

Pollen spectra of honeys from the Middle Delta of the Paraná River (Argentina) and their environmental relationship*

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Abstract

The aim of this study was to characterize honeys from the Middle Delta of Paraná River (Argentina) through pollen analysis and basic sensorial analysis, as well as assessing the vegetation contribution of this freshwater wetland as a source of nectar for *Apis mellifera*. Sixty-five samples were studied, out of which 109 pollen types, belonging to 53 plant families, were identified. The families Asteraceae and Leguminosae provided the greatest diversity of pollen types. Multifloral honeys were predominant (> 70%). Monofloral honeys were from Type *Polygonum hydropiperoides* (7), *Sagittaria montevidensis* (5), *Eupatorium* spp. (3), Pontederiaceae (2) and *Salix humboldtiana* (1). Regional honeys are mistakenly called «Catay honeys» (*Polygonum* spp.) due to the wide distribution of species of this genus and the intense activity of honey bees observed on them. However, in the samples studied, Type *Polygonum hydropiperoides* varied from < 1% to 81% showing similar sensorial characteristics, among which the absence of crystals stands out. Pollen spectra of the «Catay honeys» reflect the surrounding freshwater wetland vegetation; when they present a value of > 25% taxa that do not belong to this ecosystem, they differ in their sensorial characteristics, particularly the presence of crystals. Pollen types such as Type *Polygonum hydropiperoides*, *Sagittaria montevidensis*, *Eupatorium* spp., Pontederiaceae, *Nymphoides indica*, *Mimosa vellosiella*, *Vigna luteola*, *Cleome* sp. and Type *Solanum glaucophyllum*, are suggested as geographical markers. According to the current knowledge, we propose to characterize these honeys considering their geographical origin instead of their botanical origin.

Additional key words: botanical origin, Catay honey, freshwater wetland, geographical origin, Melissopalynology.

Resumen

Espectro polínico de mieles del Delta Medio del Río Paraná (Argentina) y su relación medioambiental

El objetivo del presente trabajo fue caracterizar mieles provenientes del Delta Medio del Río Paraná, mediante análisis polínicos y análisis sensoriales básicos, así como también evaluar la contribución de la vegetación de este humedal de agua dulce como recurso nectarífero para *Apis mellifera*. Fueron estudiadas 65 muestras, en las cuales se identificaron 109 tipos polínicos pertenecientes a 53 familias botánicas. La mayor diversidad de tipos polínicos correspondió a las familias Asteraceae y Leguminosae. Predominaron las mieles multiflorales (> 70%); las monofloras resultaron de Tipo *Polygonum hydropiperoides* (7), *Sagittaria montevidensis* (5), *Eupatorium* spp. (3), Pontederiaceae (2) y *Salix humboldtiana* (1). Las mieles regionales son erróneamente denominadas «de Catay» (*Polygonum* spp.) debido a la amplia distribución de las especies de este género y la intensa actividad de las abejas melíferas observada en ellas. Sin embargo, en las muestras estudiadas, el Tipo *Polygonum hydropiperoides* varió entre < 1% y 81%, presentando similares características sensoriales, entre las que se destaca la ausencia de cristales. El espectro polínico de las «mieles de Catay» refleja la vegetación del humedal de agua dulce; cuando presentan un valor > 25% de taxones que no pertenecen a este ecosistema, difieren en sus características sensoriales, especialmente por la presencia de cristales. Tipos polínicos como Tipo *Polygonum hydropiperoides*, *Sagittaria montevidensis*, *Eupatorium* spp., Pontederiaceae, *Nymphoides indica*, *Mimosa vellosiella*, *Vigna luteola*, *Cleome* sp. y Tipo *Solanum glaucophyllum*, se proponen como marcadores geográficos. En base al conocimiento actual, se propone caracterizar estas mieles considerando su origen geográfico en lugar de su origen botánico.

Palabras clave adicionales: humedal de agua dulce, Melisopalynología, miel de Catay, origen botánico, origen geográfico.

* Dedicated to the memory of Prof. Marta Caccavari.

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Received: 30-10-08; Accepted: 10-02-10.

Introduction

Argentina is the third largest producer of honey in the world, after China and the United States. It accounts for 70% of the honey produced in South America, 25% of America, and 6% of the world total. In beekeeping, Argentina plays an important role in the international market, since most of the production of honey and other hive products (wax, propolis, and live material) is exported. The global export of honeys is of approximately 360,000 tons, and Argentina participates with a share of just over 20% of the total market, making it the main honey exporter in the world. It exports over 95% of its production, and its main destinations are Germany, followed by the United States, Italy, the United Kingdom, and Spain (SAGPyA, 2008).

The Paraná River Delta can be defined as a macro-mosaic area, mainly subordinated to the hydrological regime. The flood pulse generates conditions that determine a variability that offers greater possibilities for the colonization of species. The ecological characteristics of the area combined with human intervention, have modified the various sectors to a greater or lesser degree. Due to these characteristics, this heterogeneous floristic region offers sources that allow for the development of various alternatives in beekeeping (Gurini, 2002). The region known as Middle Delta allows the development of an intensive beekeeping activity, obtaining the largest yields in the country, with averages of 60-70 kg hive⁻¹ yr⁻¹, and similar to the highest in the world (SAGPyA, 2008). Thanks to the region's particular characteristics, large volumes of organic honey are produced there (Grupo «Islas del Delta», 2009). The honeys from this region are mistakenly called «Catay honeys» (*Polygonum* spp.), due to the intensive honey bee activity observed over its flowers, and because of their sensory properties, among which the absence of crystals stands out (Grupo «Islas del Delta», 2009).

