Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: http://www.tandfonline.com/loi/sgra20

Botanical and geographical origin of honey from the dry and humid Chaco ecoregions (Argentina)

Cristina R. Salgado-Laurenti, Maria Cristina Tellería & Juan Manuel Coronel

To cite this article: Cristina R. Salgado-Laurenti, Maria Cristina Tellería & Juan Manuel Coronel (2017): Botanical and geographical origin of honey from the dry and humid Chaco ecoregions (Argentina), Grana, DOI: 10.1080/00173134.2016.1276619

To link to this article: http://dx.doi.org/10.1080/00173134.2016.1276619

Published online: 04 May 2017.

Grana, 2017
https://doi.org/10.1080/00173134.2016.1276619

Taylor & Francis
Taylor & Francis Group

Botanical and geographical origin of honey from the dry and humid Chaco ecoregions (Argentina)

CRISTINA R. SALGADO-LAURENTI[1], MARIA CRISTINA TELLERÍA[2] & JUAN MANUEL CORONEL[3]

[1]*Departamento de Ciencias Básicas Agronómicas, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina,* [2]*Laboratorio de Sistemática y Biología Evolutiva (LASBE), Museo de La Plata, Argentina,* [3]*Departamento de Biología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina*

Abstract
To characterise the botanical and geographical origin of honey from the Chaco region (Argentina), 189 samples of honey were examined for pollen content using standard methodology. A general feature was the high representation of pollen from native arboreal plants, mainly of Fabaceae and the scarcity of pollen from herbaceous plants. One hundred and twenty-three samples proved to be unifloral. The most common were from: *Prosopis alba*, *Helianthus annuus*, *Sarcomphalus mistol*, *Eugenia uniflora* and *Schinopsis balansae* followed by those of *Copernicia alba*, *Trithrinax schizophylla*, *Tessaria integrifolia*, *Baccharis-Eupatorium*, *Cynophalla retusa*, *Schinopsis lorentzii*, *Gleditsia amorphoides*, *Heimia salicifolia*, *Pisonia zapallo*, *Sagittaria montevidensis* and *Bulnesia sarmientoi*. The pollen spectrum allowed the Dry and Humid Chaco ecoregions to be distinguished. The presence of pollen from cultivated crops and naturalised plants reflects a transitional complex between both ecoregions.

Keywords: *honey, pollen,* Apis mellifera*, Chaco region, native flora*

Argentina, which produces some 60 000 t of honey a year, is ranked as one of the most prominent honey exporters worldwide. The province of Chaco contributes 800–850 t per year, from which only a small fraction of the entire production is harvested under organic certification protocol. The administrative province of Chaco is located between 24° 00′ S, 58° 00′ W and 28° 00′ S, 63° 00′ W, and belongs to the phytogeographic Chaco region (Figure 1; Pennington et al. 2000). This is one of the few areas in the world where the transition between the Tropics and the temperate belt does not occur in the form of a desert but rather as a semi-arid forest and woodlands. In this region, both the richness of the vegetation and the adaptability of honeybees *Apis mellifera* var. *mellifera* L. to the climatic conditions are favourable for apiculture. In addition, flowering of native vegetation offers nectar and pollen almost all the year round (Salgado et al. 2014; Salgado 2016).

In contrast to the decrease in biodiversity due to the increasing area under soybean production in Argentina, apiculture is an environment-friendly activity. Through pollinator activity, honeybees play an important role in maintaining ecosystems and conserving biodiversity and at the same time provide employment to local people. In the province of Chaco, apiculture is mainly practised by descendants of immigrants, but in the north and central region, members of the Wichi and Toba Native People also practise this activity for extra income. The floral diversity of the region allows many different types of honey to be produced, with a multitude of colours, tastes and aromas. Thus, there is interest in establishing the pollen content of the honeys produced in the Chaco region and in classifying them to obtain better prices in the international market.

There are only a few studies on honey and honeybee plants in the Chaco region. Salgado et al. (2014)

Correspondence: Cristina R. Salgado, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE), Sgto. Cabral 2131, Corrientes 3400, Argentina. E-mail: *crsalgado2009@gmail.com*

(Received 15 July 2016; accepted 21 November 2016)

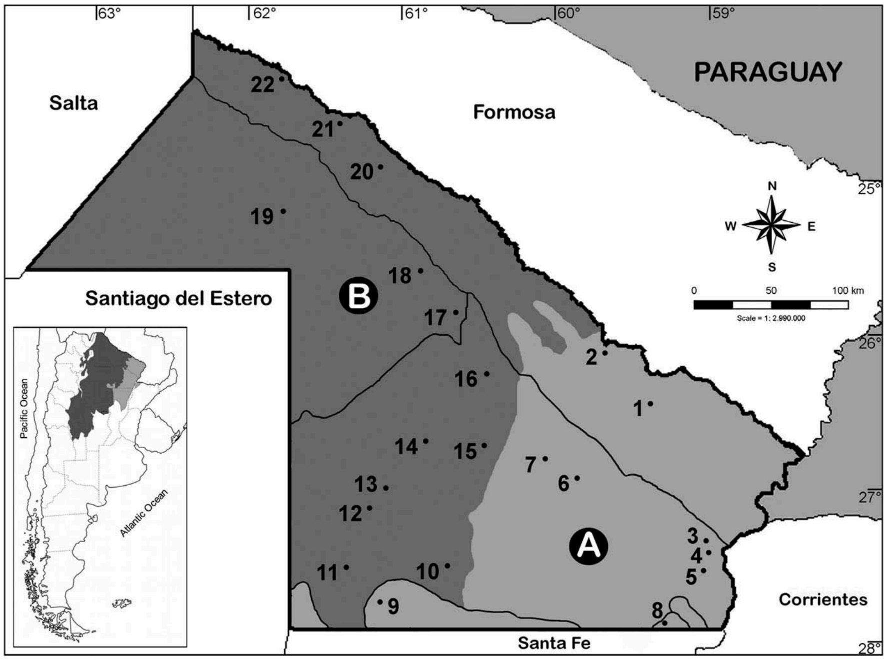

Figure 1. Study area and location of apiaries sampled. A, Humid Chaco, B, Dry Chaco. Apiary locations: 1, General San Martín; 2, Presidencia General Roca; 3, Resistencia; 4, Barranqueras; 5, Los Palmares; 6, Presidencia de la Plaza; 7, Machagai; 8, Basail; 9, Santa Sylvina; 10, Hermoso Campo; 11, Villa Ángela; 12, Charata; 13, Las Breñas; 14, Campo Largo; 15, Presidencia Roque Sáenz Peña; 16, Tres Isletas; 17, Juan José Castelli; 18, Miraflores; 19, Fuerte Esperanza; 20, Interfluvio; 21, El Sauzalito and 22, Tartagal. Apiaries 10–16 are placed in Subhumid Chaco (transition area within the Dry Chaco).

studied the flowering phenology in connection with apiarian activity, Salgado and Maidana (2014) studied the physical-chemical features of honeys and Salgado (2016) investigated the nectar resources and the quality of honey. The aim of this study was to characterise botanical and geographical origin of honeys from the Dry and Humid Chaco ecoregions through the identification and quantification of the pollen contained in the honeys.

