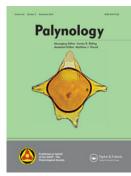


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Melissopalynology in Brazil: a map of pollen types and published productions between 2005 and 2017

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ABSTRACT

We analysed scientific production related to melissopalynology in Brazil and surveyed the pollen types described in publications, producing a melissopalynological database. We inventoried articles published between 2005 and 2017 indexed in the Web of Science (WoS) and Scientific Electronic Library Online (SciELO). Searches in WoS involved researching the terms (1) mel?ssopalynolog*, (2) pollen analy?* AND honey, (3) bee* pollen, (4) pollen NEAR/15 bee*, (5) pollen analysis of honey, and (6) pollen analy?* NEAR/15 honey; in SciELO, we used the search terms (1) melissopalinologia, (2) pólen AND mel, (3) pólen AND abelha, (4) pólen apícola, and (5) análise polínica AND mel. Additionally, we consulted the resume of the principal authors of melissopalynology research in Brazil to identify articles published during that period but not appearing in other database searches. We uncovered a total of 133 publications distributed among 56 journals, with a mean of 4.92 authors/publication. A total of 1,362 pollen types were identified, representing 130 botanical families. Among those, the most well represented in terms of numbers of pollen types were Fabaceae (270), Asteraceae (89), Euphorbiaceae (61), Rubiaceae (58), Myrtaceae (51), Malvaceae (51), Bignoniaceae (49) and Arecaceae (48). Fifty-nine bee species were mentioned in the studies, distributed among 19 genera. Apis mellifera was the most frequently mentioned bee species (73 publications), followed by the native bee species Tetragonisca angustula (13 publications). The pollen types most frequently mentioned in the articles were (in decreasing order): Eucalyptus, Myrcia, Cecropia, Mimosa caesalpiniifolia, Vernonia, Poaceae type and Croton. The inventoried publications comprised all five geographical regions of Brazil, especially the northeastern region of that country, with 59 publications. There are still large areas of Brazil without any melissopalynological studies, however, principally the central-western and southern regions. As such, more research will be necessary to fill gaps in our knowledge of Brazilian palynodiversity.

KEYWORDS Pollen; bees; floral resources; Brazil; scientometrics; state of the art

1. Introduction

Melissopalynological studies comprise that part of palynology focusing on pollen grains found in the sediments of products elaborated by eusocial bees (Hymenoptera: Apoidea), such as honey, pollen, propolis, and royal jelly, to help determine their botanical and geographical origins (Louveaux et al. 1970; Jones and Bryant 1996). Palynology is an important tool for identifying the floral resources preferentially used by bee populations, as qualitative and quantitative analyses of pollen grains can reveal aspects of their feeding behaviours and thus contribute to ecological and conservation studies of those insects (Novais et al. 2009; Vossler et al. 2014; Alves-dos-Santos et al. 2016).

Melissopalynological studies contribute to the identification of plant species important to bee nutrition and to botanical information concerning the products derived from the labours of those insects, and can aggregate commercial value. The first studies of melissopalynology in Brazil were undertaken by Santos (1964), who investigated the pollen types present in products such as honey and the pollen masses collected from Africanised bee colonies in the region surrounding Piracicaba, São Paulo State. Subsequent studies by Barth (1969a, 1969b, 1970a, 1970b, 1971a, 1971b, 1973) were undertaken in different regions of Brazil.

In an effort to examine the melissopalynology studies undertaken in Brazil, Barth (2004) published a short review of the findings to date, including analyses of honey, propolis, and pollen loads. A decade later, Freitas and Novais (2014) compiled studies concerning melissopalynology in the Amazon region, focusing on the pollen types identified and, principally, on the botanical species involved and their relationships to the bee species being studied. Those authors analysed 28 articles published between 1977 and 2013 and compiled an extensive database containing 610 pollen types and 49 bee species.

Supplemental data for this article can be accessed <u>here</u>.



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Subsequent to the reviews published by Barth (2004) and Freitas and Novais (2014), numerous additional studies have appeared focusing on the pollen types encountered in apicultural and melliponine products in Brazil. As such, we inventoried the scientific literature concerning melissopalynology in Brazil published between 2005 and 2017 and indexed in the Web of Science and Scientific Electronic Library Online databases, characterising Brazilian scientific production in that area, especially in terms of their temporal and spatial distribution, considering the five geographic regions of that country.

