Rodriguésia 72: e00902020. 2021
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202172100

Original Paper

Pollen analysis of honeys from *Apis mellifera* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae) in the Upper Paraná Atlantic Forest, Argentina

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Abstract

Nectariferous and polliniferous resources are key to the survival of social bees, so identifying the origin sources allows the implementation of management actions aimed at a greater supply of nutrients for the colonies. Besides, defining the floral origin of honeys contributes to their characterization and commercialization. The objective of our work was to identify the plants that provide nectar to the bees *Apis mellifera* and *Tetragonisca fiebrigi* in northern Misiones, through palynological analysis of honey samples collected between 2006 and 2008. Both bees showed a polylectic foraging habit. Richness of pollen types per sample ranged between 10 and 34 (mean = 20.5 ± 7.7) for *A. mellifera*, and between 13 and 43 (mean = 24.8 ± 7.1) for *T. fiebrigi.* The 15 most abundant pollen types in the honeys of *A. mellifera* were, in decreasing order of importance, Euphorbiaceae, *Euterpe edulis*, *Holocalyx balansae*, *Calyptranthes concinna*-type, *Actinostemon*, *Salix*, *Ruprechtia laxiflora*, *Myrcianthes pungens*-type, *Thinouia mucronata*, *Allophylus edulis*, *Ilex*, *Syagrus romanzoffiana*, *Gouania latifolia*-type, *Parapiptadenia rigida*, and *Baccharis*-type, whereas in the honeys of *T. fiebrigi* the most important pollen types included *S. romanzoffiana*, *Schinus weinmannifolius*-type, *Baccharis*-type, *H. balansae*, *E. edulis*, Rhamnaceae, *Citrus*, *Leonurus japonicus*, *G. latifolia*-type, *A. edulis*, *Gomphrena perennis*-type, *Pouteria gardneriana*, *P. rigida*, *Zanthoxylum*, and *Actinostemon*.

Key words: honeybee, Melissopalynology, melliferous flora, stingless bee.

Resumen

Los recursos nectaríferos y poliníferos son clave para la supervivencia de las abejas sociales, por lo que identificar las fuentes de origen permite implementar acciones de manejo tendientes a un mayor suministro de nutrientes para las colonias. Asimismo, definir el origen floral de las mieles contribuye a la caracterización y comercialización de las mismas. El objetivo de nuestro trabajo fue identificar los recursos vegetales que proporcionan néctar a las colmenas de *Apis mellifera* y *Tetragonisca fiebrigi* en el norte de Misiones, a partir del análisis polínico de las muestras de miel colectadas entre 2006 y 2008. Ambas especies mostraron un hábito de alimentación poliléctico. La riqueza de tipos polínicos por muestra osciló entre 10 y 34 (media = 20,5 ± 7,7) para *A. mellifera*, y entre 13 y 43 (media = 24,8 ± 7,1) para *T. fiebrigi*. Los 15 tipos polínicos más abundantes en las mieles de *A. mellifera* fueron, en orden decreciente de importancia, Euphorbiaceae, *Euterpe edulis*, *Holocalyx balansae*, tipo *Calyptranthes concinna*, *Actinostemon*, *Salix*, *Ruprechtia laxiflora*, tipo *Myrcianthes pungens*, *Thinouia mucronata*, *Allophylus edulis*, *Ilex*, *Syagrus romanzoffiana*, tipo *Gouania*

See supplementary material at <https://doi.org/10.6084/m9.figshare.16689481.v1>

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latifolia, *Parapiptadenia rigida* y tipo *Baccharis*, mientras que en las mieles de *T. fiebrigi* incluyeron a *S. romanzoffiana*, tipo *Schinus weinmannifolius*, tipo *Baccharis*, *H. balansae*, *E. edulis*, Rhamnaceae, *Citrus*, *Leonurus japonicus*, tipo *G. latifolia*, *A. edulis*, tipo *Gomphrena perennis*, *Pouteria gardneriana*, *P. rigida*, *Zanthoxylum* y *Actinostemon.*

Palabras clave: abeja melífera, Melisopalinología, flora melífera, abejas sin aguijón.

Introduction

Nectariferous and polliniferous resources are key to the survival of social bees and their colonies (Michener 2007). The use of these resources can be analysed by observing the flora and its visitors (Souza *et al.* 2016) or by pollinic analysis of food sources (honey and pollen) stored in nests (Vossler 2019). The pollen present in the nests can be a botanical and geographical indicator of their provenance (Barth 1989; Soares *et al.* 2017; Alves & Santos 2018; Bandeira & Novais 2020). Nowadays, it is recommended that this information, along with the description of the sensory and nutraceutical characteristics of honeys, be included on the labels of honeys for commercialization purposes (Mădaş *et al.* 2019).

In Argentina, among the honey-producing species we can mention to *Apis mellifera* L. (Apidae, Apini, Moure 2012) and native stingless bees belonging to the Meliponini tribe (Apidae, Camargo & Pedro 2013). *A. mellifera* is the most widespread honey-producing species in the world and the number of hives is increasing (Potts *et al.* 2016) despite the fact that in some countries of the Northern Hemisphere has undergone the loss of colonies due to Colony Collapse Disorder (CCD, Vanengelsdorp *et al.* 2017).

On the other hand, 37 species of stingless bees have been identified in Argentina; they belong to 18 genera and are distributed mainly in the ecoregions of Yungas, Chaco and Atlantic Forest (Álvarez 2016). Some of these species have been traditionally exploited in different regions of the country (Arenas 2003; Cebolla-Badie 2009; Zamudio *et al.* 2010; Flores *et al.* 2018). For example, *Tetragonisca fiebrigi* (Schwarz) is an eusocial insect used by local communities; stores honey and pollen in permanent colonies generally located within pre-existing cavities, like tree holes (Michener 2013). Recently, their honeys have been included in the Argentine Food Code (<https://www.boletinoficial.gob.ar/detalleAviso/primera/206764/20190502>), a fundamental step that adds value to this product and ensures adequate mechanisms for quality control and fair trade.

In Argentina, the food sources of *A. mellifera* have been frequently studied (Salgado & Pire 1998; Tamame & Naab 2003; Fagúndez & Caccavari 2006; Salgado-Laurenti *et al.* 2017; Sánchez & Lupo 2017; Reyes *et al.* 2019; among others).

Previous studies of the floral origin of *A. mellifera* honeys from the Argentine Atlantic Forest (Misiones province), revealed a dominance of the families Asteraceae, Anacardiaceae, Fabaceae, Myrtaceae, Rutaceae and Sapindaceae, and the abundant presence of pollen types belonging to native tree species (*e.g.*, *Anadenanthera colubrina* (Vell.) Brenan, *Balfourodendron riedelianum* (Engl.) Engl., *Cordia trichotoma* (Vell.) Arráb. *ex* Steud., *Eugenia uniflora* L., *Ilex paraguariensis A*. St.-Hil., *Parapiptadenia rigida* (Benth.) Brenan, *Peltophorum dubium* (Spreng.) Taub., *Schinus terebinthifolia* Raddi, *Sebastiania brasiliensis* Spreng., *Syagrus romanzoffiana* (Cham.) *Zanthoxylum petiolare* A.St.-Hil. & Tul.), exotic trees (*e.g.*, *Citrus* sp., *Hovenia dulcis* Thunb., *Lagerstroemia indica* L., *Psidium guajava* L.) and herbaceous species (*e.g.*, *Chamissoa altissima* (Jacq.) Kunth, *Leonurus japonicus* Houtt., *Sida rhombifolia* L.) (Aquino *et al.* 2015; Miranda *et al.* 2010, 2018).

Likewise, in the same ecoregion, studies of honeys and pollen loads from southeastern Brazil showed that the main botanical families used as resources were Anacardiaceae, Arecaceae, Asteraceae, Euphorbiaceae, Fabaceae, Myrtaceae, Solanaceae, among others. The main pollen types were *Allophylus petiolulatus*, *Baccharis*, *Campomanesia guazumifolia*, *Citrus*, *Cocos nucifera*, *Eucalyptus*, *Ilex paraguariensis*, *Matayba elaeagnoides*, *Mikania*, *Myrcia*, *Vernonanthura* and *Syagrus romanzoffiana* (Ramalho *et al.* 1991; Alves & Santos 2018).