Material and methods

Study area

The vegetation of Chaco characterises a vast plain of northern and central Argentina, southern Bolivia, western and central Paraguay and central and northwestern Brazil (Figure 1; Pennington et al. 2000). It encompasses a region of more than 800 000 km^2 covered by dry forest. The rainfall declines from the east to west, with a dry season in the winter and spring and a rainy season in the summer. The vegetation in this region is subjected to low soil moisture and freezing in the dry season and water logging and extremely high air temperature during part of the rainy season – 4.4 °C to 44.8 °C (INTA 2016).

The vegetation of Chaco is characterised by a closed canopy dominated by Fabaceae, Anacardiaceae, Capparidaceae and Bignoniaceae, amongst others, and a sparse ground flora with few grasses (Figure 2). A variety of aquatic plants may be found in rivers and seasonal swamps. According to the most recent ecological study (Morello et al. 2012), the Chaco region is divided into two ecoregions: Dry and Humid (Figure 1). In the Humid Chaco rain is abundant, *c.* 1200 mm per year whereas in the Dry Chaco, rain rarely exceeds 500 mm per year. *Aspidosperma quebracho-blanco* and *Schinopsis* sp., amongst other trees, are grown in both ecoregions; *Schinopsis lorentzii* is emblematic of the Dry Chaco and *Schinopsis balansae* of the Humid Chaco. On the basis of a combination of physiognomies or landscapes, Morello et al. (2012) recognised eight different complexes of ecosystems in the area; in the context of the present study, only the Sub-humid Central Chaco complex will be considered. Although this complex is located in the Dry Chaco, it represents a transition area between Dry and Humid ecoregions where some native species from both ecoregions grow together. This area is also characterised by its intense agricultural activity with sunflower and soybean the most important crops.

A total of 189 honey samples obtained by centrifuging were taken from 20 different apiaries (Figure 1) during the years 2005–2008; 65 samples were obtained from the Dry Chaco, 64 from the Subhumid Central Chaco complex and 60 from the Humid Chaco (Table I). Pollen grains were identified by comparing them with a reference pollen collection obtained from plants collected during the two principal flowering periods for apiculture in Chaco: September–December and February–late March (Salgado et al. 2014). This reference collection contained pollen from 282 species belonging to 65 angiosperm families. Pollen collected from both plant and honey samples were acetolysed (Erdtman 1960), mounted in glycerine–gelatine and sealed with paraffin. Specimens collected were deposited in the

Figure 2. Apiary in the Dry Chaco. Note the bare soil surface.

Herbarium of the Instituto de Botánica del Nordeste, Corrientes, Argentina (CTES), and the pollen slides in the palynotheca of the Universidad Nacional del Nordeste, Corrientes, Argentina (UNNE; PAL-CTES). A Leitz Diaplan CME microscope was used for identification and quantification of pollen grains.

For scanning electron microscopy (SEM), acetolysed pollen grains were suspended in 90% ethanol, then mounted on stubs and examined with a Jeol JSM 5800 LV at the Secretaría General de Ciencia y Tecnología – Universidad Nacional del Nordeste, Corrientes, Argentina (SGCyT-UNNE). Qualitative analysis of honey samples was carried out according to Louveaux et al. (1978). The frequency classes of pollen grains were given as dominant pollen (> 45%), secondary pollen (15–45%) and important minor pollen (3–15%). Honeys were considered unifloral if the representation of one taxon was > 45%. The minimal number of pollen grains counted in honey samples was established according to Vergeron (1964); for this, counts were continued up to percentage stabilisation. Three hundred pollen grains were the minimum number that contained the pollen species representative of the honey samples and 700 the maximum.

Data analysis

A cluster analysis (CA) was performed to determine the possibility of group formation on the basis of pollen content of honey samples and, principal component analysis (PCA) was conducted to explain the variability among data sets and to analyse the relationships among samples. Hierarchical cluster analysis was conducted using Euclidean distance according to Ward's methods. The programme STATISTICA (StatSoft 1999) was used.

Results

Qualitative analysis

The honey samples studied contained pollen from 120 different taxa, belonging to 47 families. Eighty-

Table I. Apiary numbers in the ecoregions of Chaco and identifier numbers of the analysed samples.

Ecoregion	References of apiaries	Number of samples
Humid Chaco	1. General San Martín	2–11, 44, 141–146
	2. Presidencia General Roca	126–140
	3. Resistencia	12, 13, 46
	4. Barranqueras	3
	5. Los Palmares	177–179
	6. Presidencia de la Plaza	147–164
	7. Machagai	165–167
	8. Basail	43
	9. Santa Sylvina	34
Sub-humid Central Chaco complex (within Dry Chaco)	10. Hermoso Campo	1, 30–33, 35, 36, 38, 39, 40
	11. Villa Ángela	173–176
	12. Charata	4, 17–24, 50
	13. Las Breñas	25–29, 47
	14. Campo Largo	48, 74, 75, 181–184
	15. Presidencia Roque Sáenz Peña	69, 70–73, 102, 180
	16. Tres Isletas	99–104
	17. Juan José Castelli	41, 54,55, 105–113, 169–172
Dry Chaco	18. Miraflores	45, 51, 52, 65, 66, 78, 84, 87, 90, 103
	19. Fuerte Esperanza	15, 16, 42, 37,76–81,185
	20. Interfluvio	49, 53, 56–64, 82–89, 91–98, 114–125, 168, 186–189
	21. El Sauzalito	14
	22. Tartagal	67, 68, 88