In Argentina there are many melissopalynologic studies which mainly focus on the central-east region, the leading producer of honey. This corresponds to the Pampean Phytogeographic Province (Tellería, 1988, 1992, 1996a,b; Andrada *et al.*, 1998a,b, 1999; Irurueta *et al.*, 2001; Basualdo *et al.*, 2006) and the Espinal Phytogeographic Province (Costa, 1982; Naab, 1993; Costa *et al.*, 1995; Valle *et al.*, 1995, 2004; Tellería, 1996c; Andrada, 2001; Andrada and Tellería, 2002; Fagúndez and Caccavari, 2003, 2006; Valle *et al.*, 2007, among others) close to the studied region. Honeys

produced in the Parana River Delta are poorly studied, and they mainly comprise the area of the lower Delta (Basilio and Romero, 1996; Basilio, 1998), and, to a lesser extent, the upper Delta (Lusardi *et al.*, 2005).

In the present work, a palynological analysis and a basic sensorial analysis were carried out in order to characterize the honeys from the Middle Delta of Paraná River, and to evaluate the freshwater wetland vegetation contribution as source of nectar for *Apis mellifera* L.

Material and methods

Characteristics of the study area

The Paraná River, formed in the South American tropical regions, flows over Brazil, Paraguay and Argentina, in a wide basin, through alluvial plains. It extends for 4,000 km in a north to south direction and empties into the Río de la Plata, forming an extensive delta (17,500 km²) located in a subtropical region (Fig. 1) (Malvárez *et al.*, 1999).

The delta is the result of sedimentary particles carried by the river in an alluvial valley, forming lowland prairies with high margin islands that determine a mosaic of freshwater wetlands (Malvárez, 1999). Every year, the river receives two seasonal streams and islands of Middle Delta (230,000 ha) remain swamped for a long time (Malvárez, 1999) (Fig. 1). These events prevent the formation of typical subtropical forests, which can be found in the upper and lower delta regions, and whose vegetation is composed of grasses, shrubs and abundant aquatic and marshy native species (Burkart, 1957).

Polygonum spp. is one of the most abundant taxa, but other species typical of freshwater wetlands can also be found: aquatic or marshy (*Sagittaria montevidensis*, *Pontederia* spp., *Eichhornia* spp., *Nymphoides indica*, *Ludwigia* spp., *Panicum* spp., *Bidens laevis*), terrestrial herbaceous (*Cleome hassleriana*, *Vigna luteola*, *Mikania micrantha*), several shrubs (*Eupatorium* spp., *Solanum glaucophyllum*, *Mimosa vellosiella*, *Sesbania virgata*, and sporadic trees (*Salix humboldtiana*, *Tessaria integrifolia*, *Sapium haematospermum*, *Albizzia inundata* and *Ocotea acutifolia*) (Burkart, *op. cit.*).

Sample collection

Sixty-five honey samples produced by *Apis mellifera* were collected from different apiaries located in

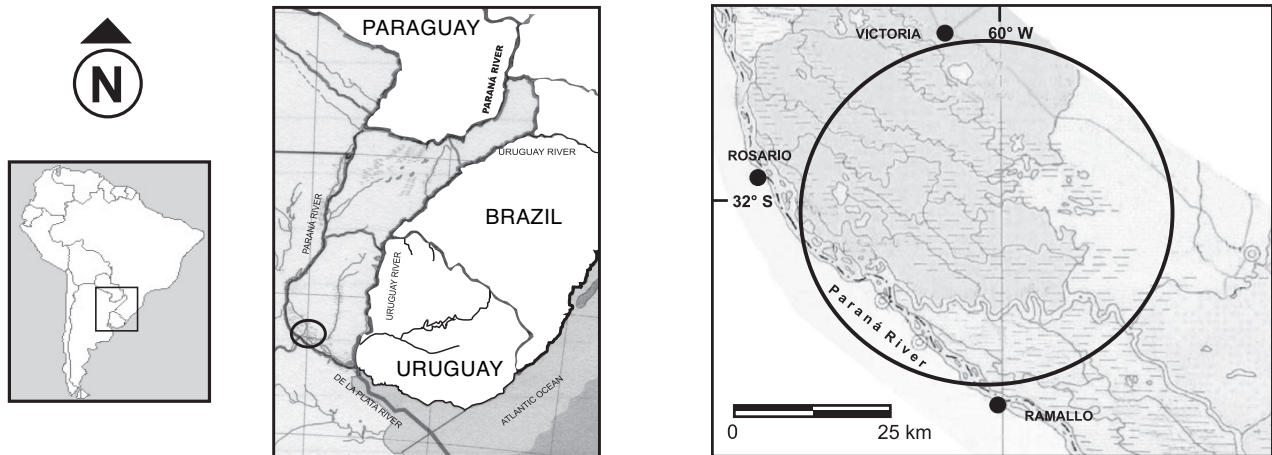


Figure 1. Geographical location of the study area.

the Middle Delta region of the Paraná River, between the years 2003 and 2005 (Fig. 1). A previous study of the surrounding flora of apiaries was also carried out. From identified taxa, acetolyzed pollen samples (Erdtman, 1969) were prepared for reference purposes.