In Argentina, the floral resources used by stingless bee species have received attention only in recent years. In the north of Chaco province and the northwest of Córdoba province they were extensively studied by Vossler (Vossler *et al.* 2010, 2014; Vossler 2019, among others) and Geisa (Geisa 2020), respectively. The nectariferous resources used by *T. fiebrigi* was studied through the pollen characterization of its honeys for the first time in the Yungas (northwestern Argentina) by Flores & Sánchez (2010), and in the Argentine Atlantic Forest by Fernández *et al.* (2015), and a preliminary work (including two honey samples) by Miranda *et al.* (2018). In some of these works the species is referred to as *T. angustula* Latreille; this species is synonym of *T. fiebrigi*, which is the only species of the genus present in Argentina (Álvarez 2016). Fernández *et al.* (2015) identified Apiaceae, Asteraceae, Fabaceae and Solanaceae as the main botanical families, and *Hovenia dulcis*, *Cecropia pachystachya*, *Lagerstroemia indica*, *Ammi*-type and Poaceae as the pollen types with the highest frequency of occurrence in the samples. On the other hand, Miranda *et al.* (2018) highlighted the botanical families Anacardiaceae, Apiaceae, Arecaceae and Asteraceae, and the pollen types *Syagrus romanzoffiana*, *Trema micrantha*, *Cecropia pachystachya* and *Schinus terebinthifolia.*

Therefore, because there is a local interest in knowing the floral resources used by *A. mellifera* and *T. fiebrigi* and in order to contribute to the list of main nectariferous species in northern Misiones and the ecorregion, the pollen content of their honeys was analysed and the most abundant botanical families and pollen types are discussed for each bee species, providing substantial information about their diet.

Materials and Methods

Study area

The study was conducted in different localities of General Manuel Belgrano, Iguazú, and San Pedro Departments, in the north of Misiones province (Fig. 1), belonging to the Upper Paraná Atlantic Forest (Galindo-Leal & Camara 2003). The inhabitants of the study area belong to diverse cultural groups: Mbya-Guaraní ethnic groups, Europeans, Asians, Brazilians, and Paraguayans, as well as people from other Argentine provinces (Gallero & Krautstofl 2010; Zamudio *et al.* 2010). At present, the landscape is composed of protected areas, and urban and rural areas where forestry-agro-livestock production systems of different sizes coexist (Furlan *et al.* 2015). Among the most widespread crops are

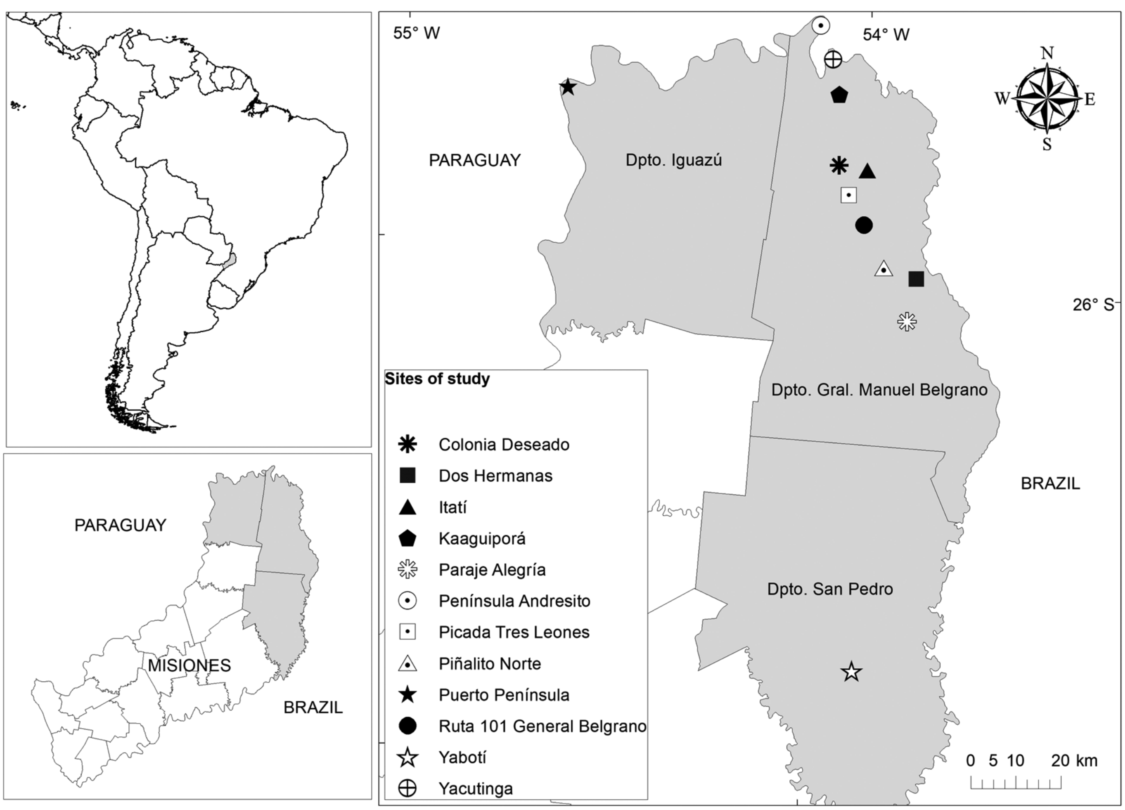

Figure 1 – Collection sites of honeys of *Apis mellifera* and *Tetragonisca fiebrigi.*

"yerba mate" (*Ilex paraguariensis* A. St.-Hil.), tobacco (*Nicotiana tabacum* L.), citrus (*Citrus* sp.) and, to a lesser extent, corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.), soybean (*Glycine max* (L.) Merr.), and tea (*Camellia sinensis* (L.) Kuntze). In addition, cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas* (L.) Lam) and fruit trees are cultivated in households, complemented with animal husbandry (Zamudio & Hilgert 2011; Furlan *et al.* 2015). In this context, the bees *A. mellifera* and *T. fiebrigi* were selected, within the framework of ethnobiological studies carried out in the region and according to their importance of use by local communities (Zamudio *et al.* 2010; Kujawska *et al.* 2012). The sample collection sites were selected according to the use of honey from both bees in the same production unit.

Honey sampling

Between November 2006 and November 2008, 14 honey samples of *A. mellifera* and 39 honey samples of *T. fiebrigi* were collected from man-made hives (Tab. 1). In *A. mellifera*, 250 mL of honey per hive were collected using a conventional beekeeping extractor. In *T. fiebrigi*, 10 mL of honey per hive were extracted with a sterile syringe (the average annual production per hive is 1 L).

Laboratory processing and microscopic analysis

Each honey sample was homogenized and processed according to Louveaux *et al.* (1978) and the sediment was acetolyzed (Erdtman 1960). The obtained microscope slides were deposited in the reference collections of Laboratory of Palynology of Universidad Nacional de Jujuy (PAL-JUA) and Instituto de Biología Subtropical of Universidad Nacional de Misiones (PAL-ETNO). Observations were made under Leica DM 500 optical microscope and microphotographs were taken with a Leica ICC50 digital camera. For quantification of the pollen types, at least 1,000 pollen grains were counted from each sample; they were determined and classified as predominant (more than 45% of

Table 1 – Number of honey samples collected from different localities of northern Misiones.

Locality	*Apis mellifera*		*Tetragonisca fiebrigi*	
	N°	**Sample code (collection month)**	**N°**	**Sample code (collection month)**
Colonia Deseado	2	A4, A5 (October-2007)	2	Y7 (October-2007), Y8 (November-2007)
Dos hermanas	1	A12 (September-2007)	1	Y19 (January-2008)
Itatí	1	A3 (October-2007)	1	Y5 (November-2007)
Kaaguiporá	2	A9 (November-2007), A14 (January-2008)	2	Y10 (June-2007), Y21 (January-2008)
Paraje Alegría	1	A13 (January-2008)	1	Y1 (November-2006)
Península Andresito	3	A1, A2, A6 (October-2007)	10	Y4 (August-2007), Y6, Y9 (October-2007), Y11 (November-2007), Y22, Y23, Y24, Y25, Y26, Y27 (March-2008)
Picada Tres Leones	1	A10 (December-2007)	7	Y14 (November-2007), Y15, Y17 (December-2007), Y20 (January-2008), Y32, Y33, Y34 (March-2008)
Piñalito Norte	1	A11 (December-2007)	5	Y16 (December-2007), Y28, Y29, Y30, Y31 (March-2008)
Puerto Península	-	-	1	Y18 (July-2007)
Ruta 101 General Belgrano	-	-	1	Y13 (December-2007)
Yabotí	2	A7, A8 (November-2007)	2	Y12 (November-2007), Y35 (December-2007)
Yacutinga	-		6	Y2, Y3 (January-2007), Y36, Y37, Y38, Y39 (November-2008)
Total	14		39	

the pollen grains counted), secondary (16–45%), important minor pollen (3–15%), and minor pollen (less than 3%). Honeys were classified as unifloral if there was a predominant pollen type, except for *Citrus* sp. with 10% (Louveaux *et al.* 1978) and *Eucalyptus* sp. with 70% (Resolution 274/95; SAGPyA 1995). The frequency of occurrence (FO; Feller-Demalsy *et al.* 1987) was established as the percentage of a pollen type present in all the samples: very frequent (> 50%), frequent (20–50%), less frequent (10–20%) and rare (< 10%). Pollen types of wind-pollinated or nectarless plants were excluded from the frequency calculations because these plants do not contribute to honey production and their presence samples can distort botanical characterization (Louveaux *et al.* 1978; Flores *et al.* 2015). Pollen types were identified using the reference collection of the study area and the existing literature (Markgraf & D'Antoni 1978; Pire *et al.* 1998, 2001, 2006, 2013). The nomenclature of the pollen types follows the criterion of De Klerk & Joosten (2007).