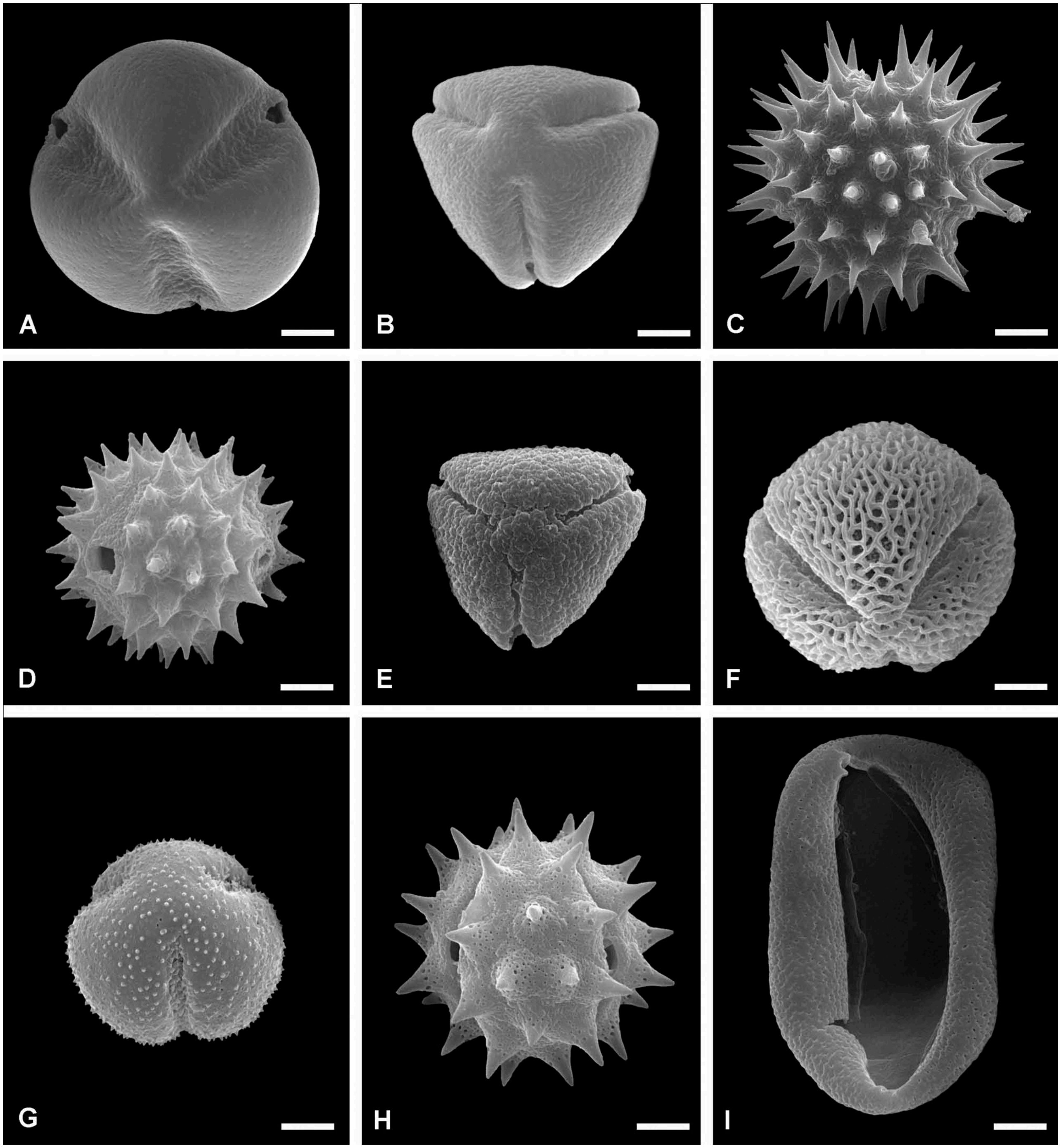

Figure 3. SEM photographs of some dominant pollen types. **A.** *Prosopis alba*. **B.** *Sarcomphalus mistol*. **C.** *Helianthus annuus*. **D.** *Baccharis-Eupatorium*-type. **E.** *Eugenia uniflora*. **F.** *Schinopsis balansae*. **G.** *Cynophalla retusa*. **H.** *Tessaria integrifolia*. **I.** *Trithrinax campestris*. Scale bars – 5 μm.

five were identified at species level, 28 at genus level, six at family level and two were assigned as 'types' (Table II, see also Online Supplementary Material, OSM). These types included regional taxa indistinguishable on pollen features such as the *Baccharis-Eupatorium-* and *Croton-Jatropha*-types. Eighteen different types of pollen were present in more than 20% of samples, 20 in 10–20% and 83 in less than 10% of total pollen. Native species, particularly species of Fabaceae, the family most represented in honey samples (more than 50%), were intensely used (Table II). This family has an important diversity of species, some of which belong to the subfamilies Mimosoideae (*Acacia aroma*, *Acacia bonariensis*, *Acacia caven*, *Acacia curvifructa*, *Acacia praecox*, *Albizia inundata*, *Enterolobium contortisiliquum*, *Inga uruguen-*

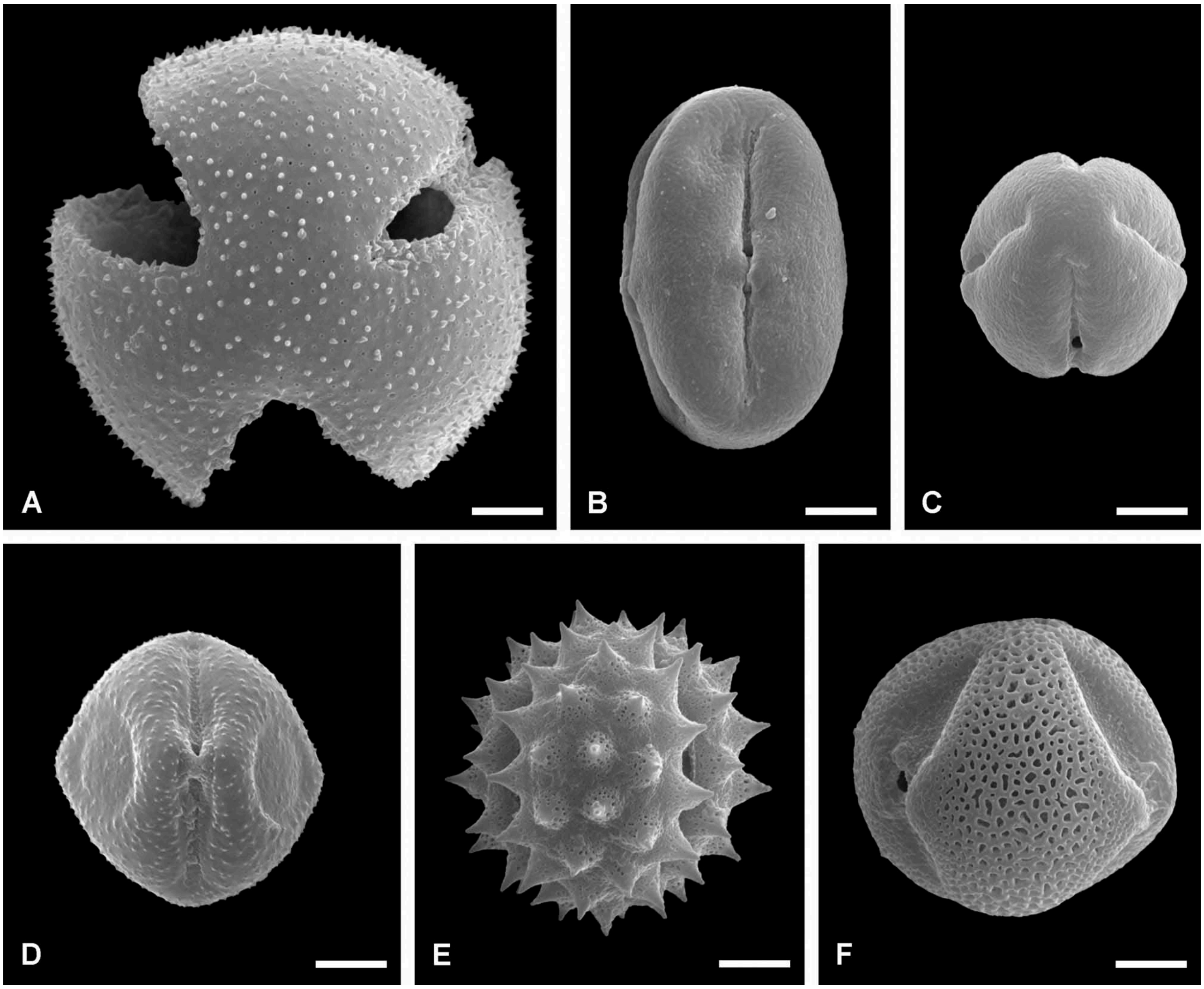

Figure 4. SEM photographs of some secondary pollen types. **A.** *Pisonia zapallo*. **B, C.** *Sideroxylon obtusifolium*. **D.** *Acicarpha tribuloides*. **E.** *Senecio grisebachii*. **F.** *Gleditsia amorphoides*. Scale bars – 5 µm.

