the prevailing types identified in the stingless bee honey and pollen samples of both provinces seen with a magnification of 400. Genus *Trifolium* (**a**); Genus *Alternanthera* (**b**); *Mimosa pudica* (**c**); *Bursera simaruba* (**d**) and *Psidium guajava* (**e**). Scale bar: 20 µm.

stingless bee livestock of both provinces has a predilection for collecting pollen grains of small size (varying from 10 to 25 µm) and explain the highest representation of the species *M. pudica* in the Mayabeque honey (it measures 10 µm) (Figure 2).

The main floral resources used by the livestock of Matanzas province (Fig 3) were, first, *M. pudica*, with a mean percentage of 55,24%, dominant in three of the five samples, followed by *B. simaruba* (31,11%) and *P. guajava* (25,50%). Fabaceae was the most represented family in Matanzas and Mayabeque (Fig 4).

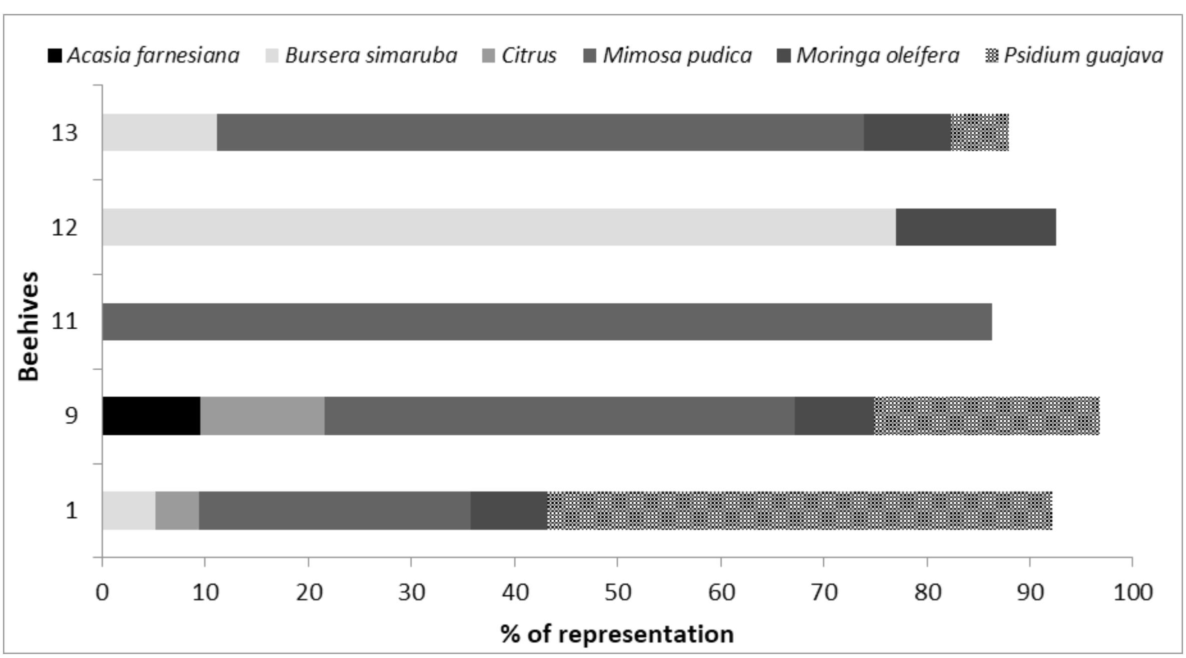

Fig 3. Main floral resources identified in the pollen collected from Matanzas province. Dominant pollen (>45%), companion or secondary pollen (16-45%), isolated pollen (4-15%).