For pollen analysis, honey samples were extracted by centrifugation. Twenty grams of heated natural honey were diluted and stirred in 100 mL of acidified water (0.1 mL of sulphuric acid in 100 mL of distilled water) and two sub-samples were obtained for qualitative and quantitative analysis. Samples were very rich in crystalline matter (0-1 mL), which dissolves with diluted sulphuric acid.

Qualitative analysis was done following the method of Louveaux *et al.* (1978). A honey sample (= 10 g) was acetolyzed, mounted in 80% glycerine, and sealed with paraffin. A Leitz Laborlux microscope at 400X or 1,000X magnification was used for microscopic observation. Samples were centrifuged for 10 min in a Rolco CM 2036 centrifuge at 2,500 rpm with a centre-rotor radius of 15.4 cm (Pendleton, 2006). Louveaux's method was followed during the whole sampling period to enable the comparison between results.

The pollen grain frequency classes were designated as: predominant pollen, «D» (> 45%); secondary pollen, «S» (16-45%); important minor pollen, «M» (3-15%); minor pollen, «m» (between > 1 and < 3%); and present, «+» (≤ 1) (Louveaux, *op. cit.*). The samples in which one pollen type represented $\geq 45\%$ were classified as monofloral, and those in which no pollen type reached this percentage were classified as mixed (Louveaux, *op. cit.*). Estimation of the relative frequency of pollen types was carried out on a minimum

of 1,200 pollen grains from two slides of the same sample.

Pollen types were identified to species level when possible, or otherwise at genus or family level, or simply as pollen type. In the latter, the genus or species to which the pollen in question could be attributed was added in parentheses, underlining those most likely to be represented in the spectrum (Ortiz, 1990; Ortiz and Fernández, 1992). The palynothesca from the Center of Scientific and Technology Transfer Research to Production (CICyTTP) and also bibliography (Caccavari, 1983, 1985; Erdtman, 1983; Roubik and Moreno Patiño, 1991; Basilio, 1996; Dutra and Barth, 1997; Pire *et al.*, 1998, 2002, 2006; Fagúndez, 2001, 2003) were consulted for material identification.

The frequency of appearance of pollen types for the whole of the samples was determined through the method used by Feller-Demalsy *et al.* (1987).

Quantitative analysis to determine the absolute number of pollen grains per gram of honey was calculated using tablets of *Lycopodium clavatum* L. spores (Stockmarr, 1971) following the methodology proposed by Moar (1985). A subsample of 10 g of honey was dissolved in distilled water and two *Lycopodium clavatum* tablets (dissolved in 10 mL of 10% hydrochloric acid), each containing $10,679 \pm 953$ spores, were added. The sediment was concentrated by repeated centrifugation at 2,500 rpm for 10 min, mounted in glycerine gelatine, and sealed with paraffin. Pollen richness was classified according to Maurizio (1939). Unacetolyzed samples (10 g) were also mounted to observe contaminated particles. In order to rule out the possibility that these samples could be originated from honeydew, the ash content was analysed in some representative samples.

The basic sensorial analysis was carried following the method of Gonnet and Vache (1989). Color measurement was determined in a Lovibond apparatus and converted to the Pfund scale.

Results

Pollen analysis

Quantitative analysis of the studied honeys classified them mainly into Class I (Maurizio, 1939). A total

of 109 pollen types, belonging to 53 botanical families, were identified, 70% of which appeared in a very low percentage (< 3%) (Table 1). Leguminosae and Asterales were the families with a higher representation of pollen types (23 and 16 respectively) (Table 1). Pollen from Type *Polygonum hydropiperoides* and *Sagittaria montevidensis* was found in 100% of the samples; Pontederiaceae (*Eichhornia* spp. and *Pontederia* spp.), *Vigna luteola*, *Bidens laevis*, *Eupatorium* spp., *Nymphoides indica*, Type *Solanum glaucophyllum*, Poaceae, Type *Mimosa vellosiella* and *Eucalyptus* spp., in more than 70% of samples (Fig. 2). Frequency of occurrence