sis, *Mimosa pigra*, *Mimozyganthus* sp., *Prosopis alba*, *Prosopis kuntzei*, *Prosopis nigra* and *Prosopis ruscifolia*) followed by Papilionoideae (*Erythrina crista-galli*, *Geoffroea decorticans*, *Glycine max*, *Lotus* sp., *Medicago lupulina*, *Medicago sativa*, *Melilotus albus*, *Trifolium polymorphum* and *Vicia macrograminea*) and Caesalpinioideae (*Bahuinia forficata*, *Caesalpinia paraguariensis*, *Cercidium praecox*, *Gleditsia amorphoides*, *Parkinsonia aculeata*, *Peltophorum dubium* and *Senna* sp.). Out of 189 honey samples analysed, 123 were found to be unifloral and 66 multifloral. The dominant types (> 45% of total pollen) that characterised the unifloral honey were: *Bulnesia sarmientoi*, *Cynophalla retusa*, *Copernicia alba*, *Eugenia uniflora*, *Gleditsia amorphoides*, *Heimia salicifolia*, *Helianthus annuus*, *Pisonia zapallo*, *Prosopis alba*, *Sagittaria montevidensis*, *Schinopsis balansae*, *Schinopsis lorentzii*, *Tessaria integrifolia*, *Baccharis-Eupatorium*-type, *Trithrinax schizophylla* and *Sarcomphalus mistol* (Figure 3, Table II). Secondary pollen types were mainly represented by Alismataceae (*Echinodorus grandiflorus*), Amaranthaceae (*Alternanthera* sp.), Apiaceae (*Ammi majus*), Asteraceae (*Senecio grisebachii*), Brassicaceae (*Brassica* sp.), Capparidaceae (*Sarcotoxicum salicifolium*), Calyceraceae (*Acicarpha tribuloides*), Euphorbiaceae (*Croton-Jatropha*-type, *Ricinus communis* and *Sapium haematospermum*), Fabaceae (*Acacia praecox*, *Glycine max*, *Medicago lupulina*, *Melilotus albus* and *Mimosa pigra*), Myrtaceae (*Eucalyptus* sp.), Salicaceae (*Salix humboldtiana*), Sapotaceae (*Sideroxylum obtusifolium*) (Figure 4, Table II). The contribution of pollen types from the remaining families was variable among samples and many of them were present as minor pollen types (3–15%) or traces (< 3%) (Table II). Pollen types included in both categories belonged to Achatocarpaceae, Alismataceae, Amaranthaceae, Anacardiaceae, Apiaceae, Asteraceae,

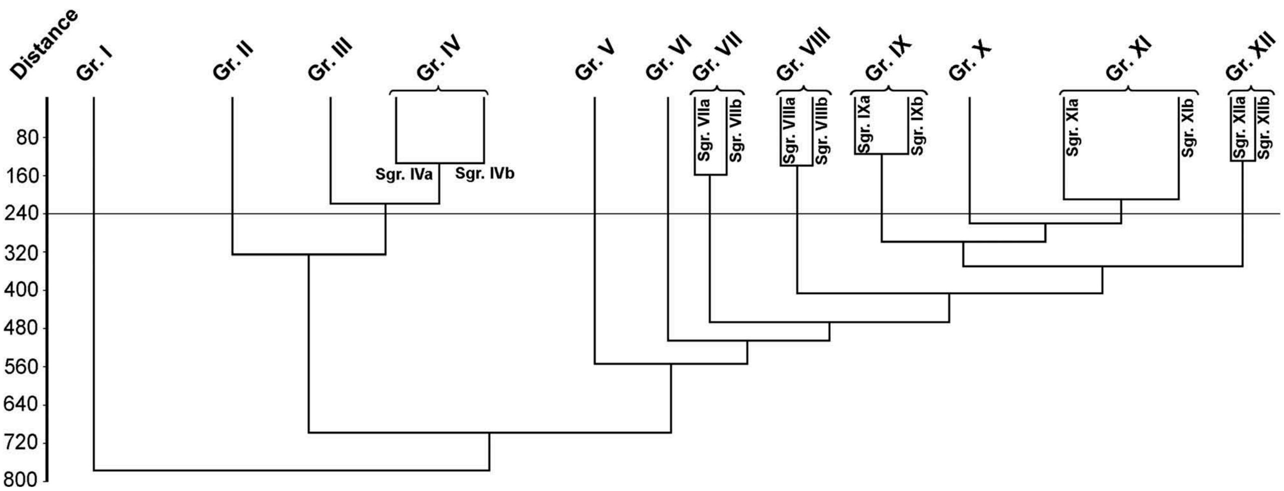

Figure 5. Abbreviated dendrogram showing 12 groups and 12 subgroups of 189 samples of honey using Euclidean distance and Ward's methods.

Boraginaceae, Brassicaceae, Cactaceae, Cannabaceae, Capparidaceae, Casuarinaceae, Celastraceae, Chenopodiaceae-Amaranthaceae (Chen-Am), Commelinaceae, Cyperaceae, Fabaceae, Lamiaceae, Lauraceae, Malpighiaceae, Malvaceae, Menyanthaceae, Nyctaginaceae, Nymphaeaceae, Onagraceae, Polygonaceae, Pontederiaceae, Portulacaceae, Rhamnaceae, Ranunculaceae, Rubiaceae, Rutaceae, Salicaceae, Sapindaceae, Solanaceae, Ulmaceae and Verbenaceae (Table II, OSM).

Results of CA and PCA

Twelve groups of honey were resolved in the CA at the Euclidean distance of 240: eight formed by unifloral honey and four by multifloral honey, but with a particular botanical association (Figure 5; see details of pollen content by sample in OSM). Within unifloral honeys, Group I was characterised by 23 honey samples of *Helianthus annuus* (No. 17–22, 24–26, 36, 44, 70–73, 173–176, 180–182, 184); Group II by 15 honey samples of *Sarcomphalus mistol* (No. 59, 61–68, 78–81, 96, 113); Group IV by 28 honey samples of *Prosopis alba* separated into two subgroups: one including nine honeys with *Sarcomphalus mistol* as secondary pollen (No. 76, 77, 105, 106, 110, 116–118, 124) and the other including 19 samples without secondary pollen (No. 7, 8, 14, 15, 32, 53, 54, 74, 75, 84, 103, 107, 108, 112, 119, 123, 125, 170, 185); Group V by 15 honey samples of *Eugenia uniflora* (No. 102, 137, 141, 145, 146, 149, 150, 155, 157, 159, 160, 162–165); Group VI by six honey samples of *Trithrinax schizophylla* (No. 45, 52, 82, 86, 87, 90); Group VII by two subgroups: subgroup VIIa, including two honeys of *Copernicia alba* (No. 177, 179), and subgroup VIIb, comprising 11 honeys of *Schinopsis balansae* (No. 6, 9, 12, 13, 27–29, 38, 40, 50, 69); Group VIII by two subgroups: subgroup VIIIa, including five honeys of *Tessaria integrifolia* (No. 46, 58, 186, 188, 189), and subgroup VIIIb, including five samples of the *Baccharis-Eupatorium*-type (No. 30, 41, 56, 91, 92); and Group XII also formed by two subgroups: subgroup XIIa, with three honeys of *Schinopsis lorentzii* (No. 51, 55, 95), and subgroup XIIb, with four honeys of *Cynophalla retusa* (No. 83, 88, 98, 120).