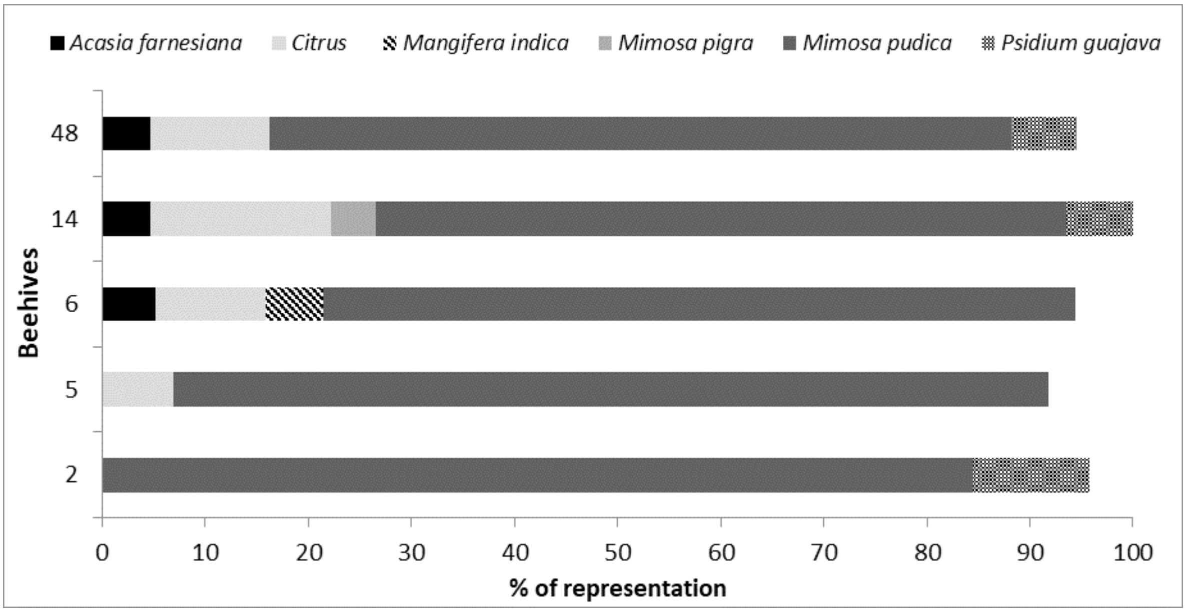

Fig 4. Main floral resources identified in the pollen collected from the Mayabeque province. Dominant pollen (> 45%), companion or secondary pollen (16-45%), isolated pollen (4-15%).

Discussion

If the criterion expressed by Pérez de Zabalza (1989) is followed, the studied honey would be classified as of low palynological richness because they represent less than 20 pollen forms. Nevertheless, this is a parameter established for European bee (*Apis mellifera* Linnaeus, 1758) honey in a Mediterranean environment which largely differs from the tropics. Studies by Imperatriz-Fonseca et al. (1989) and Ramalho (1990) in stingless bees showed some general foraging patterns for Meliponini, proving that, despite foraging on the wide diversity of available floral resources in the area, they are concentrated only on a few species. This would explain the obtained results.

Ramalho (2004) observed that the bees of the genus *Melipona* visit flowers of different shapes, sizes, and colors to obtain their food. However, there was a preference for small flowers with radial symmetry and open corollas in which access to pollen and nectar is relatively easy. Studies conducted in 18 colonies of three species of the Meliponini tribe found a prevalence of small pollen grains, suggesting the preference of these bees for small-size flowers with short pistils (Vossler, 2015).

Guimarães et al. (2021) studies confirm the results obtained in the Mayabeque honey regarding the abundance of *M. pudica pollen grains* (10 µm). These authors detected high representativeness of the *Mimosa type in the pollen content of honey samples of Melipona seminigra pernigra Moure and Kerr, 1950,* ascribable, according to Ferreira and Absy (2017), to its release in large quantities, their configuration, and the small size of pollen grains (Zappi et al., 2018).

In the case of *M. beecheii*, it has been reported that the type of opening or dehiscence of the anthers (Vossler, 2015) influences the preference for pollen types among the flora in the foraging area. Such preference favors collecting pollen types from Fabaceae, Melastomataceae, and Myrtaceae, which have poricidal openings (Biesmeijer, 1997). Hence, the higher representation of these families in the evaluated honey of both provenances coincides with the observation made by Novais and Absy (2015) and Costa et al. (2017) in the Melionini tribe.

