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Multivariate analysis of pollen frequency of the native species *Escallonia pulverulenta* (Saxifragaceae) in Chilean honeys

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ABSTRACT – (Multivariate analysis of pollen frequency of the native species *Escallonia pulverulenta* (Saxifragaceae) in Chilean honeys). The aim of this work was the identification of geographic zones suitable for the production of honeys in which pollen grains of *Escallonia pulverulenta* (Ruiz & Pav.) Pers. (Saxifragaceae*)* can be detected. The analysis of botanical origin of 240 honey samples produced between La Serena and Puerto Mont (the IV and X Administrative Regions of Chile), allowed the detection of pollen grains of *E. pulverulenta* in 46 Chilean honeys. The geographic distribution of the honeys studied is presented together with their affinities, through factor analysis and frequency tables. The study was based on the presence of *E. pulverulenta* pollen. *Escallonia pulverulenta* pollen percentages oscillated between 0.24% and 78.5%. Seventeen of the studied samples were designated as unifloral – *i.e.* samples showing more than 45% pollen of a determined plant species. Two of these corresponded to *E. pulverulenta* (*corontillo*, *madroño* or *barraco*) honeys. The remaining unifloral honeys correspond to 8 samples of *Lotus uliginosus* Schkuhr (birdsfoot trefoil), 2 samples of *Aristotelia chilensis* (Molina) Stuntz (*maqui*) and 1 sample of *Escallonia rubra* (Ruiz & Pav.) Pers. (*siete camisas*), *Eucryphia cordifolia* Cav. (*ulmo* or *muemo*), *Weinmannia trichosperma* Cav. (*tineo*), *Rubus ulmifolius* Schott (blackberry) and *Brassica rapa* L. (turnip). Honeys with different percentages of *E. pulverulenta* pollen – statistically analyzed through correspondence analysis – could be associated and assigned to one of three geographic types, defined on the basis of this analysis. The geographical type areas defined were the Northern Mediterranean Zone (samples from the IV Region), Central Mediterranean Zone (samples from the V to the VIII regions including two samples of unifloral *Escallonia pulverulenta* honey), and Southern Mediterranean Zone (samples from the IX Region).

Key words - *Apis mellifera*, Chile, *Escallonia,* Mediterranean type climates, unifloral honey

RESUMO – (Análise multivariada da freqüência do pólen da espécie nativa *Escallonia pulverulenta* (Saxifragaceae) em méis do Chile). O objetivo do presente trabalho foi o de identificar as zonas geográficas preferidas para a produção dos méis que contêm grãos de pólen provenientes de *Escallonia pulverulenta* (Ruiz & Pav.) Pers. (Escalloniaceae), espécie essencialmente nectarífera. A análise da origem botânica de 240 méis, produzidos entre La Serena e Puerto Montt (IV e X regiões administrativas do Chile), detectou a presença de *Escallonia pulverulenta* em 46 destas amostras. A distribuição geográfica dos méis examinados é apresentada juntamente com suas afinidades morfopalinológicas, usando diagramas de freqüência e de análise de fatores (análise de correspondência). As porcentagens do pólen do *E. pulverulenta* variaram entre 0,24% e 78,5%. Dezessete das 46 amostras estudadas foram designadas como meis uniflorais, isto é, tem uma freqüência acima de 45% do pólen de uma determinada espécie de planta, mas somente duas corresponderam ao mel unifloral de *E. pulverulenta*. Dos méis uniflorais restantes, oito corresponderam a *Lotus uliginosus* Schkuhr (nome vulgar *lotera*, *alfalfa chilota*), dois a *Aristotelia chilensis* (Molina) Stuntz (*maqui*) e um a cada uma das espécies de *Escallonia rubra* ('siete camisas'), *Eucryphia cordifolia* Cav. (*ulmo*, *muermo*), *Weinmannia trichosperma* Cav. (*tineo*), *Rubus ulmifolius* Schott (*mora*) e de *Brassica rapa* L. (*yuyo*). Os méis com freqüência variável de pólen de *E.pulverulenta,* analisados estatisticamente usando a análise de correspondência, puderam ser associados e designados para cada uma das três zonas geográficas definidas com base nesta análise: a zona norte mediterrânea (amostras da IV região administrativa, a zona central mediterrânea (amostras entre a IV e VIII regiões administrativas, incluindo uma das amostras uniflorais de *Escallonia pulverulenta* e a zona sul mediterrânea (amostras da IX região administrativa).