Data analysis

For each sample, the following parameters were calculated:

a) Pollen type richness (S), *i.e.*, the number of recorded pollen types;

b) Index of species importance (IS_j) = Mean relative abundance of j × Number of samples with j / Total number of samples

Where j is each pollen type;

c) Index of botanical family importance (IF_i) = Sum of mean relative abundance of species of i × Number of samples with i / Total number of samples.

Where i is the botanical family (Nates-Parra *et al.* 2013; Vossler 2019). The proposed indexes were calculated using the Microsoft Excel program.

For the pollen diagram, data analysis and dendrogram the TILIA 1.7.16 software package was used (Grimm 2011). Standardised coefficients of dissimilarity were applied using the Edwards & Cavalli-Sforza coefficient and non-constrained (without sample ordination). Groups were organized according to abundance and frequency of their pollen types. Pollen types in class frequency of less than 3% were not included, because these types are present throughout the pollen spectrum and do not contribute to the differentiation of the groupings. In this way, sample groupings were established presenting affinity among them.

Results

Botanical origin and pollen richness of samples

A total of 117 pollen types were found; of them, 76 types were present in the *A. mellifera* samples, 104 in the *T. fiebrigi* samples, and 63 in the samples of both species. In *A. mellifera*, one pollen type was identified at division (Liliopsida), 14 at family, 22 at genus, and 38 at species levels, and one was undetermined. Richness varied between 10 and 34 pollen types, with an average of 20.5 ± 7.7. In the honeys of *T. fiebrigi*, one pollen type was identified at botanical division (Liliopsida), 18 at family, 30 at genus, and 54 at species levels, and one was undetermined. Richness per sample varied between 13 and 43 pollen types, with an average of 24.8 ± 7.1 (Tab. 2; see also Appendix S1 and S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.16689481.v1).

In the total of samples 14 pollen types of wind-pollinated or nectarless plants were found. The pollen types more frequents in the honeys of *T. fiebrigi* were *Trema micrantha*, *Cecropia pachystachya*, *Celtis iguanaea*, Moraceae and *Piper*, and in the honeys of *A. mellifera* were *C. pachystachya*, Solanaceae-type, *C. iguanaea* and *Lycium morongii*-type (Tab. 2).

Frequency classes of pollen types and classification of honeys

In honeys of *A. mellifera*, 3% of the pollen types were classified as predominant (present in 7 -*i.e.* 50%- samples), 6% as secondary, 27% as important minor, and 64% as minor pollen. In this bee species, 50% (7 samples) of the honeys were unifloral. In honeys of *T. fiebrigi*, 3% of the pollen types were classified as predominant (present in 23 -*i.e.* 59%- samples), 7% as secondary, 18% as important minor, and 72% as minor pollen. In this bee species, 59% of the honeys (23 samples) were classified as unifloral (Fig. 2).

Index of species and botanical family importance

According to the index of species importance, the pollen type Euphorbiaceae were the most abundant and frequent in the samples of *A. mellifera* followed for *Euterpe edulis*, *Holocalyx balansae*, *Calyptranthes concinna*-type, *Actinostemon*, *Salix*, *Ruprechtia laxiflora*, *Myrcianthes pungens*-type, *Thinouia mucronata*, *Allophylus edulis*,

Table 2 – Pollen types identified in honey samples of *Apis mellifera* and *Tetragonisca fiebrigi*, and their frequency classes and frequency of occurrence in northern Misiones.

Family	Pollen type	*Apis mellifera*					*Tetragonisca fiebrigi*				
		p	s	im	m	FO	p	s	im	m	FO
Acanthaceae	*Justicia*				1	7					
Amaranthaceae	*Alternanthera aquatica*								1	5	15
Amaranthaceae	*Amaranthus muricatus*								1	6	18
Amaranthaceae	*Amaranthus*									4	10
Amaranthaceae	*Chamissoa acuminata*				1	7				16	41
Amaranthaceae	*Gomphrena perennis*-type						1		1	7	23
Anacardiaceae	Anacardiaceae								3	3	21
Anacardiaceae	*Astronium balansae*			1		7					
Anacardiaceae	*Schinus weinmannifolius*-type			1	5	43	4	7	11	6	79
Apiaceae	Apiaceae				1	7				14	36
Apiaceae	*Bowlesia*							1	3	5	23
Apocynaceae	Apocynaceae								1		3
Apocynaceae	*Aspidosperma*								2	3	13
Apocynaceae	*Forsteronia*				1	7				5	13
Apocynaceae	*Mandevilla angustifolia*-type				1	7				2	5
Apocynaceae	*Rauvolfia sellowii*									5	13
Aquifoliaceae	*Ilex*			5	2	50			1	20	54
Arecaceae	*Euterpe edulis*	2	2	2	3	64	2	2	2	3	23
Arecaceae	*Syagrus romanzoffiana*		1	1	3	36	8	10	8	5	79
Asteraceae	Asteraceae			1	4	36				21	54
Asteraceae	*Baccharis*-type			2	8	71	1	7	3	13	62
Asteraceae	*Bidens*				2	14					
Asteraceae	*Elephantopus*									2	5
Asteraceae	*Eupatorium*				1	7				1	3
Asteraceae	*Senecio*				1	7				1	3
Asteraceae	*Vernonia*				5	36			1	3	10
Bignoniaceae	Bignoniaceae			1	4	36			5	12	44
Brassicaceae	*Rorippa hilariana*-type				1	7			2	8	26
Bromeliaceae	Bromeliaceae									1	3
Buddlejaceae	*Buddleja stachyoides*-type									1	3
Cactaceae	Cactaceae			2	1	21					
Cactaceae	*Pereskia grandiflora*				3	21				7	18
Caricaceae	*Carica papaya*									1	3
Cucurbitaceae	*Sicyos*							1		2	8
Euphorbiaceae	*Actinostemon*		2		4	43		1	6	20	72
Euphorbiaceae	*Croton*			1		7					
Euphorbiaceae	Euphorbiaceae	2	1	5		64			4	27	79
Euphorbiaceae	*Sapium*				1	7					

Family	**Pollen type**	***Apis mellifera***					***Tetragonisca fiebrigi***				
		p	**s**	**im**	**m**	**FO**	**p**	**s**	**im**	**m**	**FO**
Euphorbiaceae	*Sebastiania*				4	29				1	3
Fabaceae	*Acacia*				1	7					
Fabaceae	*Anadenanthera colubrina*				2	14					
Fabaceae	*Erythrina*									1	3
Fabaceae	Fabaceae				1	7			2	4	15
Fabaceae	*Holocalyx balansae*	1	3	3	5	86	1	5	8	15	74
Fabaceae	*Mimosa cruenta*-type				2	14				1	3
Fabaceae	*Mimosa somnians*-type				1	7				2	5
Fabaceae	*Mimosa velloziana*-type				1	7				2	5
Fabaceae	*Parapiptadenia rigida*		1	2	6	64		1	7	24	82
Fabaceae	*Peltophorum dubium*				3	21			1	7	21
Lamiaceae	*Hyptis australis*-type									1	3
Lamiaceae	*Hyptis elegans*-type									1	3
Lamiaceae	*Leonurus japonicus*			3	3	43	1	1	2	22	69
Lauraceae	Lauraceae									2	5
Lauraceae	*Cinnamomum*-type									2	5
Loranthaceae	Loranthaceae				1	7					
Malpighiaceae	*Heteropterys*									9	23
Malpighiaceae	*Galphimia brasiliensis*									1	3
Malvaceae	*Abutilon pauciflorum*-type				7	50			2	12	36
Meliaceae	*Cabralea canjerana*									4	10
Meliaceae	Meliaceae				1	7				4	10
Meliaceae	*Melia azedarach* *				1	7					
Meliaceae	*Trichilia catigua*-type				1	7			1	2	8
Melastomataceae	*Miconia*									2	8
Myrtaceae	*Calyptranthes concinna*-type	1	2	2	5	71			4	13	44
Myrtaceae	*Eucalyptus* *				1	7			1	4	13
Myrtaceae	*Myrceugenia euosma*-type				1	7				1	3
Myrtaceae	*Myrcianthes pungens*-type		1	3	1	36					
Myrtaceae	*Myrcia oblongata*-type									5	13
Myrtaceae	Myrtaceae			1	1	14				3	8
Myrsiniaceae	*Myrsine*									2	5
Polygonaceae	*Polygonum*									1	3
Polygonaceae	*Ruprechtia laxiflora*		1	4	3	57		1	3	13	44
Ranunculaceae	*Clematis campestris*-type									3	8
Rhamnaceae	*Gouania latifolia*-type			4		29	1		1		5
Rhamnaceae	*Scutia buxifolia*-type								2	1	8
Rhamnaceae	Rhamnaceae			1	6	50		3	10	16	74
Rubiaceae	Rubiaceae									3	8
Rutaceae	*Balfourodendron riedelianum*-type							1		2	8