Multifloral honeys were distributed into four groups; the most significant pollen types that helped to define these groups was the secondary pollen. Group III comprised 12 honeys with *Prosopis alba* and *Pisonia zapallo* as secondary pollen (No. 16, 42, 57, 60, 97, 109, 111, 114, 115, 121, 122, 167); Group IX comprised two subgroups that differed in their secondary pollen: subgroup IXa, with seven honeys with *Gleditsia amorphoides* (No. 2, 10, 11, 136, 144, 152, 153) and one with dominant pollen of *Gleditsia amorphoides* (No. 3), and subgroup IXb, with nine honeys with *Eugenia uniflora* (No. 138, 139, 142, 143, 147, 148, 151, 161, 166). Group X comprised 13 honeys with *Prosopis alba* and *Cynophalla retusa* as secondary pollen (No. 31, 37, 85, 89, 93, 94, 99, 100, 169, 168, 171, 172); within this group, a single unifloral honey of *Bulnesia sarmientoi* (No. 187) was distinguished. Group XI had two subgroups: subgroup XIa, with *Schinopsis balansae* as secondary pollen in 17 samples (No. 4, 5, 33–35, 39, 47, 129–135, 140, 154, 156, 178) and a single unifloral sample of *Heimia salicifolia* (No. 158); and subgroup XIb, with honeys with *Helianthus annuus* as secondary pollen and *Brassica* sp. as minor and trace pollen; these subgroups also

Table II. Pollen types and their frequency classes in the 189 analysed honey samples.

Family	Pollen type	D	S	M	T	FO
ACHATOCARPACEAE	*Achatocarpus praecox* Griseb.			4	19	12.2
ALISMATACEAE	*Echinodorus grandiflorus* (Cham. et Schltdl.) Micheli		2	9	4	7.9
	Hydrocleys nymphoides (Willd.) Buchenau			1	1	1.1
	Sagittaria montevidensis **Cham. et Schltdl.**	1	2	5	15	12.2
AMARANTHACEAE	*Amaranthus* sp.				1	0.5
	Alternanthera sp.		1		4	2.6
	Gomphrena sp.				2	1.1
	Iresine difussa Humb. et Bonpl. ex Willd.				2	1.1
ANACARDIACEAE	***Schinopsis balansae*** Engl.	11	15	20	15	29.1
	Schinopsis lorentzii (Griseb.) Engl. +	3	4	6	4	9.0
	Schinus sp.			1	4	2.6
APIACEAE	*Ammi majus* L. *		1	6	13	10.6
	Eryngium sp.			4	9	6.9
ARECACEAE	***Copernicia alba*** Morong	2	4	9	8	12.7
	Trithrinax schizophylla Drude +	6	1	7	2	8.5
ASTERACEAE	***Baccharis-Eupatorium*-type**	5	3	25	35	36.0
	Helianthus annuus L.	23	13	22	7	34.4
	Holocheilus hieracioides (D.Don) Cabrera			6	19	13.2
	Mikania sp.				2	1.1
	Senecio grisebachii Baker		3	26	16	23.8
	Solidago chilensis Meyen				1	0.5
	Tagetes minuta L.				2	1.1
	Taraxacum sp.			2	5	3.7
	Tessaria integrifolia Ruiz et Pav.	5	5	8	8	13.8
	Vernonia chamaedrys Less.			5	9	7.4
BORAGINACEAE	*Cordia americana* (L.) Gottschling et J.S.Mill.			1	2	1.6
	Heliotropium sp.			2	1	1.6
BRASSICACEAE	*Brassica* sp. *			1	13	15.3
	Rapistrum rugosum (L.) All. *				3	1.6
	Sinapis arvensis L.			1	1	1.1
CACTACEAE	*Cereus* sp.				4	2.1
CALYCERACEAE	*Acicarpha tribuloides* Juss.		4	27	16	24.9
CANNABACEAE	*Trema micrantha* (L.) Blume				2	1.1
	Celtis sp.			13	32	23.8
CAPPARIDACEAE	*Anisocapparis speciosa* (Griseb.) Cornejo et Iltis			6	4	5.3
	Capparicordis tweediana (Eichler) Iltis et Cornejo			7	5	6.3
	Cynophalla retusa (Griseb.) Cornejo et Iltis	4	8	16	9	19.6
	Sarcotoxicum salicifolium (Griseb.) Cornejo et Iltis +		1	13	13	14.3
CASUARINACEAE	*Casuarina cunninghamiana* Miq. *			1	1	1.1
CELASTRACEAE	*Maytenus spinosa* (Griseb.) Lourteig et O'Donell +				3	1.6
	Maytenus vitis-idaea Griseb.			12	5	9.0
CHENOP.-AMAR.				5	13	9.5
COMMELINACEAE	*Commelina erecta* L.			2	2	2.1
CUCURBITACEAE	*Cucurbita* sp.				2	1.1
CYPERACEAE					4	2.1
EUPHORBIACEAE	*Croton-Jatropha*-type		1	5	16	11.6
	Ricinus communis L. *		1	3		2.1
	Sapium haematospermum Müll. Arg.			1	53	48.1
FABACEAE	*Acacia aroma* Hook. et Arn.			15	59	39,2
	Acacia bonariensis Hook. et Arn.			1	19	10.6
	Acacia caven (Molina) Molina				2	1.1
	Acacia curvifructa Burkhart +			1	6	3.7
	Acacia praecox Griseb.		2	10	12	12.7
	Albizia inundata (Mart.) Barneby et J.W.Grimes			5	27	16.9
	Bahuinia forficata Link			3	6	4.7
	Caesalpinia paraguariensis (D.Parodi) Burkart				4	2.1
	Cercidium praecox (Ruiz et Pav. ex Hook.) Harms +			8	5	6.9
	Enterolobium contortisiliquum (Vell.) Morong				4	2.1
	Erythrina crista-galli L.			1	2	1.6
	Geoffroea decorticans (Gillies ex Hook. et Arn.) Burkart				2	1.1
	Gleditsia amorphoides (Griseb.) Taub.	1	10	23	6	21.2

(*Continued*)

Table II. (*Continued*).