We then constructed a database that includes information about the pollen types identified in those studies, the focal bee species, and the products analysed (honey, pollen, propolis etc.). The present work therefore sought to promote future melissopalynological research in Brazil and indicate the geographical areas that have been only poorly investigated.

2. Materials and methods

Two databases were consulted: the Web of Science (WoS) and the Scientific Electronic Library Online (SciELO). WoS was chosen as it comprises 16 separate databases that, together, consider scientific studies published in more than 12,000 scientific journals – thus constituting the principal database for global scientific research (Mugnaini et al. 2017); SciELO includes research studies published in more than 1000 scientific journals, with significant participation of Brazilian and Latin American researchers (Packer et al. 2014).

Consultations of WoS used six search terms in the TOPIC field: (1) *mel?ssopalynolog**, (2) *pollen analy?** AND *honey*, (3) *bee* pollen*, (4) *pollen* NEAR/15 *bee**, (5) *pollen analysis of honey*, and (6) *pollen analy?** NEAR/15 *honey*, with the subsequent use of filters that considered only papers published between 2005 and 2017 in Brazil. Search operators, such as AND and NEAR, and wildcards (unknown characters), such as the asterisk (* – which can represent any group of characters, including no character) and the question mark (? – which represents any single character), were used to combine terms in order to either broaden or narrow retrieval.

We also consulted the SciELO database for publications not listed by WoS, principally articles published in Portuguese through searches undertaken using the following terms: (1) *melissopalinologia*, (2) *pólen* AND *mel*, (3) *pólen* AND *abelha*, (4) *pólen apícola*, and (5) *análise polínica* AND *mel*. The searches identified 108 articles, of which 20 were considered relevant to the objectives of the present study; certain studies were excluded if they presented overlapping results with searches using WoS.

Additionally, we consulted the 'Lattes' platform (lattes.cnpq.br) to access the resume of the main Brazilian authors of papers found in the WoS and SciELO searches (if they were registered on that platform) to determine whether there were additional publications of similar content that did not appear in the two primary databases. The Lattes platform is the principal site for the resume of Brazilian researchers. From those results, an additional 33 papers were added to our database.

The scientific publications identified were characterized according to the following descriptors: numbers of authors

per article; numbers of articles published per year and per region; and the journals in which they appeared. The data gleaned from the publications were arranged in tables using Microsoft Excel® software, according to the following descriptors: botanical family, pollen type, bee species, locality (region), and the bibliographical reference of the article. The online databank of the Missouri Botanical Garden (www.tropicos.org) was consulted to update the family, generic, and species names of the plants. A taxonomic revision of the names of the bee species was performed by consulting the Moure Catalog of Bees (moure.cria.org.br/catalogue).

3. Results and discussion

3.1. Characterisation of the scientific production

Within the set of searches using all of the WoS terms, and after the use of filters and the posterior exclusion of overlaps (identical articles that appear in more than one search), 760 publications were individually consulted to determine whether they were appropriate to the objectives of the present study. We selected only articles that directly considered the theme of melissopalynology through studies of the pollen grains collected by the bees or encountered in the products they produced (such as honey, pollen, propolis and royal jelly). Following that filtering, the WoS search resulted in 80 publications.