Family	Pollen type	*Apis mellifera*					*Tetragonisca fiebrigi*				
		p	s	im	m	FO	p	s	im	m	FO
Rutaceae	*Citrus* *				3	21	3		5	12	51
Rutaceae	*Zanthoxylum*				6	43	1	1		7	23
Salicaceae	*Salix*		1	2	4	50			2	1	8
Sapindaceae	*Allophylus edulis*			7	3	71		2	6	13	54
Sapindaceae	*Allophylus guaraniticus*				1	7				3	8
Sapindaceae	*Matayba elaeagnoides*			2	5	50			1	17	46
Sapindaceae	*Paullinia meliifolia*				1	7				2	5
Sapindaceae	*Serjania*				2	14			1	10	28
Sapindaceae	*Thinouia mucronata*	1				7				2	5
Sapotaceae	*Chrysophyllum gonocarpum*									3	8
Sapotaceae	*Chrysophyllum marginatum*									8	21
Sapotaceae	*Pouteria fragrans*							1	3	11	44
Sapotaceae	*Pouteria gardneriana*				2	14		1	4	2	18
Sapotaceae	*Pouteria salicifolia*								1	1	5
Sapotaceae	*Sideroxylon obtusifolium*								1	1	5
Simaroubaceae	*Castela tweediei*-type				2	14				1	3
Ulmaceae	*Phyllostylon rhamnoides*				1	7				2	5
Urticaceae	*Urera baccifera*				1	7			2	8	26
Urticaceae	Urticaceae									2	5
Valerianaceae	*Valeriana*									1	3
Verbenaceae	*Acantholippia*				1	7					
Verbenaceae	Verbenaceae			1		7					
Vitaceae	*Cissus*				1	7				1	3
Pollen types of wind-pollinated or nectarless plants											
Cannabaceae	*Celtis iguanaea*					43					82
Cannabaceae	*Trema micrantha*					21					100
Cecropiaceae	*Cecropia pachystachya*					79					82
Cyperaceae	Cyperaceae										3
Moraceae	Moraceae					29					62
Moraceae	*Sorocea bonplandii*										21
Oleaceae	*Fraxinus* *										21
Piperaceae	*Peperomia*					29					10
Piperaceae	*Piper*					21					51
Poaceae	Poaceae					21					33
Poaceae	*Zea mays* *					36					44
Solanaceae	*Lycium morongii*-type					43					5
Solanaceae	Solanaceae-type					57					23
Solanaceae	*Solanum*										10

Frequency classes: p = predominant pollen (> 45%); s = secondary pollen (16-45%); im = important minor pollen (3-15%); m = minor pollen (<3%); FO = frequency of occurrence; * = Pollen types of exotic species.

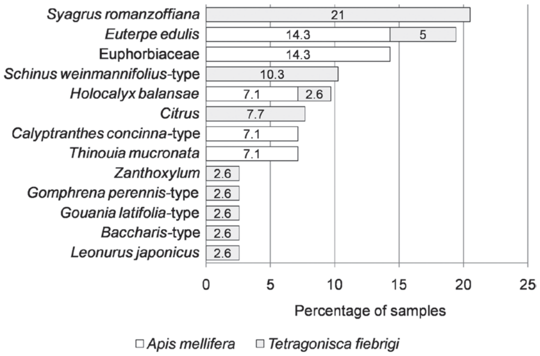

Figure 2 – Predominant pollen types in unifloral honeys of *Apis mellifera* and *Tetragonisca fiebrigi* in Misiones, Argentina.

Ilex, *Syagrus romanzoffiana*, *Gouania latifolia*-type, *Parapiptadenia rigida*, and *Baccharis*-type (Tab. 3; Figs. 3; 4). On the other hand, in the samples of *T. fiebrigi* the pollen type most important was *S. romanzoffiana* followed for *Schinus weinmannifolius*-type, *Baccharis*-type, *H. balansae*, *E. edulis*, Rhamnaceae, *Citrus*, *Leonurus japonicus*, *Gouania latifolia*-type, *A. edulis*, *Gomphrena perennis*-type, *Pouteria gardneriana, P. rigida, Zanthoxylum*, and *Actinostemon* (Tab. 4; Figs. 3; 4).

In the samples of *A. mellifera*, pollen types belonging to 34 botanical families were recorded, with Fabaceae presenting the highest pollen richness. However, according to the index of family importance, Fabaceae was the third most important (15%), after Euphorbiaceae (21%) and Arecaceae (17%). On the other hand, the pollen types of *T. fiebrigi* honeys belonged to 44 botanical families, with Fabaceae also exhibiting the highest pollen richness. Likewise, when considering the index of family importance, Fabaceae also was third, after Arecaceae (27%) and Anacardiaceae (17%) (Fig. 5).

Pollen diagram

Two main groups were distinguished in the pollen diagram prepared for *A. mellifera* honeys (Fig. 6). The first of them (I) concentrates samples collected in September, October and November 2007 (samples A12 to A8). These are characterized by a high presence of *Holocalyx balansae*, followed by the pollen types *Calyptranthes concinna*-type, Euphorbiaceae, *Euterpe edulis*, *Actinostemon*, *Ruprechtia laxiflora*, *Myrcianthes pungens*-type, *Allophylus edulis*, *Ilex*, *Leonurus japonicus*, *Gouania latifolia*-type, and *Matayba elaeagnoides.*

Group II gathers samples from December 2007 (A11 and A10) and January 2008 (A11 to A14), where *Euterpe edulis* stood out for its high presence in the samples, accompanied by *Calyptranthes concinna*-type and *Thinouia mucronata* and to a lesser extent by *Salix*, *Syagrus romanzoffiana*, *Parapiptadenia rigida* and Euphorbiaceae.

On the other hand, three main groups were distinguished in the *T. fiebrigi* honey samples (Fig. 7, groups III, IV and V). In the first of them, the samples from 2006 (Y1, November) and 2007 (Y2 and Y3, January) presented a high abundance of the arboreal pollen type *Syagrus romanzoffiana* accompanied by *Leonurus japonicus*, *Baccharis*-type, Bignoniaceae and *Rorippa hilariana*-type. In the same group and with samples corresponding to several months of the year 2007 (samples Y10 to Y35) a high presence of the pollen type *Holocalyx balansae* was observed, accompanied to a lesser extent by the pollen types *Schinus weinmannifolius*-type, *S. romanzoffiana*, *L. japonicus*, *Pouteria fragrans*, *P. gardneriana*, *Parapiptadenia rigida*, *Citrus*, *Gouania latifolia*-type, *Gomphrena perennis*-type, *Zanthoxylum*, Rhamnaceae, Bignoniaceae, *Euterpe edulis*, *Balfourodendron riedelianum*-type, *Aspidosperma*, Anacardiaceae, *Allophylus edulis* and *Actinostemon*.

In group IV, with samples from January, March and November 2008 (samples Y19 to Y37), a high presence of the pollen types *S. romanzoffiana*, *Schinus weinmannifolius*-type, *Baccharis*-type and to a lesser extent stand out Rhamnaceae, *H. balansae*, *A. edulis*, *Citrus*, and *E. edulis* were observed. While group V, with samples from November 2008 (Y38 and Y39), *E. edulis* stood out, followed for *P. gardneriana*, *P. rigida*, *S. weinmannifolius*-type and Euphorbiaceae.

Discussion

The pollen richness observed in the honey samples reflects the polylectic foraging habit of the studied bee species. These results agree with findings of studies conducted in Argentina for *A. mellifera* (Fagúndez & Caccavari 2006; Salgado-Laurenti *et al.* 2017; Sánchez & Lupo 2017; Reyes *et al.* 2019) and *T. fiebrigi* (Flores & Sánchez 2010; Vossler *et al.* 2014; Fernández *et al.* 2015). This behaviour was also observed in other members of the genus *Tetragonisca*, such as *T. angustula* in Bolivia (Saravia-Nava *et al.* 2018), Brazil (Iwama & Melhem 1979; Novais *et al.* 2013), Colombia (Obregón *et al.* 2013), and Mexico (Martínez-Hernández *et al.* 1994). In turn, it is a foraging

Table 3 – Pollen types of nectariferous plants in honey samples of *Apis mellifera* in northern Misiones.