Family	Pollen type	D	S	M	T	FO
	Glycine max (L.) Merr. *		2	3		2.6
	Inga uruguensis Hook. et Arn.			1	1	1.1
	Lotus sp. *				3	1.6
	Medicago sativa L. *			9	9	9.5
	Medicago lupulina L.		3	12	10	13.2
	Mimosa pigra L.		2	4	8	7,4
	Melilotus albus Medik. *		4	21	8	17.5
	Mimozyganthus sp.			3	6	4,8
	Parkinsonia aculeata L.			6	3	4.8
	Peltophorum dubium (Spreng.) Taub.				1	0.5
	Prosopis alba Griseb.	28	29	61	19	70.4
	Prosopis kuntzei Harms ex Kuntze				3	1.6
	Prosopis nigra (Griseb.) Hieron.	1	2	14	17	18.0
	Prosopis ruscifolia Griseb.			3	3	3.2
	Senna sp.			1	3	2.1
	Trifolium polymorphum Poir.				2	1.1
	Vicia macrograminea Burkart			18	16	18.0
LAMIACEAE	*Hyptis lappacea* Benth.				4	2.1
	Leonurus sibiricus L.			4	7	5.8
	Mentha pulegium L. *				2	1.1
	Salvia cardiophylla Benth.				2	1.1
LAURACEAE	*Persea americana* Mill. *				2	1.1
LYTHRACEAE	***Heimia salicifolia*** Link	1	2	26	17	24.3
MALPIGHIACEAE	*Mascagnia* sp.				7	3.7
MALVACEAE	*Abutilum* sp.			1	4	2.6
	Sphaeralcea bonariensis (Cav.) Griseb.			5	15	10.6
	Wissadula densiflora R.E.Fr.				1	0.5
MENYANTHACEAE	*Nymphoides indica* (L.) Kuntze			1	3	2.1
MYRTACEAE	*Eucalyptus* sp. *		3	3		3.2
	Eugenia uniflora L.	15	8	23	4	27.5
NYCTAGINACEAE	*Bougainvillea campanulata* Heimerl				4	2.1
	Pisonia zapallo Griseb.	1	12	23	29	36.0
NYMPHAEACEAE	*Nymphaea* sp.				3	1.6
ONAGRACEAE	*Ludwigia* sp.				5	2.6
	Oenothera sp.				5	2.6
PINACEAE	*Pinus* sp. *				1	0.5
POACEAE					37	19.6
POLYGONACEAE	*Polygonum* sp.			4	10	7.4
PONTEDERIACEAE	*Eichhornia crassipes* (Mart.) Solms			3	7	5.3
	Pontederia cordata L.			6	9	7.9
PORTULACACEAE	*Portulaca* sp.				4	2.1
RHAMNACEAE	*Scutia buxifolia* Reissek			1	2	1.6
	Sarcomphalus mistol (Griseb.) Hauenshild +	15	13	25	13	35.4
RANUNCULACEAE	*Clematis* sp.			5	12	9.0
RUBIACEAE					6	3.2
	Borreria verticillata (L.) G.Mey				1	0.5
	Richardia brasiliensis Gomes				2	1.1
RUTACEAE	*Citrus* sp.*				9	4.8
	Zanthoxylum rhoifolium Lam.				1	0.5
SALICACEAE	*Salix humboldtiana* Willd.		1		4	2.6
	Xylosma venosa N.E.Br.				1	0.5
SAPINDACEAE				2	16	9.5
SAPOTACEAE	*Sideroxylon obtusifolium* (Roem. et Schult.) T.D.Penn.		2	20	35	30.2
SIMAROUBACEAE	*Castela coccinea* Griseb.			2		1.1
SOLANACEAE	*Salpichroa origanifolia* (Lam.) Baill.				3	1.6
VERBENACEAE	*Aloysia* sp.			3	3	3.2
	Lippia sp.				4	2.1
	Phyla nodiflora (L.) Greene			17	28	23.8
ZYGOPHYLLACEAE	***Bulnesia sarmientoi*** Lorentz ex Griseb. +	1		6	9	8.5

Note: D, dominant pollen (> 45 %) in bold; S, secondary pollen (15–45 %); M, minor pollen (15–3 %); T, trace (< 3 %); FO, percentage of frequency; +, characteristic species of the Dry Chaco; *, exotic species.

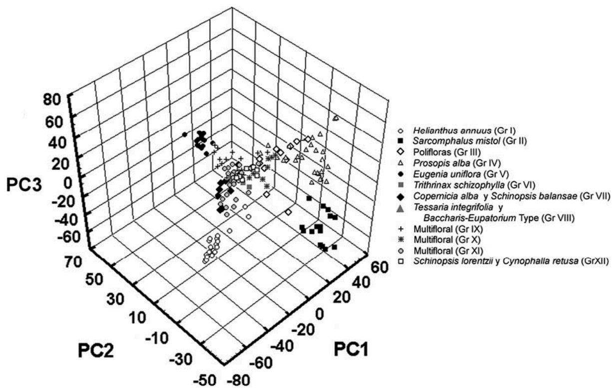

Figure 6. Scatter plot of the first three components from the principal component analysis (PCA). The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1 = 22.06%, PC 2 = 15.14%, PC3 = 11.46%.

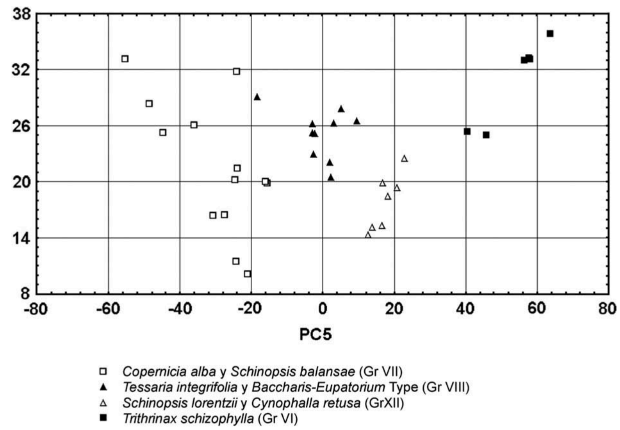

Figure 7. Scatter plot of the PC 4 and PC 5 components from the principal component analysis (PCA). The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 4 = 9.55%, PC 5 = 6.32%.

comprised samples with high percentages of pollen of *Sagittaria montevidensis* (No. 126–128).

The results of PCA agreed with the classification by CA. The first five principal components explained 64.53% for the total variance in differentiating honeys. The first principal component accounted 22% of total variance, the second principal component accounted for 15% of the total variance, showing high correlation with *Eugenia uniflora* (0.57), and the third principal component accounted for 11.5% of the total variance. The three-dimensional scatter plot resulting from the PCA (Figure 6) indicated that all the honey samples analysed could be divided into four groups, belonging to the unifloral honeys of *Helianthus annuus* (Group I), *Sarcomphalus mistol* (Group II), *Prosopis alba* (Group IV) and *Eugenia uniflora* (Group V). The multifloral honeys remained in the centre of the graph. After eliminating the groups of unifloral and polyfloral honeys, the remaining honeys were analysed using the fourth and fifth principal component, which accounted for 9.5% and 6.3% of the total variance (Figure 7).

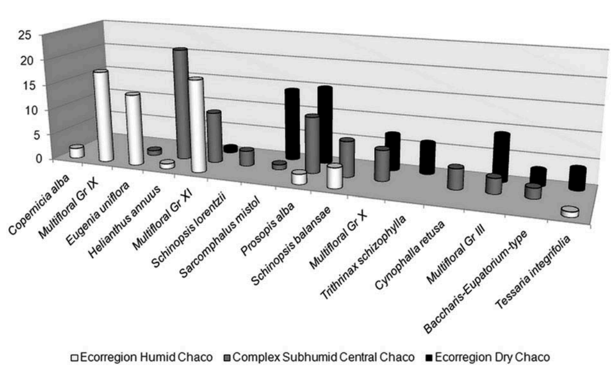

Figure 8. Honey types produced in Dry, Humid and Subhumid Chaco.

The four groups of honeys were clearly separated (Figure 5): Group VI was formed by six monofloral honeys of *Trithrinax schizophylla*, with two subgroups formed on the basis of the percentages of pollen: subgroup VIa, with more than 80% of pollen of *Trithrinax schizophylla* (No. 45, 52, 82, 90), and subgroup VIb, with less than 70% (No. 86, 87); Group VII had two subgroups: subgroup VIIa, with unifloral honeys of *Schinopsis balansae* with more than 60% of pollen (No. 12, 27, 40, 50, 69), and subgroup VIIb, with honeys that had similar percentages of pollen of*Schinopsis balansae* and *Copernicia alba* (No. 6, 9, 13, 38, 69) or *Schinopsis balansae* and *Helianthus annuus* (No. 28, 29); Group VIII formed by unifloral honeys of *Baccharis-Eupatorium*-type (No. 30, 41, 56, 91, 92) and *Tessaria integrifolia* (No. 46, 58, 186, 188, 189); Group XII was formed by two subgroups of unifloral honeys: subgroup XIIa, with unifloral honey of *Schinopsis lorentzii* (No. 51, 55, 95), and subgroup XIIb, with unifloral honey of *Cynophalla retusa* (No. 83, 88, 98, 120).