SciELO searches resulted in 108 articles, of which 20 were considered appropriate to the objectives of the present study. The consultation of the resume available on the Lattes platform resulted in an additional 33 articles not encountered in the two earlier searches. As such, 133 publications encountered in the different databases are discussed here (Supplemental material 1). The articles are organised according to the year of publication (between 2005 and 2017), their references, the identification code of the article in the Supplemental material and the geographical origin of the type of product examined as well as the bee species studied (Abreu et al. 2014, Aleixo et al. 2013, Almeida et al. 2005, Almeida-Anacleto et al. 2012, Almeida-Muradian et al. 2005, Almeida-Muradian et al. 2013, Almeida-Muradian et al. 2014, Alves and Santos 2014, Alves and Santos 2016, Alves et al. 2006, Andrade et al. 2009, Antonini et al. 2006, Araújo et al. 2013, Araújo et al. 2017, Arruda et al. 2013, Azevedo et al. 2017, Barth 2005, Barth 2006, Barth & Luz 2009, Barth et al. 2005, Barth et al. 2009, Barth et al. 2010, Barth et al. 2013a, Barth et al. 2013b, Boff et al. 2011, Borges et al. 2014, Borsato et al. 2014a, Borsato et al. 2014b, Bosco and Luz 2017, Braga et al. 2012, Carneiro-Neto et al. 2017, Carpes et al. 2009, Carpes et al. 2013, Costa et al. 2009, Costa et al. 2015, Cruz et al. 2015, D'Apólito et al. 2010, Deveza et al. 2015, Dárea et al. 2009, Dórea et al. 2010a, Dórea et al. 2010b, Dórea et al. 2010c, Dórea et al. 2013, Dórea et al. 2017, Faria et al. 2012, Ferreira and Absy 2013, Ferreira and Absy 2015, Ferreira and Absy 2017a, Ferreira and Absy 2017b, Ferreira et al. 2010, Freire et al. 2012, Freitas et al. 2010a, Freitas et al. 2010b, Freitas et al. 2011, Freitas et al. 2013, Freitas et al. 2015, Freitas et al. 2017, Frias et al. 2016, Jesus et al. 2014, Jong et al. 2009, Kadri et al. 2016, Lima et al. 2017, Lima Neto et al.

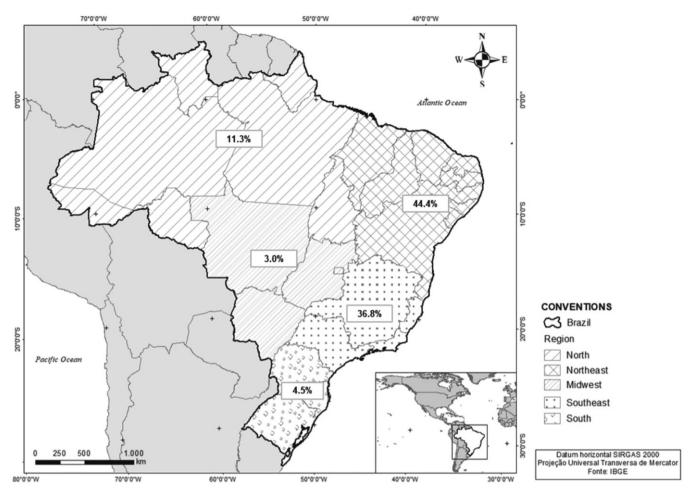
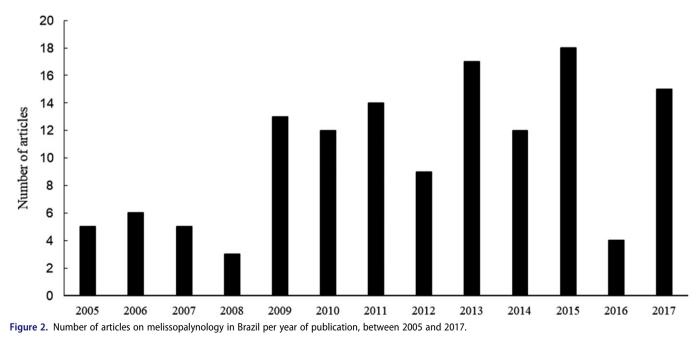


Figure 1. Map of Brazil, highlighting the percentages of articles focused on melissopalynology per Brazilian region between 2005 and 2017.



2017, Lucas et al. 2017, Luz and Barth 2012, Luz et al. 2007, 2008a, Men

Luz et al. 2009, Luz et al. 2017, Luz and Barti 2012, Luz et al. 2007, Luz et al. 2009, Luz et al. 2010, Luz et al. 2011, Marques et al. 2011, Marques-Souza et al. 2007, Marques-Souza 2010, Martins et al. 2011, Matos and Santos 2015, Matos and Santos 2016, Matos et al. 2014, Melo et al. 2009, Mendonça et al. 2008a, Mendonça et al. 2008b, Modro et al. 2007, Modro et al. 2009, Modro et al. 2011, Moreti et al. 2005, Morgado et al. 2011, Nascimento et al. 2009, Nascimento et al. 2015a, Nascimento et al. 2015b, Novais and Absy 2013, Novais and Absy 2015, Novais et al. 2006, Novais et al. 2009, Novais et al. 2009,

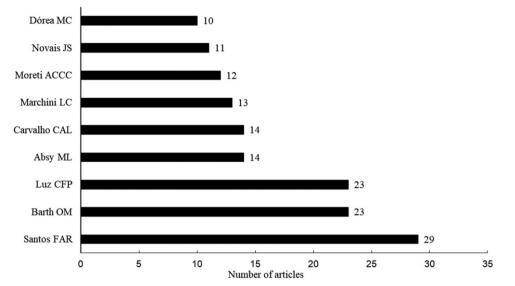


Figure 3. Numbers of articles on melissopalynology in Brazil according to the authors who contributed most to that area, between 2005 and 2017.