N°	Pollen types	IS_j	FO	N°	Pollen types	IS_j	FO
1	Euphorbiaceae	14.9	64	34	*Mandevilla angustifolia*-type	0.10	7
2	*Euterpe edulis*	14.7	64	35	*Mimosa cruenta*-type	0.093	14
3	*Holocalyx balansae*	13	86	36	*Mimosa somnians*-type	0.064	7
4	*Calyptranthes concinna*-type	9	71	37	*Pereskia grandiflora*	0.064	21
5	*Actinostemon*	5	43	38	*Melia azedarach*	0.057	7
6	*Salix*	4.9	50	39	*Citrus*	0.043	21
7	*Ruprechtia laxiflora*	4.7	57	40	Fabaceae	0.036	7
8	*Myrcianthes pungens*-type	4	36	41	*Anadenanthera colubrina*	0.029	14
9	*Thinouia mucronata*	3.6	7	42	*Myrceugenia euosma*-type	0.029	7
10	*Allophylus edulis*	3.1	71	43	*Bidens*	0.029	14
11	*Ilex*	2.8	50	44	*Castela tweediei*-type	0.021	14
12	*Syagrus romanzoffiana*	2.5	36	45	*Serjania*	0.021	14
13	*Gouania latifolia*-type	2.2	29	46	*Acacia*	0.021	7
14	*Parapiptadenia rigida*	1.9	64	47	*Acantholippia*	0.021	7
15	*Baccharis*-type	1.8	71	48	*Eucalyptus*	0.014	7
16	Liliopsida	1.4	21	49	*Pouteria gardneriana*	0.014	14
17	*Matayba elaeagnoides*	1.1	50	50	*Trichilia catigua*-type	0.014	7
18	*Leonurus japonicus*	1.1	43	51	*Senecio*	0.014	7
19	Myrtaceae	0.8	14	52	*Mimosa velloziana*-type	0.014	7
20	Cactaceae	0.61	21	53	*Paullinia meliifolia*	0.014	7
21	Rhamnaceae	0.58	50	54	*Forsteronia*	0.014	7
22	Asteraceae	0.54	36	55	*Allophylus guaraniticus*	0.007	7
23	Bignoniaceae	0.51	36	56	*Phyllostylon rhamnoides*	0.007	7
24	*Schinus weinmannifolius*-type	0.47	43	57	*Urera baccifera*	0.007	7
25	Verbenaceae	0.35	7	58	*Chamissoa acuminata*	0.007	7
26	*Astronium balansae*	0.31	7	59	*Eupatorium*	0.007	7
27	*Zanthoxylum*	0.30	43	60	*Justicia*	0.007	7
28	*Sebastiania*	0.26	29	61	*Rorippa hilariana*-type	0.007	7
29	*Croton*	0.26	7	62	*Cissus*	0.007	7
30	*Abutilon pauciflorum*-type	0.19	50	63	Loranthaceae	0.007	7
31	*Peltophorum dubium*	0.14	21	64	Apiaceae	0.007	7
32	*Sapium*	0.11	7	65	Meliaceae	0.007	7
33	*Vernonia*	0.10	36				

IS_j = Index of species importance; FO = frequency of occurrence.

mode commonly observed in stingless bees and *A. mellifera* of other Neotropical regions (Ramalho *et al.* 1990, 1991).

Arecaceae was the most abundant family for *T. fiebrigi* and the second most abundant for *A. mellifera*, especially with the pollen types of *Syagrus romanzoffiana* and *Euterpe edulis*. Similarly, the relevance of *S. romanzoffiana* was indicated for the west and centre of Misiones province (Aquino *et al.* 2015) and the states of Rio

Grande do Sul and São Paulo (southern Brazil) (Ramalho *et al.* 1991; Hilgert-Moreira *et al.* 2014), and of *E. edulis* for the states of Bahia and São Paulo, Brazil (Barroso *et al*. 2010; Bandeira & Novais 2020). Likewise, the high presence of *Euterpe*/*Syagrus* pollen type in honeys of *A. mellifera* was highlighted from Porto Velho, São Paulo (Bosco & da Luz 2018).

In general, the role of palm trees in supplying resources to bees is associated with entomophilia, the most widespread pollination syndrome of this angiosperms family (Henderson 1986; Barfod *et al.* 2011). Likewise, the predominance of the family was also documented in studies on honeys, corbicular loads and pollen pots of *A. mellifera*, and stingless bees from Brazil (Oliveira *et al.* 2009; Rech & Absy 2011; Freitas & Novais 2014; Hilgert-Moreira *et al.* 2014; Bandeira & Novais 2020). Other palm trees, such as *Copernicia alba* Morong and *Trithrinax schizophylla* Drude, are important nectar and pollen resources for *A. mellifera* and *T. fiebrigi* in the Chaco ecoregion of Argentina (Vossler *et al.* 2014; Salgado-Laurenti *et al.* 2017).

The presence of *Syagrus romanzoffiana* and *Euterpe edulis*, key elements of the Upper Paraná Atlantic Forest, is probably associated with the abundance of these trees in areas surrounding family production units and the extended flowering period of these species. Approximately 5 months in *E. edulis* (Silva & Reis 2018) and 9 months in *S. romanzoffiana* (Garcia & Barbedo 2016). In particular, *E. edulis* is a profitable non-timber forest resource from which “palmito” or “cogollo” and fruit pulp -Jejy’a- are obtained for food purposes, and its seeds for ornamental purposes (Chediack 2008; Campanello *et al.* 2019; Villagra *et al.* 2019).

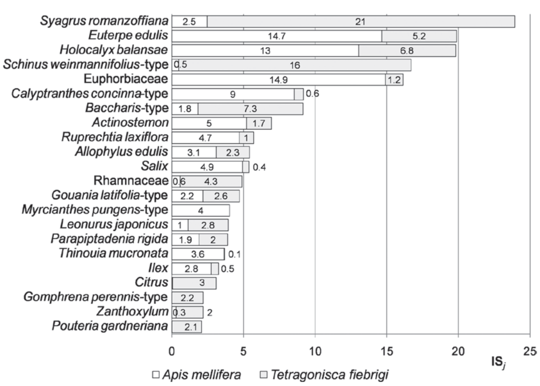

Figure 3 – Index of species importance (IS$_j$). Only pollen types with an index equal to or higher than 2% are represented.

Fabaceae was abundant in the honeys of both species, as indicated by the richness of pollen types and the index of botanical family importance. This high presence could be linked to the representativeness of the family in the province of Misiones (Zanotti *et al.* 2020) and in the Neotropics (Ramalho *et al.* 1990). The predominance of Fabaceae was also observed in studies of *A. mellifera* conducted in other departments of Misiones (Miranda *et al.* 2018) and other areas of Argentina (Fagúndez & Caccavari 2006; Malacalza *et al*. 2007; Salgado-Laurenti *et al.* 2017; Sánchez & Lupo 2017; Reyes *et al.* 2019). In northwestern Argentina, this family was relevant in honeys of *T. fiebrigi* (Flores & Sánchez 2010) and *Plebeia intermedia* Wille (Flores *et al.* 2015). In the Neotropics, Fabaceae is important for *A. mellifera* (Alves & Santos 2018) and *Melipona scutellaris* Latreille in Brazil (Carvalho *et al.* 2001), and for *T. angustula* in Bolivia (Saravia-Nava *et al.* 2018), different localities of Brazil (Cortopassi-Laurino & Gelli 1991; Carvalho *et al.* 1999; Barth *et al.* 2013; Freitas & Novais 2014; Novais *et al.* 2013, 2015), and southern Mexico (Martínez-Hernández *et al.* 1994).

Holocalyx balansae is a frequent tree of the medium stratum of the Atlantic Forest (Martínez-Crovetto 1963) that provides important services to the local fauna; indeed, the natural hollows of this tree are used as nesting sites by stingless bees (Colleselli & Gómez-Olivera 2008). In this study, its importance as a nectar resource for the studied bees was recorded.

Parapiptadenia rigida, with a frequency of occurrence higher than 60% in honey samples, is a conspicuous tree in Upper Paraná Atlantic Forest (Martínez-Crovetto 1963) and intensely visited by *A. mellifera* in the northwest of Misiones (Miranda *et al.* 2010). The present results agree with findings reported for honeys of *A. mellifera* from other areas of Misiones (Aquino *et al.* 2015) and for honeys of *Scaptotrigona jujuyensis* (Basilio *et al.* 2013) from Formosa province. In addition, the botanical genus includes other species of melliferous importance, such as *P. excelsa* (Griseb.) Burkart for stingless bees (Flores & Sánchez 2010; Flores *et al.* 2015) and *A. mellifera* in northwestern Argentina (Sánchez & Lupo 2017); and *P. zehntneri* (Harms) M. P. Lima & H. Lima C., a resource visited by *A. mellifera* and stingless bees from northeastern Brazil (Santos *et al.* 2006).