Discussion

Botanical origin

Within Argentinean honeys, those produced in the Chaco region are highlighted by the contribution of nectar from native plants, as observed in other areas of Argentina including Delta del Paraná (Gurini & Basilio 1995), Monte (Tamame 2011) and Caldenal (Andrada & Tellería 2002). Nevertheless, the honey from Chaco contained distinctive pollen; a total of 46 angiosperm families were represented by diverse pollen types, notably by the Fabaceae which was represented by 30 different taxa (Table II). The honey samples studied contained pollen from woody and shrubby plants, together with pollen from a variety of herbaceous plants, including aquatics. In particular pollen of arboreal plants, mostly woody ones, characterised most of the unifloral honey and had high frequencies in multifloral honeys. The families most represented were Anacardiaceae (*Schinopsis balansae* and *Schinopsis lorentzii*), Arecaceae (*Copernicia alba* and *Trithrinax schizophylla*), Capparidaceae (*Cynophalla retusa*), Celastraceae (*Maytenus* sp.), Fabaceae (*Prosopis alba* and *Prosopis nigra*), Myrtaceae (*Eugenia uniflora*), Nyctaginaceae (*Pisonia zapallo*) and Rhamnaceae (*Sarcomphalus mistol*). Flowering of these trees commonly occurs at the end of winter and during spring (Salgado et al. 2014); they are abundant in the region and offer large quantity of rewards to pollinators through their dense inflorescences.

The melliferous value of *Prosopis* has been previously assessed in melissopalynological studies of honeybees of other regions (e.g. Andrada & Tellería 2002; Cabrera 2006) and stingless bees of Chaco (Vossler et al. 2010). Herbaceous and subshrub plants offer nectar mainly at the end of spring and during summer, coinciding with the rainy period, although some of them such as *Acicarpha tribuloides* and *Sagittaria montevidensis*, flower almost all year round (Salgado et al. 2014). Pollen from some of these herbaceous plants, such as those of family Alismataceae (*Sagittaria montevidensis*), Asteraceae (*Baccharis-Eupatorium*-type and *Tessaria integrifolia*) and Lythraceae (*Heimia salicifolia*), was also present in unifloral honeys. Honeys of *Baccharis-Eupatorium*-type and *Tessaria integrifolia* were the most common; honey of *Sagittaria montevidensis* and *Heimia salicifolia* occurred only once during the sampling. Pollen from *Sagittaria* is very common in honeys of the Pampean region (Tellería 1988, 1992) and is frequent in those produced in the central region of the Espinal phytogeographical province (Caccavari & Fagúndez 2010). Although the percentage of pollen in honey studied suggested a unifloral origin (Louveaux et al. 1978), it is important to note that the 'real' contribution of nectar of this aquatic plant to the honey has never been investigated. *Sagittaria* has separate female and male flowers and both are visited by honeybees. Pollen from another Alismataceae (*Hydrocleis nymphoides*) together with pollen from other aquatic species belonging to the families Menyanthaceae (*Nymphoides indica*), Nymphaeaceae (*Nymphaea* sp.), Onagraceae (*Ludwigia* sp.), Pontederiaceae (*Eicchornia crassipes* and *Pontederia cordata*) was also recognised in honeys.

Other herbaceous and shrubby native plants that are present in honey belong to the families Asteraceae (*Holocheilus hieracioides*, *Mikania* sp., *Senecio grisebachii*, *Solidago chilensis*, *Tagetes minuta* and *Vernonia chamaedrys*), Boraginaceae (*Heliotropium* sp.), Malvaceae (*Sphaeralcea* sp. and *Wissadula densiflora*) and Polygonaceae (*Polygonum* sp.). Pollen from several plants that do not produce nectar was found in the honey samples; these belonged to the following families: Amaranthaceae (*Amaranthus* sp. and *Pfaffia* sp.), Commelinaceae (*Commelina erecta*), Nymphaeaceae (*Nymphaea* sp.) and the anemophilous Poaceae and Plantaginaceae (*Plantago* sp.).

Geographical origin

The pollen spectra of the honey samples analysed reflected the rich and diverse flora of the Chaco region. In comparison with honey from other regions of Argentina, a common feature was the richness of the family Fabaceae and the scarcity of Asteraceae. These two families are generally mentioned, among others, as the most important for honeybees (e.g. Crane 1991). Within Fabaceae, the presence of the subfamily Mimosoideae was remarkable. Pollen from *Albizzia inundata*, *Enterolobium contortisiliquum*, *Inga uruguensis*, *Mimosa pigra* as well as pollen from different species of *Acacia* and *Prosopis* were identified in honeys (Table II). The high frequency of Mimosoideae together with that of native plants and the scarcity of Asteraceae are only comparable with that found in honeys from the Caatinga Region in Brazil (Barth 2004; Oliveira et al. 2010). However, in honey from Chaco, *Prosopis* and diverse species of *Acacia* were the most abundant Mimosoideae, whereas in honey from the Caatinga, a variety of *Mimosa* species were dominant.

Although some unifloral honeys from Chaco were produced from the flowering of *Baccharis-Eupatorium*-type and *Tessaria integrifolia*, in general, the diversity of pollen of Asteraceae was low compared with honey from other regions of Argentina such as Pampean (Tellería 2009) and Espinal (Fagúndez & Caccavari 2006). On a large scale, honeys from Chaco may be characterised by a combination of dominant and secondary types of pollen belonging to native representatives of diverse families: Alismataceae (*Sagittaria montevidensis* and *Echinodorus grandiflorus*), Amaranthaceae (*Alternanthera* sp.), Anacardiaceae (*Schinopsis balansae* and *Schinopsis lorentzii*), Arecaceae (*Copernicia alba* and *Trithrinax schizophylla*), Asteraceae (*Baccharis-Eupatorium*-type, *Tessaria integrifolia* and *Senecio grisebachii*), Calyceraceae (*Acicarpha tribuloides*), Capparidaceae (*Cynophalla retusa* and *Sarcotoxicum salicifolium*), Lythraceae (*Heimia salicifolia*), Euphorbiaceae (*Croton-Jatropha*-type and *Sapium haematospermum*), Fabaceae (*Acacia praecox, Gleditsia amorphoides, Mimosa pigra* and *Prosopis alba*), Myrtaceae (*Eugenia* *uniflora*), Nyctaginaceae (*Pisonia zapallo*), Salicaceae (*Salix humboldtiana*), Sapotaceae (*Sideroxylum obtusifolium*), Rhamnaceae (*Sarcomphalus mistol*) and Zygophyllaceae (*Bulnesia sarmientoi*).

However, on a more detailed scale, honeys from both the Dry and Humid ecoregions, as well as from the Sub-humid Central complex, could be recognised from the pollen content. Unifloral honeys, which represented more than 50% of total honeys and were clearly separated by the CA and strongly supported by PCA, are useful to illustrate the geographical origin not only by the dominant types but also by associations of particular pollen types. Unifloral and multifloral honeys with high occurrence of pollen of *Prosopis alba*, *Gleditsia amorphoides* and *Pisonia zapallo* are common in the whole region. However, in honey from the Dry Chaco, the species that characterised the unifloral honeys include *Schinopsis lorentzii*, *Trithrinax schizophylla*, *Tessaria integrifolia*, *Cynophalla retusa*, *Pisonia zapallo* and *Sarcomphalus mistol*. The most diverse honeys were produced in the Sub-humid Central Chaco complex, a transition area between both ecoregions (Figure 8).