2010, Novais et al. 2013, Novais et al. 2014, Novais et al. 2015, Oliveira et al. 2009, Oliveira et al. 2010, Oliveira et al. 2017, Pinto et al. 2015, Poderoso et al. 2012, Ramalho et al. 2007, Rech and Absy 2011a, Rech and Absy 2011b, Ribeiro et al. 2013, Ribeiro et al. 2016, Santana et al. 2011, Santos et al. 2013, Sekine et al. 2013, Sereia et al. 2011, Serra et al. 2012, Silva and Santos 2014, Silva et al. 2006, Silva et al. 2012, Silva CRB et al. 2013, Silva IAA et al. 2013, Silva TMS et al. 2013, Silva GR et al. 2014, Silva TMG et al. 2014, Silva AS et al. 2015, Silva SJR et al. 2015, Silva et al. 2007, Solré et al. 2008, Souza et al. 2015, Tette et al. 2017, Ueira-Vieira et al. 2013, Vieira et al. 2011).

The north-eastern region of Brazil was the area most intensively studied melissopalynologically, accounting for 44.4% of the publications within the selected time period; Bahia State (situated within that region) was the best represented, with 34 publications. In second place was the southeastern region of the country, with 36.8% of the publications; within that region, São Paulo State stood out with 30 publications. The northern region of Brazil was responsible for 11.3% of the publications, with the predominance of Amazonas State (12 articles). The southern region accounted for 4.5% of the published articles, with five of them having been undertaken in Paraná State. The smallest number of publications originated from the central-western region, with only 3% of the articles, with three studies having been undertaken in Mato Grosso do Sul State (Figure 1).

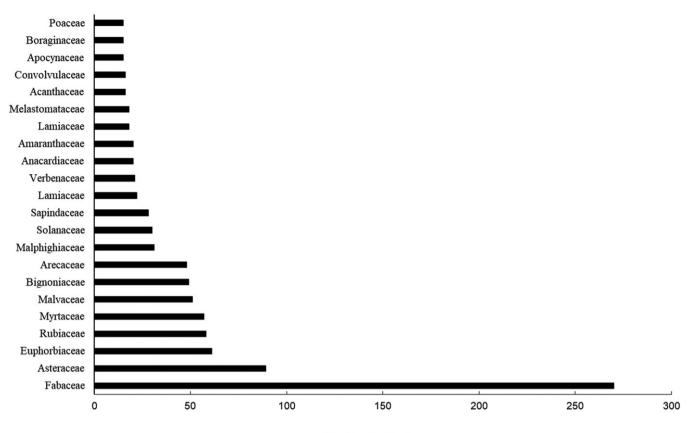
The study by Barth (2004) indicated that pollen analyses of honey produced in the southern region of Brazil were quite infrequent, which was corroborated in the present research – starkly contrasting with the quantities of honey commercially produced in that region. According to Vidal (2018), 39,600 tons of honey were produced in Brazil in 2016, of which 17,100 tons were produced in its southern region, but with only low numbers of melissopalynological studies from that area.

Brazil is composed of 26 states and one Federal District. Among these localities, the states of Acre, Alagoas, Amapá, Goiás, Mato Grosso, Paraíba, Pernambuco, Rio Grande do Norte, Rondônia, and Tocantins, as well as the Federal District, were not represented in any study between 2005 and 2017. In relation to the year of publication (2005–2017), 14, 13 and 12 articles were published in the years 2011, 2009, and 2010/2014, respectively, while 15 or more articles were published in each of 2013, 2015 and 2017. The lowest number of publications (three) was observed in 2008. The mean number of annual publications was 10.15.