The pollen characteristics observed for *Schinus weinmannifolius*-type suggest that it

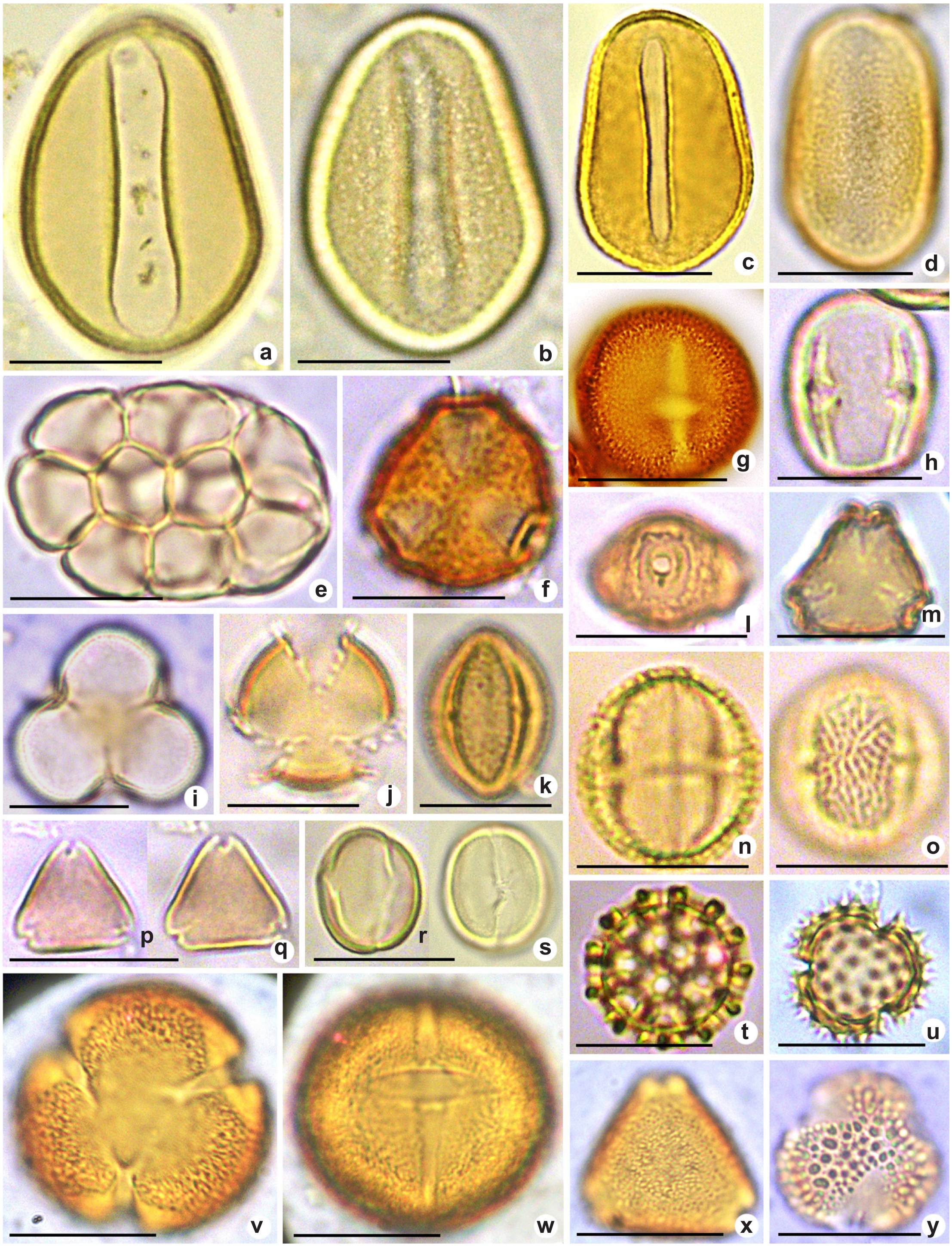

Figure 4 – a-y. Main pollen types identified in honey samples of *Apis mellifera* and *Tetragonisca fiebrigi* – a-b. *Syagrus romanzoffiana* (Arecaceae); c-d. *Euterpe edulis* (Arecaceae); e. *Parapiptadenia rigida* (Fabaceae); f. *Thinouia mucronata* (Sapindaceae). g. *Citrus* (Rutaceae). h. *Pouteria gardneriana* (Sapotaceae). i. *Actinostemon* (Euphorbiaceae); j. *Leonurus japonicus* (Lamiaceae); k. *Schinus weinmannifolius*-type (Anacardiaceae); l-m. Rhamnaceae; n-o. *Zanthoxylum* (Rutaceae); p-q. *Calyptranthes concinna*-type (Myrtaceae); r-s. *Holocalyx balansae* (Fabaceae); t. *Gomphrena perennis*-type (Amaranthaceae); u. *Baccharis*-type (Asteraceae); v-w. Euphorbiaceae; x. *Allophylus edulis* (Sapindaceae); y. *Ilex* (Aquifoliaceae). Scale bar = 20 µm.

Table 4 – Pollen types of nectariferous plants in honey samples of *Tetragonisca fiebrigi* in northern Misiones.

N°	Pollen types	IS_j	FO	N°	Pollen types	IS_j	FO
1	*Syagrus romanzoffiana*	21	79	46	Myrtaceae	0.15	8
2	*Schinus weinmannifolius*-type	16	79	47	*Alternanthera aquatica*	0.13	15
3	*Baccharis*-type	7.3	62	48	*Chrysophyllum marginatum*	0.12	21
4	*Holocalyx balansae*	6.8	74	49	*Heteropterys*	0.10	23
5	*Euterpe edulis*	5.2	23	50	*Rauvolfia sellowii*	0.10	13
6	Rhamnaceae	4.3	74	51	*Clematis campestris*-type	0.092	8
7	*Citrus*	3	51	52	*Chrysophyllum gonocarpum*	0.090	8
8	*Leonurus japonicus*	2.8	69	53	*Myrcia oblongata*-type	0.079	13
9	*Gouania latifolia*-type	2.6	5	54	*Amaranthus*	0.067	10
10	*Allophylus edulis*	2.3	54	55	*Allophylus guaraniticus*	0.062	8
11	*Gomphrena perennis*-type	2.2	23	56	*Paullinia meliifolia*	0.062	5
12	*Pouteria gardneriana*	2.1	18	57	*Cabralea canjerana*	0.059	10
13	*Parapiptadenia rigida*	2	82	58	*Castela tweediei*-type	0.059	3
14	*Zanthoxylum*	2	23	59	*Forsteronia*	0.056	13
15	*Actinostemon*	1.7	72	60	Meliaceae	0.054	10
16	*Pouteria fragrans*	1.5	44	61	*Galphimia brasiliensis*	0.051	3
17	Euphorbiaceae	1.23	79	62	*Thinouia mucronata*	0.051	5
18	Bignoniaceae	1.17	44	63	*Pereskia grandiflora*	0.049	18
19	Anacardiaceae	1.14	21	64	Urticaceae	0.046	5
20	*Bowlesia*	1.11	23	65	*Cinnamomum*-type	0.033	5
21	*Ruprechtia laxiflora*	0.99	44	66	*Cissus*	0.033	3
22	*Baulfourodendron riedelianum*-type	0.73	8	67	*Sebastiana*	0.031	3
23	*Calyptranthes concinna*-type	0.64	44	68	Lauraceae	0.028	5
24	*Urera baccifera*	0.62	26	69	*Myrsine*	0.026	5
25	*Rorippa hilariana*	0.61	26	70	Liliopsida	0.023	3
26	*Aspidosperma*	0.54	13	71	*Buddleja stachyoides*-type	0.018	3
27	*Sicyos*	0.52	8	72	Rubiaceae	0.015	8
28	*Ilex*	0.49	54	73	*Hyptis australis*-type	0.015	3
29	Fabaceae	0.47	15	74	*Mimosa cruenta*-type	0.013	3
30	*Salix*	0.44	8	75	*Elephantopus*	0.010	5
31	*Eucalyptus*	0.44	13	76	*Mimosa velloziana*-type	0.010	5
32	Asteracae	0.43	54	77	*Mandevilla angustifolia*-type	0.008	5
33	*Matayba elaeagnoides*	0.36	46	78	*Mimosa somnians*-type	0.008	5
34	*Abutilon pauciflorum*-type	0.34	36	79	*Miconia*	0.008	5
35	*Scutia buxifolia*-type	0.32	8	80	*Eupatorium*	0.008	3
36	*Serjania*	0.27	28	81	*Carica papaya*	0.008	3
37	Apiaceae	0.26	36	82	*Phyllostylon rhamnoides*	0.005	5
38	*Vernonia*	0.25	10	83	*Valeriana*	0.005	3
39	*Sideroxylon obtusifolium*	0.21	5	84	*Erythrina*	0.003	3