Palavras-chave - *Apis mellifera*, Chile, climas Mediterrâneos, *Escallonia*, mel unifloral

Introduction

Honey is produced by the honeybee *Apis mellifera* L. by the transformation of plant nectar and other sweet substances produced by the living parts of plants, or left on their surfaces by aphid insects (Codex Alimentarius Commission 1987, Bogdanov & Martin 2002). The botanical origin of a particular honey is one of the most important quality parameters which can add value to the product (Krell 1996, Montenegro *et al.* 2003). This can be determined using a melissopalynological method (Louveaux *et al.* 1978, Montenegro *et al.* 2003) which consists in the qualitative (identification) and quantitative

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(count and proportion calculation) analysis of the pollen grains content. Unifloral honeys – defined as those in which the dominant pollen grain is more than 45% of the total – have higher economical value than multifloral honeys since their sensorial properties (aroma, color and taste) and nutritive characters present greater consistency over time (Andrade *et al*. 1999, Montenegro *et al*. 2005a).

Applying the Chilean Regulation of Honey Denomination via Melisopalynological Analysis which was published recently in this country (Montenegro *et al*. 2005a), it is possible to classify a honey as unifloral, bifloral or multifloral.

Chile produces a limited number of unifloral honeys with native plant origin. These are mainly derived from *Quillaja saponaria* Molina (*quillay*) in the central zone, and *Eucryphia cordifolia* Cav. (*ulmo*) in the southern zone (Ramírez & Montenegro 2004, Montenegro *et al*. 2005b). Given their excellent qualities, these honeys are greatly appreciated in the international market and generate better returns than non differentiated honeys. However, unifloral honeys are also produced from other native Chilean plant species, such as *Gevuina avellana* Molina (*avellano*) and species of the genus *Escallonia*. For this reason the botanical origin of the honey produced by local beekeepers should be certified, identifying the species composition of the differentiated honey's pollen spectra. In Chile the genus *Escallonia* comprises thirteen species in the continental territories and one in the Juan Fernandez archipelago (Sleumer 1968). Various species of the genus (*E. pulverulenta* (Ruiz et Pav.) Pers., *E. illinita* K. Presl, *E. revoluta* (Ruiz et Pav.) Pers., *E. rosea* Griseb. and *E. rubra* (Ruiz et Pav.) Pers.) have been detected in the pollen spectrum of Chilean honeys. However the most important contribution is from *E. pulverulenta* (Montenegro *et al*. 2005a). The morphology of the pollen grain of *E. pulverulenta* – monad, isopolar, radiosymmetric, tricolporate, colpi length, recessed, pores short, circular, located in the area where colpi are mildly depressed, circular-subprolate, amb circular, exine *ca.* 1 µm in thickness, tectate, columellate, collumellae distinct, finely reticulate – is distinctive for the genus (Heusser 1971). The grain is commonly smaller than other *Escallonia* species, with 20-22 µm in equatorial axis and 19-21 µm in polar axis.

In botanical terms, *E. pulverulenta*, as well as being a nectar source for honey production, is a main resource in the production of propolis. This plant is known, amongst other names, as *barraco* – which in Spanish means *boar* – due to its penetrating aroma. It is a shrub with resinous leaves and large inflorescences in spiciform racemes which are very attractive to pollinators such as *A. mellifera* (Bonod *et al*. 2003). It has been demonstrated that *E. pulverulenta* accumulates iridoids such as asperuloside, dafiloside and geniposide as well as other compounds which include the flavanone ether of 7-methylacacetine, kaempherol, rutine and chlorogenic acid (Plouvier 1956, Tomassini *et al*. 1993, Taskova *et al*. 2001). Given that *E. pulverulenta* is an endemic plant in Chile, the aim of this work was to analyze its pollen frequency in Chilean honeys, and undertake a multivariate analysis in order to determine the importance of this species in the geographical production of Chilean honeys.

Materials and methods

Hive locations – Of a total sample of 240 honeys, harvested from hives located between 30° and 39° S, and 70° and 72° W, whose botanical origin had been determined previously (Montenegro *et al*. 2003, Ramírez & Montenegro 2004, Montenegro *et al*. 2005b), 46 honeys which contained *E. pulverulenta* in their botanical composition were selected for analysis. The zone is characterized by a Mediterranean-type climate, with a dryness gradient from arid to perhumid trend (Di Castri & Hajek, 1976). This area has different anthropization degrees, with ample agrosilvicultural landscape, and the original flora reduced to patches or forest fragments (Grez *et al*., 2006). The apiaries were selected in basis to their location, in zones dominated by native flora.