Table 1. Pollen types and their frequency in the 65 honeys analysed

Family	Pollen type	D ¹	S	M	m	+	FO ²	VA ³
Acanthaceae	Type <i>Dicliptera tweediana</i> ⁴	—	—	—	—	1	2	N-P
	Type <i>Justicia</i> ⁴	—	—	—	—	11	17	N-P
	Type <i>Ruellia</i> ⁴	—	—	—	—	1	2	N-P
Alismataceae	<i>Sagittaria montevidensis</i> ⁴	5	11	33	11	5	100	N-P
	Type <i>Echinodorus</i> ⁴	—	—	12	7	14	51	N-P
Amaranthaceae	Type <i>Alternanthera aquatica</i> ⁴	—	—	—	5	18	35	P
	Type <i>Amaranthus</i> ⁴	—	—	—	—	17	26	P
	Type <i>Gomphrena perennis</i> ⁴	—	—	—	—	3	5	P
Anacardiaceae	<i>Schinus</i> sp. (<i>S. fasciculata</i> , <i>S. molle</i>) ^{4,5}	—	—	—	—	4	6	N-P
Apiaceae	<i>Ammi majus</i>	—	—	—	4	19	35	N-P
	<i>Ammi visnaga</i>	—	—	—	—	6	9	N-P
	<i>Eryngium</i> spp. ⁴	—	—	4	12	20	55	N-P
	<i>Foeniculum vulgare</i>	—	—	—	—	1	2	N-P
	<i>Hydrocotyle</i> sp. ⁴	—	—	—	—	1	2	N-P
Asteraceae	Type <i>Ambrosia</i> ⁴	—	—	1	4	17	34	P
	Type <i>Anthemis cotula</i>	—	—	—	—	4	6	N-P
	Type <i>Aster squamatus</i> ⁴	—	2	3	8	10	35	N-P
	<i>Baccharis</i> spp. ⁴	—	4	8	8	5	38	N-P
	<i>Bidens laevis</i> ⁴	—	—	33	16	10	91	N-P
	<i>Carduus</i> spp.	—	—	—	1	17	28	N-P
	<i>Centaurea</i> sp. (<i>C. melitensis</i> , <i>C. calcitrapa</i>) ⁵	—	—	—	1	6	11	N-P
	<i>Cichorium intybus</i>	—	—	—	—	3	5	N-P
	<i>Cirsium vulgare</i>	—	—	—	—	7	11	N-P
	<i>Eupatorium</i> spp. ⁴	3	22	20	3	4	80	N-P
	Type <i>Grindelia pulchella</i> ⁴	—	1	8	8	10	42	N-P
	Type <i>Gymnocoronis spilanthoides</i> ⁴	—	1	9	13	13	55	N-P
	<i>Holocheilus hieracioides</i> ⁴	—	—	—	—	11	17	N-P
Mutisieae ⁴	—	—	—	1	3	6	N-P	
Type <i>Plagiocheilus tanacetoides</i> ⁴	—	8	23	5	7	66	N-P	
<i>Tessaria integrifolia</i> ⁴	—	—	8	12	25	69	N-P	
Betulaceae	Betulaceae	—	—	—	—	1	2	P
Bignoniaceae	Type <i>Dolichandra cynanchoides</i> ⁴	—	—	—	1	2	5	N-P
Boraginaceae	<i>Echium plantagineum</i>	—	—	2	4	27	51	N-P
	<i>Heliotropium</i> sp. ⁴	—	—	—	1	2	5	N-P
Brassicaceae	Type <i>Brassicaceae</i> (<i>Brassica campestris</i> , <i>Rapistrum rugosum</i>) ⁵	—	—	1	1	19	32	N-P

Table 1 (cont.). Pollen types and their frequency in the 65 honeys analysed

Family	Pollen type	D ¹	S	M	m	+	FO ²	VA ³
Butomaceae	<i>Hydrocleys nymphoides</i> ⁴	—	—	—	10	14	37	N-P
Cactaceae	Type <i>Opuntia</i> ⁴	—	—	—	—	1	2	N-P
Calyceraceae	<i>Acicarpha tribuloides</i> ⁴	—	—	—	1	6	11	N-P
Caparaceae	<i>Cleome</i> sp. ⁴	—	2	4	3	23	49	N-P
Casuarinaceae	<i>Casuarina cunninghamiana</i>	—	—	—	—	3	5	P
Celtidaceae	Type <i>Celtis</i> ⁴	—	—	—	—	16	25	P
Commelinaceae	Type Commelinaceae	—	—	—	1	3	6	P
Convolvulaceae	Type <i>Dichondra</i> ⁴	—	—	—	—	2	3	N-P
Cucurbitaceae	Type <i>Cayaponia</i> ⁴	—	—	—	—	1	2	N-P
Cyperaceae	<i>Cyperus</i> spp. ⁴	—	—	5	13	20	58	P
Ephedraceae	<i>Ephedra</i> sp. ⁴	—	—	—	—	1	2	P
Euphorbiaceae	<i>Sapium haemospermum</i> ⁴	—	—	—	3	29	49	N-P
Geraniaceae	Type <i>Erodium</i>	—	—	—	—	5	8	N-P
Juncaceae	Type <i>Juncus</i> ⁴	—	—	—	—	2	3	P
Lamiaceae	Type <i>Hyptis mutabilis</i> ⁴	—	—	—	—	20	31	N-P
	Type <i>Salvia</i> ⁴	—	—	—	—	1	2	N-P
	<i>Scutellaria racemosa</i> ⁴	—	—	—	3	4	11	N-P
	<i>Teucrium</i> sp. (<i>T. vesicarium</i> , <i>T. cubense</i>) ^{4,5}	—	—	—	3	4	11	N-P
Lauraceae	Type <i>Nectandra</i> ⁴	—	—	—	—	2	3	N-P
Leguminosae	<i>Acacia bonariensis</i> ⁴	—	—	—	—	2	3	N-P
	<i>Acacia caven</i> ⁴	—	—	—	—	1	2	N-P
	<i>Albizia inundata</i> ⁴	—	—	—	1	2	5	N-P
	<i>Bauhinia</i> sp. ⁴	—	—	—	—	1	2	N-P
	<i>Caesalpinia</i> sp. ⁴	—	—	—	—	2	3	N-P
	Type <i>Desmodium</i> ⁴	—	—	—	1	1	3	N-P
	<i>Geoffroea decorticans</i> ⁴	—	—	—	—	2	3	N-P
	<i>Glycine max</i>	—	—	2	1	19	34	N-P
	<i>Lathyrus</i> sp. ⁴	—	—	—	—	2	3	N-P
	<i>Lotus corniculatus</i>	—	3	5	9	18	54	N-P
	<i>Medicago sativa</i>	—	—	—	—	2	3	N-P
	<i>Melilotus albus</i>	—	—	—	9	16	38	N-P
	<i>Mimosa ostenii</i> ⁴	—	—	—	—	1	2	N-P
	Type <i>Mimosa vellosiella</i> ⁴	—	—	13	7	27	72	N-P
	<i>Mimosa strigillosa</i> ⁴	—	—	—	—	4	6	N-P
	<i>Prosopis</i> sp. ⁴	—	—	—	1	9	15	N-P
	<i>Sesbania punicea</i> ⁴	—	—	—	—	7	11	N-P
	<i>Trifolium pratense</i>	—	—	—	—	4	6	N-P
	<i>Trifolium repens</i>	—	—	—	1	4	8	N-P
	<i>Trifolium</i> sp.	—	—	—	—	10	15	N-P
	<i>Vicia</i> sp. ⁴	—	—	—	1	5	9	N-P
	<i>Vigna luteola</i> ⁴	—	1	15	11	35	95	N-P
Lentibulariaceae	<i>Utricularia</i> sp. ⁴	—	—	—	—	2	3	N-P
Liliaceae	Type <i>Iris pseudacorus</i>	—	—	—	—	8	12	N-P
Loranthaceae	Type <i>Ligaria cuneifolia</i> ⁴	—	—	—	—	1	2	N-P
Malvaceae	Type Malvaceae	—	—	—	—	9	14	N-P
Meliaceae	<i>Melia azedarach</i>	—	—	—	—	1	2	N-P