N°	Pollen types	IS_j	FO	N°	Pollen types	IS_j	FO
40	*Peltophorum dubium*	0.19	21	85	*Senecio*	0.003	3
41	Apocynaceae	0.18	3	86	Bromeliaceae	0.003	3
42	*Pouteria salicifolia*	0.18	5	87	*Hyptis elegans*-type	0.003	3
43	*Amaranthus muricatus*	0.17	18	88	*Myrceugenia euosma*-type	0.003	3
44	*Chamissoa acuminata*	0.15	41	89	*Polygonum*	0.003	3
45	*Trichilia catigua*-type	0.15	8				

IS_j = Index of species importance; FO = frequency of occurrence.

may correspond to *Astronium fraxinifolium* Schott, *Schinus terebinthifolius* Raddi or *S. weinmannifolius* Engl., three species present in the province of Misiones (Zanotti *et al.* 2020). Indeed, the family Anacardiaceae includes species that are essential nectar sources for bees in other areas of Argentina, such as *Schinopsis balansae* Engl., *S. lorentzii* (Griseb.) Engl. and *Schinus fasciculatus* (Griseb.) I.M. Johnst. in the Chaco province (Vossler *et al.* 2014; Salgado-Laurenti *et al.* 2017), *Astronium balansae* Engl. in Corrientes (Salgado & Pire 1998), and *Schinus areira* L. and other *Schinus* and *Schinopsis* species in northwest of Argentina (Sánchez & Lupo 2017; Reyes *et al.* 2019).

The Euphorbiaceae pollen type was found with high presence in the honeys of *A. mellifera*; those pollen types should be studied in depth to detect the plants that provide nectar, as *Actinostemon* in the honeys of both bee species. Other important Euphorbiaceae in the north of Argentina are *Cnidoscolus loasoides* (Pax) I. M. Johnst., *Croton argenteus* L., *Croton bonplandianus* Baill., *Croton lachnostachyus* Baill., *Jatropha* spp., *Sapium haematospermum* Müll. Arg. and *Sebastiania brasiliensis* Spreng. for *T. fiebrigi* (Flores & Sánchez 2010; Vossler *et al.* 2014) and *A. mellifera* (Sánchez & Lupo 2017).

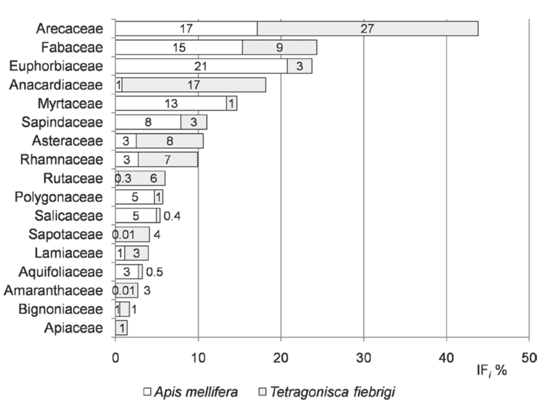

Figure 5 – Index of botanical family importance (IF_i). Families with an index equal to or higher than 1% are represented in the graph.

The *Baccharis* pollen type involves species of the genus *Baccharis*, which are abundant nectar resources in Neotropical areas (Ramalho *et al.* 1990, 1991). In the north of Argentina (Salta and Chaco provinces), *Baccharis* species are sources of nectar for the stingles bee *T. fiebrigi* and *Geotrigona argentina* Camargo & Moure (Flores & Sánchez 2010; Vossler *et al.* 2010, 2014), and for *A. mellifera* in different regions of country (Salgado & Pire 1998; Fagúndez & Caccavari 2006; Costas *et al.* 2013; Salgado-Laurenti *et al.* 2017; Sánchez & Lupo 2017). Particularly in the northwest of Misiones, *Baccharis*-type is frequent (37%) in the honeys of *A. mellifera* (Aquino *et al.* 2015) and some species of *Baccharis* (*Baccharis oxycodonta* DC., *B. tandilensis* Speg.) are visited by *A. mellifera* during the summer (Miranda *et al.* 2010).

Other species abundantly represented in this study were *Pouteria gardneriana*, *Ruprechtia laxiflora*, *Thinouia mucronata*, and pollen types of the family Myrtaceae (*Calyptranthes concinna*-type, *Myrcianthes pungens*-type); this is the first record of these species in palynological studies in Argentina. The Myrtaceae family includes important nectar species for bees, as highlighted by Ramalho *et al.* (1990).

The index of importance for *Allophylus edulis* estimated in this study was 5.4% for both bee species. This result highlights the importance of the tree as a nectar source, as reported for honeys of *T. fiebrigi* (Flores & Sánchez 2010), *Plebeia intermedia* (Flores *et al.* 2015), and *A. mellifera* from northwestern Argentina (Sánchez & Lupo 2017). However, it is not as relevant in other areas of the country, as observed in the honeys of *A. mellifera* from the Chaco province (Salgado-Laurenti *et al.* 2017).

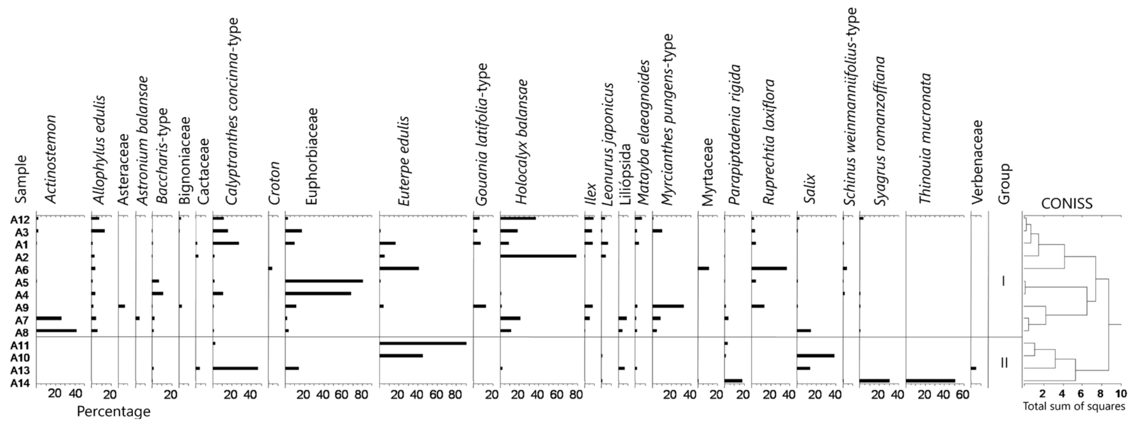

Figure 6 – Pollen diagram of honey samples of *Apis mellifera* of northern Misiones.

The index of importance of *Salix* was 5.3% for both bees, with this value being higher for the honeys of *A. mellifera* (Fig. 3). The pollen type possibly corresponds to the species *Salix humboldtiana* Willd., a tree associated with water sources (rivers, lagoons, estuaries), and documented as a frequent resource in honeys of *A. mellifera* from the Yungas (Sánchez & Lupo 2017) and the Chaco ecoregion of Argentina (Salgado-Laurenti *et al.* 2017).