It is therefore quite apparent that there were significant increases in the numbers of publications during the period examined, especially from 2009 onwards (Figure 2). According to the Research in Brazil report (produced by the analytical team of Clarivate Analytics for the Brazilian Higher Education Coordination – CAPES), scientific research in the country increased significantly between 2011 and 2016 (Cross and Sibclair 2018).

We observed that 77.4% of the articles published were written in English; the remainder (22.6%) were in Portuguese. The articles were published in 54 different journals, 33 of them with impact factors (JCR 2017) (Supplemental material 2); some journals published more than five articles on the theme during the period between 2005 and 2017: *Grana* (13 publications), *Sociobiology* (12), *Acta Botanica Brasilica* (10), *Palynology* (8), *Anais da Academia Brasileira de Ciências* (6), and the *Journal of Apicultural Research* (6). These results indicated an increase in the internationalisation of scientific research in Brazil. More than 60% of the melissopalynological studies were published in journals with impact factors, which probably reflects the heightened requirements of graduate programmes in Brazil.

There were 79 different authors of the 133 publications inventoried, with a mean of 4.9 authors per publication. The authors with the greatest numbers of publications during the study period (in decreasing order) were: F.A.R. Santos (29 articles), C.F.P. Luz (23), O.M. Barth (23), M.L. Absy (14), C.A.L. Carvalho (14), L.C. Marchini (13), A.C.C.C. Moretti (12), J.S. Novais (11) and M.C. Dórea (10) (Figure 3).



Number of pollen types

Figure 4. The most frequent plant families, based on the number of pollen types (> 15), listed in articles on melissopalynology in Brazil, published between 2005 and 2017.

3.2. A map of the pollen types cited in melissopalynology studies in Brazil

A total of 1362 pollen types were cited in melissopalynology studies, distributed among 130 botanical families (see Supplemental material). The family Fabaceae demonstrated the greatest number of pollen types in the database (270), followed by the families Asteraceae (89), Euphorbiaceae (61), Rubiaceae (58), Myrtaceae (51), Malvaceae (51), Bignoniaceae (49), and Arecaceae (48) (Figure 4). Those data for all of Brazil corroborated the work of Freitas and Novais (2014), who reported very similar results for the Brazilian Amazon region.

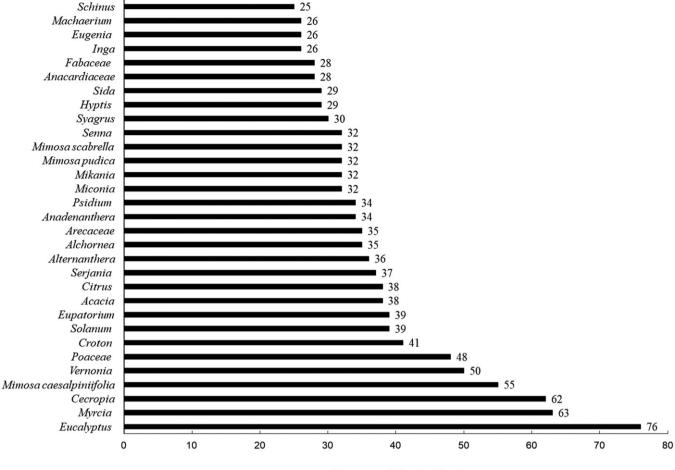
In terms of the pollen types encountered in bee products, *Eucalyptus* (Myrtaceae) was the most frequent, being cited in 77 of the inventoried texts; that pollen type was followed by *Myrcia* (Myrtaceae) (63), *Cecropia* (Urticaceae) (63), *Mimosa caesalpiniifolia* (55) (Fabaceae), *Vernonia* (51) (Asteraceae), Poaceae (49) and *Croton* (42) (Euphorbiaceae) (Figure 5). The high percentage of *Eucalyptus* pollen types in honey samples from Brazil reflects the advance of this monoculture in several Brazilian states. According to Aguiar et al. (2003), monoculture planting of *Eucalyptus* began in Brazil many decades ago, such as in the states of Espírito Santo (in the 1960s) and Bahia (in the 1980s). Since then, the cellulose industry has expanded operations into the Atlantic Rainforest.