Table 1 (cont.). Pollen types and their frequency in the 65 honeys analysed

Family	Pollen type	D ¹	S	M	m	+	FO ²	VA ³
Menyanthaceae	* <i>Nymphoides indica</i> ⁴	—	1	11	9	30	79	N-P
Myrtaceae	<i>Eucalyptus</i> spp.	—	3	5	7	31	71	N-P
Oleaceae	<i>Ligustrum</i> sp.	—	—	—	—	7	11	N-P
Onagraceae	Type <i>Ludwigia peploides</i> ⁴	—	—	1	1	33	54	N-P
Papaveraceae	Type <i>Argemone subfusiformis</i> ⁴	—	—	—	—	3	5	N-P
Plantaginaceae	<i>Plantago</i> sp. ⁴	—	—	—	—	2	3	P
Poaceae	Poaceae	—	—	10	13	24	88	P
	Type <i>Panicum</i> ⁴	—	—	—	1	7	12	P
Polygalaceae	Type <i>Polygala</i> ⁴	—	—	—	—	3	5	N-P
Polygonaceae	Type <i>Polygonum hydropiperoides</i> ⁴	7	28	23	5	2	100	N-P
	Type <i>Rumex</i> ⁴	—	—	—	—	6	9	N-P
Pontederiaceae	Type Pontederiaceae (<i>Eichhornia azurea</i> , <i>E. crassipes</i> , <i>Pontederia</i> spp.)	2	8	16	23	13	94	N-P
Ranunculaceae	Type <i>Clematis</i> (<i>C. bonariensis</i> , <u><i>C. montevidensis</i></u>) ^{4,5}	—	—	—	1	3	6	N-P
Rhamnaceae	<i>Scutia buxifolia</i> ⁴	—	—	—	3	4	11	N-P
Rubiaceae	Type <i>Borreria</i> ⁴	—	—	3	7	17	42	N-P
Rutaceae	Type <i>Citrus</i> ⁴	—	—	1	1	2	6	N-P
Salicaceae	<i>Salix humboldtiana</i> ⁴	1	1	3	6	30	63	N-P
	Type <i>Populus</i> ⁴	—	—	—	—	3	5	P
Sapindaceae	Type <i>Serjania</i> ⁴	—	—	—	—	7	11	N-P
Sapotaceae	<i>Pouteria salicifolia</i> ⁴	—	—	—	—	1	2	N-P
Solanaceae	<i>Cestrum parqui</i> ⁴	—	—	—	—	2	3	N-P
	<i>Lycium vimineum</i> ⁴	—	—	1	2	4	11	N-P
	Type <i>Solanum glaucophyllum</i> ⁴	—	1	11	15	23	77	N-P
Typhaceae	<i>Typha</i> sp. ⁴	—	—	—	—	4	6	P
Verbenaceae	<i>Verbena</i> sp. ⁴	—	—	—	—	4	6	N-P
	<i>Phyla</i> sp. ⁴	—	—	—	—	2	3	N-P
Vitaceae	<i>Cissus</i> sp. ⁴	—	—	—	—	3	5	N-P

¹ Frequency classes: values indicate the number of samples in which the different pollen types appeared at the following percentages: D, predominant pollen (>45%); S, secondary pollen (16-45%); M, important minor pollen (3-15%); m, minor pollen (>1-<3%) and "+", present pollen (≤1%). ² FO: frequency of occurrence percentage. ³ VA: apicola value. P: polliniferous plants. N-P: nectariferous-polliniferous plants. ⁴ Native species. ⁵ Underline: specie most likely to be represented in the spectrum.

percentage (FO) showed that these 11 pollen types together with other 10 were very frequent (>50% of samples) (Fig. 2). Twenty-one pollen types were frequent types (20-50%), 15 pollen types were less frequent (10-20%) and 52 were rare (<10%) (calculated from Table 1) (Fig. 3).

Eighty-five per cent of pollen types belong to nectariferous taxa and the 82% are native. Honeydew elements were practically void.