The results of the diversity index (H´) for the Matanzas honey (Fig 1) coincide with the value range from 1.23 to 2.00, obtained by Pérez-Sato et al. (2018) in *A. mellifera* honey produced in the High Plateau of the Puebla State in Mexico. However, they are below the ones reached by Soto (2007) for the stingless bee species *Scaptotrigona pectoralis* Dalla Torre, 1896, in Guatemala; and the ones reported by Rezende et al. (2020) in three *Melipona* species in Brazil. For this same index, the Mayabeque provenance was shown in correspondence with those obtained by Novais et al. (2013) for the stingless bee species *Tetragonisca angustula* Illiger, 1806, in Brazil, with ranges comprised from 0.36 to 2.55.

The diversity index (H´) is a tool that determines the breadth of the trophic niche bees through the number of botanical taxa present in the honey samples. Ferreira and Absy (2017) concluded that the breadth of the niche is determined by the movements of massive foraging of bee species with a higher number of workers, which consequently collect more trophic resources. On the other hand, colonies that have less population tend to compete with lower intensity for trophic resources, as we observed for *M. beecheii* in Matanzas (Table 2). Yet, both localities show a breadth of trophic niches in correspondence with the results of Hilgert-Moreira et al. (2013) for the genus *Melipona* Illiger, 1806.

The evenness index (J´) values were low in Matanzas (0.45) and Mayabeque (0.28). According to Espinoza-Toledo et al. (2018), these results can be explained by the dominance of some species that causes (J´) to show a strong trend toward zero, as observed in the analyzed honey. This fact is markedly proven in the Mayabeque honey (Table 4), where the dominant species in all the samples was *Mimosa pudica*. In addition, the values reached for this index indicate that the bees from both localities showed oligolectic behavior.

According to Biesmeijer (1997), bees classified as oligolectic or specialists behave by visiting and collecting pollen from a very restricted group of plants, which applies to *M. beecheii*. On the other hand, polylectic or generalist bees utilize pollen and nectar from different plant species in their feeding. In turn, Ramalho et al. (1990) and Rezende et al. (2020) ascribe this characteristic to the fact that the beehives of this stingless bee species are composed of a small number of individuals compared to other species. That is why they focus on few available resources and exploit them intensely.

The most represented family in Matanzas and Maybeque (Figure 4) was Fabaceae. Similar results were obtained by Ferreira and Absy (2013) and Barquero-Elizondo et al. (2019) in stingless bee species. They ascribe this fact to the relevance of this family within the ecosystem for its diversity (small-size plants, lianas, shrubs, and trees) and abundant flowerings throughout the year.

Within this family (Fabaceae), the species that stood out was *Mimosa pudica*, reported as a pollen source for bees of the *Melipona* genus in El Salvador (Biesmeijer, 1997), Colombia (Rodríguez, 2005) and other neotropical habitats. In Cuba, in previous studies conducted by Leal-Ramos and Sánchez (2013), they observed a high frequency of the pollen type of *M. pudica* in pollen samples obtained from the species *M. beecheii*.

Conclusions

A specialist feeding habit characterizes the Cuban stingless bee livestock of the Matanzas and Mayabeque provinces. It has a diet based on a few plant species, among which *Bursera simaruba* (L.) Sarg., *Mimosa pudica* L., *Psidium guajava* L., *Trifolium* spp., and *Alternanthera* spp. stand out. In addition, a marked preference for small pollen grains from 10 to 25 µm was observed, especially those of *Mimosa pudica* in the Mayabeque honey.

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Authors' Contribution

LFC: Conceptualization, methodology, investigation, software, validation, formal analysis, resources, data curation, writing-original draft, writing-review & editing, visualization, supervisión, project administration.
WLP: Conceptualization, investigation, resources
JDL: Conceptualization, investigation, resources
MDS: Conceptualization, investigation, resources
DMQ: Conceptualization, investigation, software, resources.
DRC: Methodology, investigation, software, validation, formal análisis, resources, writing-original draft, writing-review & editing.
JSS: Methodology, investigation, validation, resources.
ESR: Methodology, investigation, validation¸ resources.

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