As mentioned earlier, the inventoried texts considered five regions of Brazil (Figure 1). In the northern region, the pollen types most collected by bees as food resources (according to the 15 published papers examined) were from *Alchornea*, Byrsonima, Cecropia, Euterpe precatoria, Miconia, Mimosa pudica, Protium, Schefflera morototoni and Tapirira guianensis. In the north-east, among the 59 publications analysed, the pollen types most collected were from Borreria verticillata, Croton, Cecropia, Eucalyptus, Mikania, Mimosa caesalpiniifolia, Myrcia, Poaceae type, Solanum and Schinus. In the south-east, among the 49 publications analysed, the pollen types most collected were from Anadenanthera, Cecropia, Citrus, Eucalyptus, Eupatorium, Mimosa caesalpiniifolia, Myrcia and Poaceae type. In the central-western region, only four melissopalynological publications were encountered, with the most frequent pollen types being from Citrus, Elephantopus, Eucalyptus and Tabebuia.

Barth (2004) reported that publications from the southern region of Brazil were very infrequent – a situation that persisted into the next decade, with only six publications between 2005 and 2017. It is worth noting that the 1362 pollen types distributed among 130 botanical families in the 133 scientific publications inventoried here reflect the richness of the Brazilian flora encountered in its different phytogeographical domains, including the Amazon, Cerrado, Atlantic Forest, Caatinga and Pantanal, among others. Each domain has a distinct flora that is adapted to the regional environmental conditions.

3.3. The bees studied and their products

A total of 56 bee species were studied in the scientific publications inventoried here, distributed among 19 genera:



Ocorrence (%) of pollen types

Figure 5. Occurrence (%) of pollen types cited in more than 40 articles on melissopalynology in Brazil, published between 2005 and 2017.

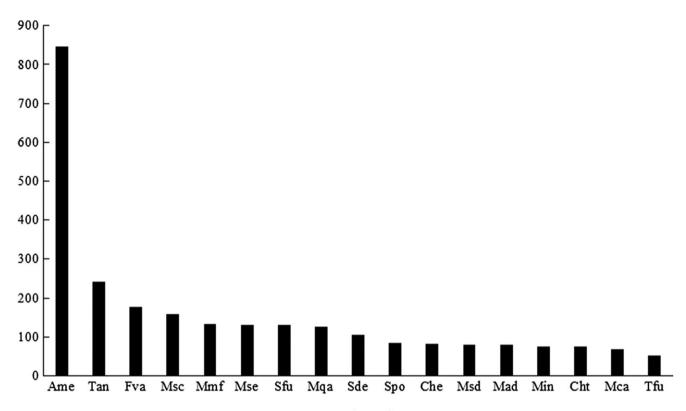
Aparatrigona Moure, 1951 (1 sp.); Apis L., 1758 (1 sp.); Centris Fabricius, 1804 (5 spp.) Cephalotrigona Schwarz, 1940 (2 spp.); Frieseomelitta Lepeletier, 1836 (1 sp.); Lestrimelitta Smith, 1863 (1 sp.); Melipona Illiger 1806 (16 spp.); Nannotrigona Lepeletier, 1836 (1 sp.); Nogueirapis Moure 1953 (1 sp.); Oxytrigona Cockerell, 1917 (1 sp.); Partamona Schwarz, 1939 (6 spp.); Plebeia Schwarz, 1938 (1 sp.); Ptilotrigona Moure, 1951 (1 sp.); Scaptotrigona Moure 1942 (6 spp.); Scaura Schwarz, 1938 (2 spp.); Schwarzula Moure 1946 (1 sp.); Tetragona Lepeletier & Serville, 1828 (1 sp.); Tetragonisca Moure 1946 (2 spp.) and Trigona Jurine, 1807 (6 spp.) (Supplemental material 3).

Overall, 74 publications studied only the bee species *Apis mellifera*; one publication focused on *A. mellifera* and *Melipona* (*Michmelia*) *scutellaris* (Latreille, 1811) (Ramalho et al. 2007), and another focused on *A. mellifera* and *Tetragonisca angustula* (Barth et al. 2013). *Tetragonisca angustula* (Barth et al. 2013). *Tetragonisca angustula* (Latreille, 1811) was the native bee species most studied (13 publications), followed by *Melipona scutellaris* (Latreille, 1811) (nine publications), and *Melipona (Melipona) quadrifasciata anthidioides* (Lepeletier, 1836) and *Melipona (Melipona) subnitida* (Ducke, 1910) with eight publications each.