Eighteen samples were typified as monofloral (Fig. 4); 7 from Type *Polygonum hydropiperoides*, 5 from *Sagittaria montevidensis*, 3 from *Eupatorium* spp., 2 from Pontederiaceae, and 1 from *Salix humboldtiana*. These taxa also occur as secondary pollen, together with Type *Aster squamatus*, *Baccharis* spp., Type *Grindelia pulchella*, Type *Gymnocoronis spilanthoides*, Type *Plagiogcheilus tanacetoides*, *Cleome* sp., *Adesmia* spp., *Lotus corniculatus*, *Vigna luteola*, *Nymphoides indica*, *Euca-*

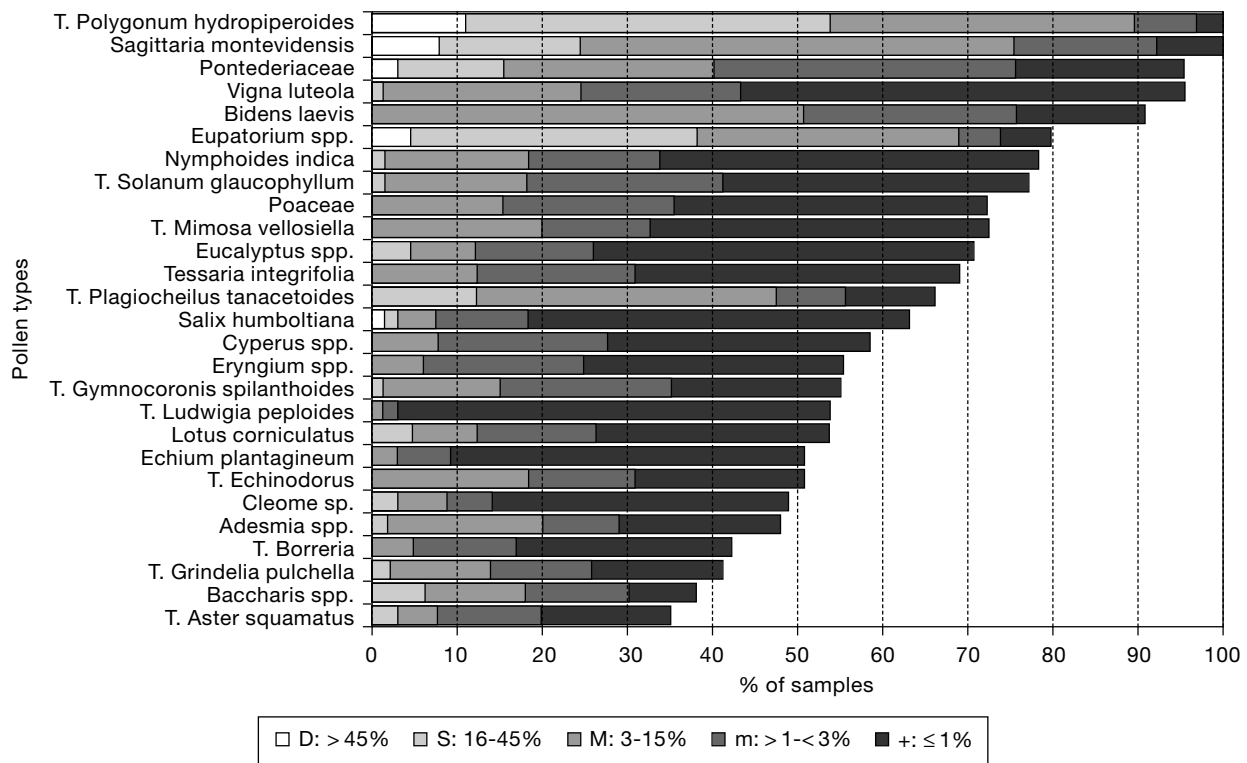


Figure 2. Pollen types with highest values of frequency of occurrence (FO) and frequency classes.

lyptus spp. and Type *Solanum glaucophyllum* (Table 1, Fig. 2).

The number of pollen types identified per sample varied between 11 and 47, with an average of 26 pollen types per sample. In Type *Polygonum hydropiperoides* honeys, 22 types were observed, varying between 16 and 33. In *Sagittaria montevidensis* honeys, there were 16, varying between 11 and 20; in *Eupatorium* honeys, 17 types, with a variation between 14 and 21; and in *Pontederiaceae* honeys, 37, varying between 36 and 39. The honey from *Salix humboldtiana* had 25 pollen

types and the multifloral honeys presented an average number of 27 types.

Generally, honey samples present a higher diversity of pollen types in spring than in summer. The occurrence of pollen from species that are not characteristic of this ecosystem is very common in low percentages, either from cultivated (*Lotus corniculatus*, *Melilotus albus*, *Glycine max*, *Eucalyptus* spp.) or native species (*Baccharis* spp.). Exceptionally, in 5 samples, the percentages were higher than 25%.

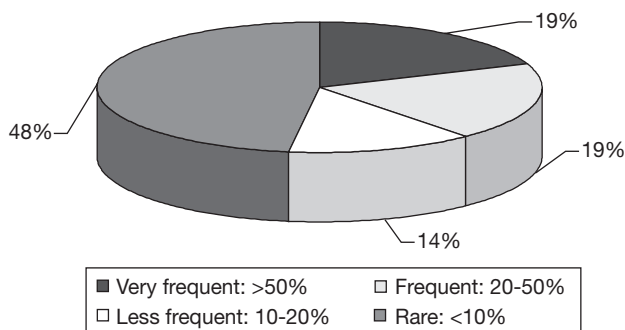


Figure 3. Frequency of appearance of 109 pollen types determined in the samples.