The Rhamnaceae pollen type was also abundant, showing a high frequency (> 50%) in the studied honeys. The members of the family are good sources of nectar for bees. For example, *Condalia microphylla* Cav., *Discaria Americana* Gillies & Hook, *Sarcomphalus mistol* (Griseb.) Hauenshild and *Scutia buxifolia* Reissek are floral resources visited for *A. mellifera*, *G. argentina* and *T. fiebrigi* in other ecoregions of Argentina (Tamame & Naab 2003; Tellería *et al.* 2006; Vossler *et al.* 2010, 2014; Salgado-Laurenti *et al.* 2017; Reyes *et al.* 2019). Likewise, *Scutia/Condalia*-type is the dominant pollen type in honeys of *A. mellifera* in northwestern Argentina (Sánchez & Lupo 2017). In Misiones and the state of Santa Catarina, Brazil, *Hovenia dulcis* Thunb. is a key resource for *A. mellifera* (Miranda *et al.* 2018) and *T. angustula* (Freitas *et al.* 2010). In fact, in this work a unifloral honey of *Gouania latifolia*-type was found; given the observed pollinic characters, it would correspond to *Gouania latifolia* Reissek or *G. ulmifolia* Hook. & Arn., two species present

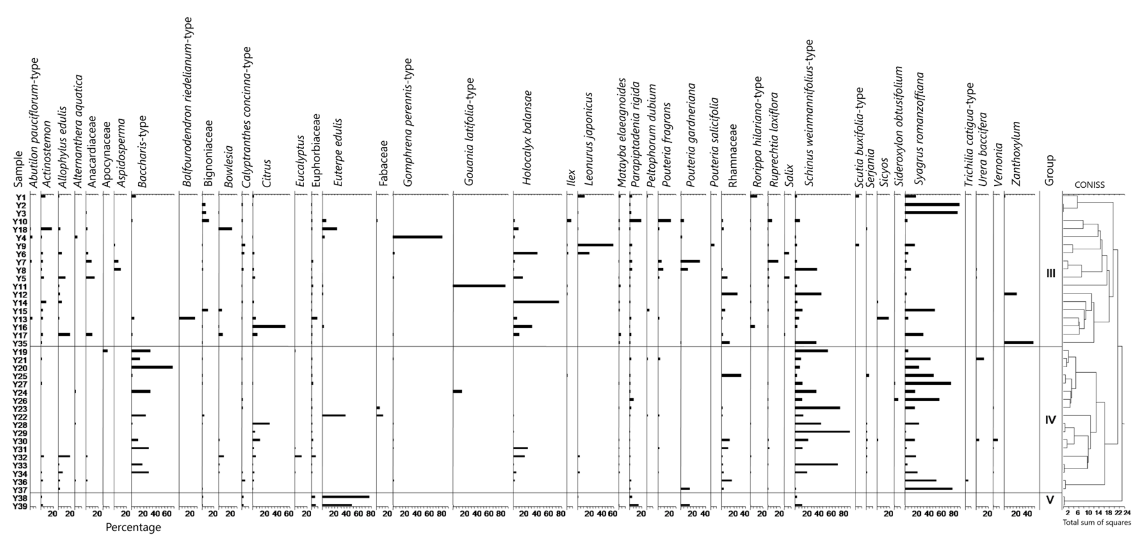

Figure 7 – Pollen diagram of honey samples of *Tetragonisca fiebrigi* of northern Misiones.

in Misiones province (Zanotti *et al.* 2020). In the centre of Misiones province, *G. ulmifolia* was found in honeys of *T. fiebrigi* (Miranda *et al.* 2018) and in the forests of Brazil, *Gouania* cf. *latifolia* is considered an attractive species for *A. mellifera* (Polatto *et al.* 2014), as *Gouania lupuloides* (L.) Urb. in Chiapas, Mexico for *T. angustula* (Martínez-Hernández *et al.* 1994).

Among herbs, *Leonurus japonicus* had a high presence in the studied honeys. Individuals of this species grow scattered in disturbed sectors and their fragrant flowers are used by *A. mellifera* in the northwest of Misiones between February and April (Miranda *et al.* 2010). Studies of honeys from north Argentina reported this species as a minor resource for *T. fiebrigi* (Flores & Sánchez 2010) and for *A. mellifera* (Salgado-Laurenti *et al.* 2017).

The *Citrus* pollen type represents wild and cultivated species present in farms (Stampella *et al.* 2013), which are sources of nectar and pollen for bees, as confirmed for different regions (Ramalho *et al.* 1990, 1991; Martínez-Hernández *et al* 1994; Flores & Sánchez 2010; Miranda *et al.* 2010; Novais *et al.* 2013; Aquino *et al.* 2015; Flores *et al.* 2015; Salgado-Laurenti *et al.* 2017; Sánchez & Lupo 2017; Reyes *et al.* 2019). Another abundant Rutaceae genus in the honeys was *Zanthoxylum*, which was also be observed in the honeys of *A. mellifera* (Sánchez & Lupo 2017) and *Plebeia intermedia* (Flores *et al.* 2015) from northwestern Argentina, and in corbicular and stored pollen of *Tetragonisca angustula* from Bolivia (Saravia-Nava *et al.* 2018). In Argentina, the genus includes botanical species that are frequent sources of nectar for *A. mellifera*, such as *Zanthoxylum coco* Gillies *ex* Hook. F. & Arn. in Yungas and Chaco ecoregions (Costas *et al.* 2013; Sánchez & Lupo 2017), *Z. rhoifolium* Lam. in the Chaco ecoregion (Salgado-Laurenti *et al.* 2017), and *Z. petiolare* A.St.-Hil. & Tul. in the Atlantic Forest (Miranda *et al.* 2010). A particular case was that of *Balfourodendron riedelianum* (Engl.) Engl., which was only found in *T. fiebrigi* honeys (with 8% frequency of occurrence), but is considered a tree intensely visited by *A. mellifera* (Miranda *et al.* 2010).

Another important crop for local people, such as *Ilex paraguariensis* A. St.-Hil., is possibly also important for the studied bees. The presence of the pollen type *Ilex* and an index of importance of 2.8% in honeys of *A. mellifera* support our assumption and the potential nectar contribution of the species. In previous studies, Aquino *et al.* (2015) found similar results in honeys of *A. mellifera* from Misiones and a frequency of occurrence of 74%.

This study presents the first case of a unifloral honey of *Gomphrena perennis*-type, which includes pollens of species present in Misiones, such as *Gomphrena celosioides* Mart., *G. elegans* Mart., *G. graminea* Moq., *Hebanthe eriantha* (Poir.) Pedersen, *Pfaffia glomerata* (Spreng.) Pedersen, *P. gnaphaloides* (LF) Mart. and *P. tuberosa* (Spreng.) Hicken (Zanotti *et al.* 2020). Likewise, in Brazil the predominance of *Gomphrena demissa* has been reported in a honey sample of *T. angustula* (Novais *et al.* 2015). However, it is necessary to investigate the contamination of nectar or operculated honey by pollen, since many members of the Amaranthaceae family have anemophilous pollination (Recio *et al.* 1998).

The pollen types of wind-pollinated or nectarless plants found in the samples belong to species that provide pollen to bees (Barth 1989; Silva *et al.* 2014) and their presence in nectar or honey probably is associated with the secondary and quaternary enrichment proposed by Persano-Oddo *et al.* (2007). Likewise, several of them were registered in melisopalinological studies carried out in other localities in Misiones (Fernández *et al.* 2015) and Brazil (Barth 1989; Novais *et al.* 2013, 2015; Freitas & Novais 2014).

In this study, we have observed that bees use nectar resources, belonging to wild and cultivated plants. On the other hand, it was possible to establish an association of the pollen types observed depending on the temporal origin of the honey sample. Associations that will allow us to predict the pollen content of samples in a future. For example, in the samples of both bees *Holocalyx balansae* was a frequent pollen type in the period studied and abundant in those samples collected in the spring season, accompanied by the pollen types *Actinostemon*, *Euterpe edulis*, *Leonurus japonicus*, *Matayba elaeagnoides* and *Gouania latifolia*-type. Additionally, other pollen indicators in that period were *Calyptranthes concinna*-type, Euphorbiaceae, *Ilex*, *Myrcianthes pungens*-type, *Salix* and *Ruprechtia laxiflora* in the samples of *A. mellifera* and *Allophylus edulis*, *Aspidosperma*, *Citrus*, *Parapiptadenia rigida*, *Pouteria fragrans*, *P. gardneriana*, Rhamanceae and *Zanthoxylum* in the samples of *T. fiebrigi.*

Likewise, *Syagrus romanzoffiana*, *Schinus weinmannifolius*-type and *Baccharis*-type make up an abundant group associated with the summer period, despite being found to a lesser extent and frequently in other samples of this study. Linked to them, it was possible to observe the presence of *Salix* and *Thinouia mucronata*, as well as other pollen types reported for the spring season (*A. edulis*, *C. concinna*-type, *Citrus*, *E. edulis*, Euphorbiaceae, *P. rigida* and Rhamnaceae). Lastly, *Gomphrena perennis*-type was an abundant pollen type in the winter *T. fiebrigi* samples, accompanied by *Actinostemon*, *Bowlesia*, *P. rigida*, and *Pouteria fragrans*.

The list of recorded nectariferous plants is a substantial contribution for the management of apiaries and meliponaries, allowing the local producer to select which plants to grow as well as to conserve vegetation patches containing these resources.

Acknowledgements

We thank CONICET, for the Post-Graduate Scholarship granted to Fabio Flores; to the CIES, of National Parks Administration, for allowing the researchers to working their premises in Iguazú at the beginning of these studies; to the Yacutinga Private Reserve, which partially funded this work and provided logistic support in honey collection; to the Rufford Foundation, for financial support to F. Zamudio. A special thanks to the residents of the north of Misiones who kindly provided the honey samples; and the reviewers who contributed to improving the work.

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Area Editor: Dr. Nicolay Cunha
Received in May 22, 2020. Accepted in September 04, 2020.