The pollen content of honey reflects cultivated crops with diverse introduced invasive herbs together with an overlap of native representatives from the Dry and Humid Chaco. The most common unifloral honeys were those of *Helianthus annuus* and native representatives such as *Schinopsis balansae*, *Eugenia uniflora* and *Baccharis-Eupatorium*-type, whereas in multifloral honeys, pollen from *Helianthus annuus* was frequent, together with other cultivated and introduced species such as *Medicago sativa*, *Medicago lupulina*, *Melilotus albus* and Brassicaceae.

In the Humid Chaco, unifloral honeys were dominated by pollen of *Schinopsis balansae*, *Copernicia alba*, *Eugenia uniflora* and *Heimia salicifolia*, whereas multifloral honeys contained a high frequency of pollen from aquatic plants such as *Eichornia crassipes*, *Echinodorus grandiflorus*, *Sagittaria montevidensis*, *Polygonum* sp. and *Pontederia cordata*.

Conclusion

The exhaustive inventory of vegetation of the Chaco and the analysis of honey pollen allowed for the botanical origin of honeys and the main sources of nectar to be identified. Unifloral honey were produced from flowering of native *Baccharis-Eupatorium*-type, *Copernicia alba*, *Cynophalla retusa*, *Eugenia uniflora*, *Prosopis alba*, *Sagittaria montevidensis*, *Schinopsis balansae*, *Schinopsis lorentzii*, *Tessaria integrifolia*, *Trithrinax campestris* and *Sarcomphalus mistol* and cultivated *Helianthus annuus*. The identity of the Chaco forest in honey samples was reflected through pollen from typical plant associations as 'algarrobales' (characterised by *Prosopis* sp.), 'quebrachales' (characterised by *Schinopsis* sp.) and 'palmares' (characterised by *Copernicia* sp. or *Trithrinax* sp.). The pollen spectrum allowed the honey from both the Dry and Humid Chaco ecoregions, and that from the Sub-humid Central Chaco complex to be distinguished.

Acknowledgements

The authors thank Sandra Sobrado (Departamento de Diversidad Vegetal - Universidad Nacional del Nordeste, UNNE, Corrientes, Argentina) who prepared the illustrations.

Funding

This work was supported by the CONICET, Caba, Argentina; Secretaria General de Ciencia y Técnica - Universidad Nacional del Nordeste (SGCyT), Corrientes, Argentina; [PI P002/11].

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here.

References

Andrada AC, Tellería MC. 2002. Botanical origin of honey from south of Caldén District (Argentina). Grana. 41: 58–62. doi:10.1080/00173130260045512.

Barth OM. 2004. Melissopalynology in Brazil: A review of pollen analysis of honeys, propolis and pollen loads of bees. Scientia Agricola. 61: 342–350. doi:10.1590/S0103-90162004000300018.

Cabrera MM. 2006. Caracterización polínica de las mieles de la Provincia de Formosa, Argentina. Revista del Museo Argentino de Ciencias Naturales. 8: 135–142. doi:10.22179/REVMACN.8.312.

Caccavari MA, Fagúndez G. 2010. Pollen spectra of honeys from the Middle Delta of the Paraná River (Argentina) and their environmental relationship. Spanish Journal of Agricultural Research. 8: 42–52. doi:10.5424/sjar/2010081-1142.

Crane E. 1991. The plant resources of honeybees. Apiacta. 26: 57–64.

Erdtman G. 1960. The acetolysis method. A revised description. Svensk Botanisk Tidskrift. 54: 561–564.

Fagúndez G, Caccavari MA. 2006. Pollen analysis of honeys from the central zone of the Argentine province of Entre Ríos. Grana. 45: 305–320. doi:10.1080/00173130601086636.

Gurini L, Basilio AM. 1995. Flora apícola en el Delta del Paraná. Darwiniana. 33: 337–346.

Louveaux J, Maurizio A, Vorwohl G. 1978. Methods of melissopalynology by International Commission for Bee Botany of IUBS. Bee World. 59: 139–157. doi:10.1080/0005772X.1978.11097714.

Morello J, Matteucci S, Rodriguez A, Silva M. 2012. Ecorregiones y complejos ecosistémicos argentinos. Buenos Aires: FADU GEPAMA, Editorial Orientación Gráfica Argentina.

Pennington RT, Prado DE, Pendry CA. 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. Journal of Biogeography. 27: 261–273. doi:10.1046/j.1365-2699.2000.00397.x.

Oliveira PP, Van den Berg C, Santos FAR. 2010. Pollen analysis of honeys from Caatinga vegetation of the state of Bahia, Brazil. Grana. 49: 66–75. doi:10.1080/00173130903485122.

Salgado CR, Piezsko G, Tellería MC. 2014. Aporte de la Melisopalinología al conocimiento de la flora melífera de un sector de la Provincia Fitogeográfica Chaqueña, Argentina. Boletín de la Sociedad Argentina de Botánica. 49: 513–524.

Salgado CR, Maidana JF. 2014. Physicochemical characterization of honey produced in the Chaco Province (Argentina). Revista de la Facultad de Ciencias Agrarias UNCUYO. 2: 191–201.

Salgado CR. 2016. Caracterización botánica y geográfica de las mieles producidas por *Apis mellifera* L. en la provincia del Chaco, a partir de su composición polínica y parámetros físico-químicos. PhD Thesis, Universidad Nacional del Nordeste. Facultad de Ciencias Agrarias, Universida del Nordeste (UNNE-IBONE), Corrientes, Argentina.

INTA, Instituto Nacional de Tecnología Agropecuaria. 2016. SIGA, Sistema de Información y Gestión Agrometeorologico. http://siga2.inta.gov.ar/; accessed 11 July 2016.

StatSoft. 1999. Statistica for Windows. Computer program manual. Tulsa, OK: StatSoft.

Tamame A. 2011. Estudio de la composición, disponibilidad y calidad de los recursos apícolas del noroeste de La Pampa, provincia fitogeográfica del Monte (República Argentina). PhD Thesis, Universidad Nacional del La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (UNLP), La Plata, Argentina.

Tellería MC. 1988. Analyse pollinique des miels du Nord-Ouest de la Province de Buenos Aires. Apidologie. 19: 275–290. doi:10.1051/apido:19880307.

Tellería MC. 1992. Caracterización botánica y geográfica de las mieles de la Provincia Fitogeográfica Pampeana (República Argentina) I: Distrito Oriental. Darwiniana. 31: 345–350.

Tellería MC. 2009. Asteraceae visited by honeybees in Argentina: A record from entomopalynological studies. Boletín de la Sociedad Argentina de Botánica. 44: 65–77.

Vergeron P. 1964. Intérpretation statistique des resultants en matière d'analyse pollinique des miels. Annales de l'Abeille. 7: 349–364. doi:10.1051/apido:19640407.

Vossler FG, Tellería MC, Cunningham M. 2010. Floral resources foraged by *Geotrigona argentina* (Apidae, Meliponini) in the Argentine Dry Chaco forest. Grana. 49: 142–153. doi:10.1080/00173131003694274.