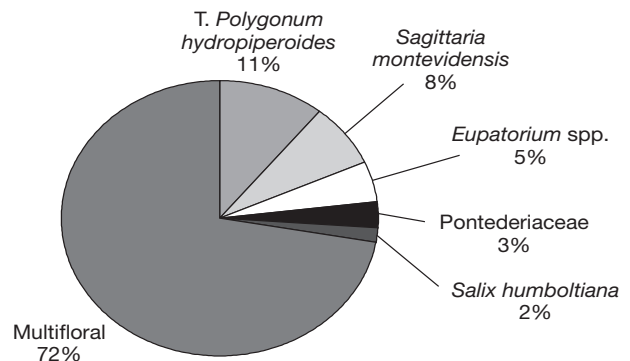


Figure 4. Botanical origin of honeys from the Middle Delta of the Paraná River (Argentina).

Sensorial analysis

In general, sensorial characteristics were similar in all the studied samples: light reddish amber color (67-76-81 mm Pfund), liquid fluidity, clear, slight fruit smell, intense sweet taste, and no crystallization. Interestingly, the five samples with more than 25% of non-regional pollen species showed crystallization.

Discussion

The high presence of nectariferous taxa together to the shortage of honeydew elements and the low ash content (0.005-0.041%) in the honeys studied shows their floral origin, which contrasts the observations made by Gurini (2002). This author mentions the possible origin of honeydew elements from *Panicum prionites*, due to the presence of monospecific communities of this species in the region, and the repeated mention of beekeepers and residents of the island that honeybees suck on it. Her observation is also based on the afore mentioned study by Gurini and Basilio (1995), who expressed the use of *Paspalum* species and *Cortaderia selloana* in the region. Mention is also made of the evidence provided by Tellería (1996c) indicating that wasps of the species *Polybia scutellaris* (Hym. Vespidae)—whose area of distribution includes Delta Paraná River— collect the sweet liquid secreted by *Paspalum dilatatum* during anthesis.

The pollen composition of studied honey provided important information about the regional flora. In the studied region, the nectar resource originated in native species, mainly herbs or shrubs. Native nectariferous taxa were dominant in the samples, indicating an optimal interaction between honey bee activity and the wetland environment vegetation. This fact also reflects the low environmental disturbance, as well as the value of this native plant species to honey bees.

Overall, the samples have a higher pollen diversity (11-47 pollen types) when compared to other honeys from similar geographical areas, such as the Brazilian mangroves (7-17 pollen types) (Barth and Fernandes Pinto Da Luz, 1998), or the Paraná River lower Delta (5-22 pollen types) (Basilio and Romero, 1996), where native trees and shrubs together with exotic cultivated species are present. This pollen diversity is possibly related to the special environment of the region, where the flora from tropical and sub-tropical wetlands comes together.

Loubreau-Callen and Damblon (1994) had expressed that the pollen spectrum of honey corresponds to the composition of the vegetation only in dry regions where the bees exploit all available species. In contrast, in tropical rainforests or tree-savannah regions the pollen spectrum of honey agrees only with the vegetation formation in the vicinity of the hives. This is inversely related with the quantity of nectar produced. In our case, where the vegetation is comprised of tropical-subtropical freshwater herbaceous elements, bees also exploit all available floral resources. Most likely, this should also apply to the low nectar production of this special vegetation.

According to Louveaux and Vergeron (1964), the number of pollen types is smaller in monofloral honeys than in multifloral honeys, with exception of Pontederiaceae honeys.

In the whole of studied samples, multifloral honeys prevailed (71%) and samples classified as monofloral were of different botanical origins.

The Middle Delta's honeys, whose main peculiarity is their special sensorial characteristics, are commonly considered monofloral, from «Catay» (*Polygonum* spp.) due to the intense honey bee activity observed in this widely extended taxon. However, the results from the pollen analysis differ: only 7 samples (11%) resulted to be monofloral from Type *Polygonum hydro-piperoides*. The remaining samples presented minor values (< 3% in 7 samples, 4-10% in 19 samples, 11-20% in 15 samples, 21-30% in 8 samples and 30-44% in 9 samples). At the same time, this study recognizes monofloral honeys from other four taxa: *Salix humboldtiana*, *Sagittaria montevidensis*, *Eupatorium* spp. and Pontederiaceae.

Honeys of Pontederiaceae and *Eupatorium* spp., from Argentina, are mentioned for the first time. *Eupatorium* spp. honeys have also been cited in Brazil (Dutra and Barth, 1997; Barth and Fernandes Pinto Da Luz, 1998).

Sensorial characteristics as well as the high fructose/glucose relation (1.5-2: commercial laboratory report) are indistinct for monofloral and multifloral honeys. The feature present in all honeys from the region is the absence of crystallization, which showed high pollen content from wetland species (mainly herbaceous). Nevertheless, some samples in this study have crystallized, and presented a pollen spectrum of more than 25% from non-regional mainland taxa. This is possibly due to the proximity of the apiaries to the coast.