Apis mellifera was the bee species associated with the greatest richness of pollen types (844) in the databases

(Figure 6), although that apparently high species richness was influenced by the fact that *A. mellifera* was the object of the greatest number of publications (76) and is the species most managed by associations, cooperatives and autonomous beekeepers for honey production. According to Rech et al. (2014), social bees are best known and studied, as many of them are commercially valuable, including the honey bee (*A. mellifera*) and various stingless bee species of the Brazilian fauna known as meliponines. Melissopalynological studies focusing on native bee species were only undertaken in the northern region of Brazil during the period investigated. The Amazon region is known as the global cradle of stingless bees, reflecting their importance as pollinators of edible crops and fruit trees (Carvalho-Zilse et al. 2005).

The melissopalynological analyses of bee products allowed diagnoses of the pollen types they contained – which were found to be highly variable depending on the flowers visited during foraging. The apicultural products analysed in the publications (in decreasing order) were: corbicula or stocked pollen (73), honey (43), propolis (8), geopropolis (4), pollen and honey (3), and post-emergence residues (2). Although the last item is not related to any bee-derived commercial product, it was included in the survey as it is important for characterising the diets of those insects (Ferreira and Absy 2013).



Bee species code

Figure 6. Number of different pollen types per bee species in articles on melissopalynology in Brazil, published between 2005 and 2017. Codes for bee species: Ame, Apis mellifera L., 1758; Tan, Tetragonisca angustula (Latreille, 1811); Fva, Frieseomelitta varia (Lepeletier 1836); Msc, Melipona (Michmelia) scutellaris Latreille, 1811; Mmf, Melipona (Melikerria) fasciculata Smith, 1854; Mse, Melipona (Michmelia) seminigra merrillae (Friese, 1903); Sfu, Scaptotrigona fulvicutis (Moure, 1964); Mqa, Melipona (Melipona) quadrifasciata anthidioides Lepeletier, 1836; Sde, Scaptotrigona depilis (Moure, 1942); Spo, Scaptotrigona postica (Latreille, 1807); Che, Centris (Heterocentris) analis (Fabricius 1801); Msd, Melipona (Melipona) subnitida Ducke, 1910; Mad, Melipona (Melipona) mandacaia Smith, 1863; Min, Melipona (Melikerria) interrupta Latreille, 1811; Cht, Centris (Heterocentris) terminata Smith, 1874; Mca, Melipona (Michmelia) capixaba Moure & Camargo, 1994; Tfu, Trigona fulviventris Guérin, 1844.

Propolis began to be melissopalynologically studied in Brazil more recently than other apicultural products (Barth 1998). Although melissopalynological studies of propolis use were reported in only eight publications (as seen in the present survey), older chemical studies were more frequent, such as those examining the use of natural medicines, and indicated that propolis was quite valuable due to its complex chemical composition (including up to 300 known compounds; Matos et al. 2014).

4. Final considerations

The analysis of scientific production in melissopalynology in Brazil based on the WoS and SciELO databases, and supplemented with consultations of the resume of Brazilian authors available on the Brazilian Lattes platform, revealed 133 publications distributed among the five regions of that country – with the greatest number (49) having been undertaken in the north-eastern region. As such, there are still gaps in our melissopalynological knowledge in certain regions, such as the central-western and southern portions of that country. Those areas should therefore be considered for priority research emphasis.

Native bees have been preferentially studied in the northern region of Brazil, while studies of Africanised bees (*Apis mellifera*) have predominated in the rest of the country. Propolis, geopropolis and royal jelly have been little studied overall, from a palynological point of view. It will be necessary to invest in research and teaching institutes in Brazil to guarantee the training of human resources for palynological studies, to strengthen existing research groups, and to initiate new melissopalynological investigations, especially in interior regions. There has also been a lack of publication of pollen catalogs in Brazil, which makes the identification of pollen types found in bee products more difficult – especially in light of the widely diverse national flora – with most pollen types having only been identified to the genus or family level.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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ROGÉRIO RIBEIRO DE SOUZA holds a licentiate's degree in biology and chemistry, with a major in biology, and an MS degree in society, environment and life quality, both from the Federal University of Western Pará, Brazil. His research interests are mainly focused on biodiversity, health and sustainability, stingless bees and melissopalynology.

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