A pool of wetland species (not only *Polygonum*) characterizes regional honeys, presenting a very high frequency of occurrence: Type *Polygonum hydropiperoides* (Fig. 5 A, B, C, D : b), *Sagittaria montevidensis* (Fig. 5 A, B: a), *Eupatorium* spp. (Fig. 5 A, B, C: f), Pontederiaceae (Fig. 5 A: d), *Nymphoides indica* (Fig. 5 B: g), Type *Mimosa vellosiella* (Fig. 5 D: m), *Vigna luteola* (Fig. 5 A, B: e), *Cleome* sp. (Fig. 5 C, D: j) and Type *Solanum glaucophyllum* (Fig. 5 B, C: i). *Panicum* pollen is frequent, indicating the activity of honey bees in flowers of this species, in accordance with Basilio and Romero (1996). Pollen from tree species is absent or in traces.

These features differentiate them from honey produced in other regions of the Paraná River Delta. The lower Delta honeys are characterized mostly by pollen from exotic species with a high frequency of trees (Basilio and Romero, 1996; Basilio, 1998). The

upper Delta honeys presented generally native tree species (Fagúndez and Caccavari, *in prep.*).

The studied honeys differ not only in their pollen spectrum, but also in sensorial characteristics, from others originated in the Argentinian Neotropical regions (Salgado and Pire, 1998; Basilio and Noetinger, 2003; Fagúndez and Caccavari, 2003, 2006; Salgado, 2006) and in the rest of the world (Sawyer, 1988; Ricciardelli D'Albore, 1997, 1998).

It is worth noting the low proportion of monoflorals and underrepresentation (=Class I Maurizio in Persano, Oddo *et al.*, 2000) of pollen, in the honeys studied.

More studies are needed in order to understand those distinctive parameters.

According to the current knowledge, we propose to characterize honeys from the Middle Delta of the Paraná River by taking into account their geographic origin instead of their botanical origin.

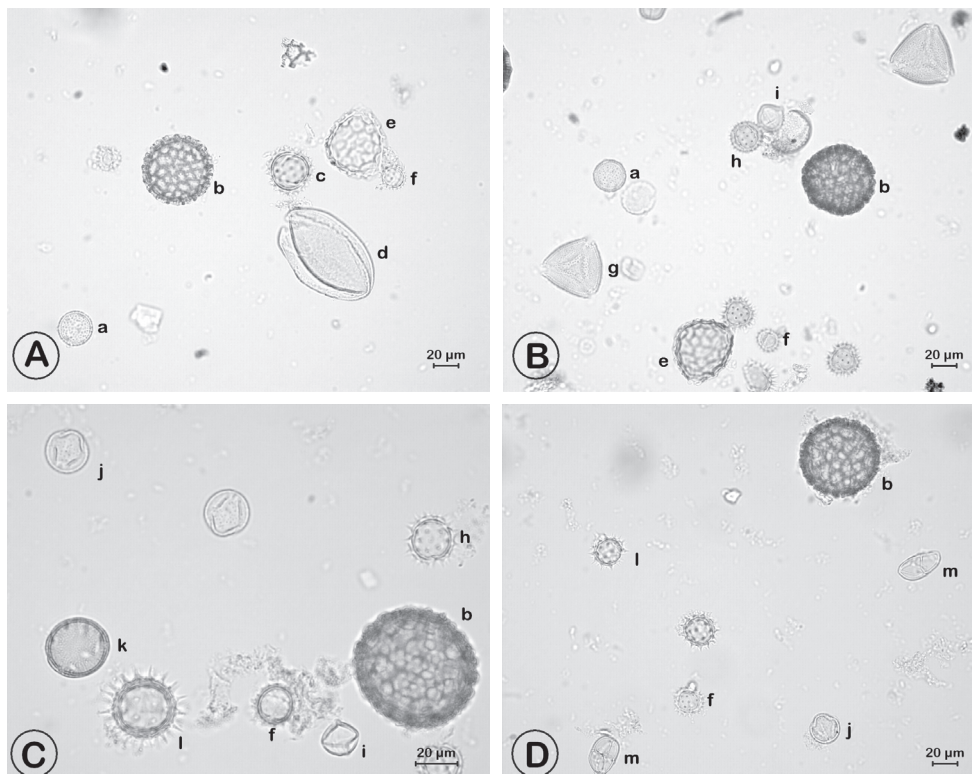


Figure 5. Characteristics of pollen types of honeys from the Middle Delta of the Paraná River (Argentina). (A): a, *Sagittaria montevidensis*; b, Type *Polygonum hydropiperoides*; c, Type *Gymnocoronis spilanthoides*; d, Pontederiaceae; e, *Vigna luteola*; f, *Eupatorium* sp. (B): a, *Sagittaria montevidensis*; b, Type *Polygonum hydropiperoides*; e, *Vigna luteola*; f, *Eupatorium* sp.; g, *Nymphoides indica*; h, Type *Plagiocheilus tanacetoides*; i, Type *Solanum glaucophyllum*. (C): b, Type *Polygonum hydropiperoides*; f, *Eupatorium* sp.; h, Type *Plagiocheilus tanacetoides*; i, Type *Solanum glaucophyllum*; j, *Cleome* sp.; k, *Borreria* sp.; l, *Bidens laevis*. (D): b, Type *Polygonum hydropiperoides*; f, *Eupatorium* sp.; j, *Cleome* sp.; m, Type *Mimosa vellosiella*; n, *Tessaria integrifolia*.

Acknowledgements

Special thanks to Guillermo Martínez for his technical support, to producers that provided the samples for the study, to Gregorio Otegui, to Horacio Moldavsky and to anonymous reviewers for the critical review of the manuscript. This work was partially supported by the SECyT-PFIP 102804 and FONCyT, PME 232.

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