The distribution of the forty-six honeys samples by administrative region was as follows (see tables 1-3 for numbers): six from the IV Region (samples 41 to 46), five from the V Region (samples 36 to 40), five from the Metropolitan Region (samples 31 to 35), eight from the VI Region (samples 23 to 30), five from the VII Region (samples 18 to 22), one from the VIII Region (sample 17), and sixteen from the IX Region (samples 1 to 16). The locations of the sampling apiaries are shown in figure 1.

Analysis of botanical origin of honeys – In order to analyze botanical origin, for each sample 20 g of honey were diluted with 20 mL of distilled water and the pollen concentrated by centrifuging at 2500 rpm for five min (Louveaux *et al.* 1978 modified). The pollen sediment was subsequently suspended with 0.1 mL of distilled water and of this suspension five aliquots of 20 µL were extracted in order to make five preparations for the optical microscope, using the method described in the literature (Louveaux *et al*. 1978), modified as in the Chilean Norm of Botanical Origin Denomination through melissopalynological method (Montenegro *et al*. 2004, Montenegro *et al*. 2005a, Montenegro & Pizarro 2006).

Frequency assessment was carried out qualitatively using comparison with palynotheque and pertinent

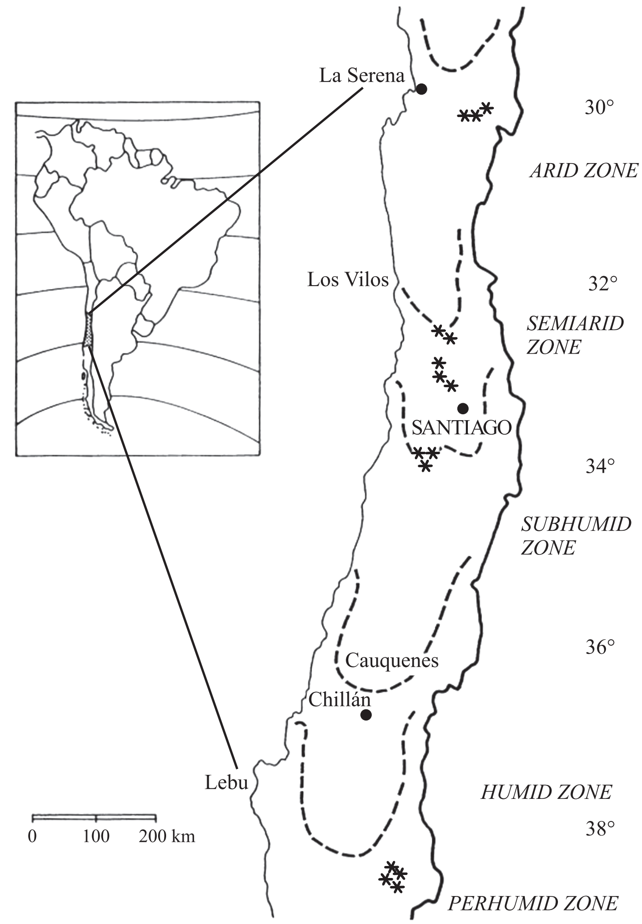

Figure 1. Map depicting the sampling areas. Sampling places are indicated by asterisk.

references (Heusser 1971, Hodges 1984, Erdtman 1986) and quantitatively following Louveaux *et al*. (1978), Moar (1985), with our modifications (Montenegro *et al*. 1992, Montenegro *et al.* 2005a). A minimum of 400 pollen grains were counted for each honey sample.

Statistical Analysis – In order to see the relationships between each honey's pollen frequency and its geographical origin, a correspondence analysis was applied, using Statistica version 6® (Stat Soft) software. For this analysis, all pollen types present in the honey's pollen spectra were considered. With the objective of verifying associations between pollen types present in the honeys' pollen spectra, a classification analysis was carried out using the cladistics program 'Hennig86' to recognize similarities between pollen types associations in the various analyzed honeys. The option 'multiple most parsimonious trees option' (mhennig*) was used. Pollen grain types percentages were coded as '0' (percentages less than 45%), '1' (ranging between 45 and 70%), and '2' (more than 70%). This analysis did not consider pollen types with percentages lower than 1%.

Table 1. Pollen types compositions of pollen spectrum of honey samples (one to sixteen). Total percentages higher or lower than 100% are caused by approximation of some pollen types percentages

Species	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acacia caven (Molina) Molina													0.2			
Acacia dealbata (Link.) F. J. Mull.																0.7
Acacia melanoxylon R. Br.					0.1											
Aextoxicon punctatum Ruiz & Pav.														0.1		
Alnus glutinosa (L.) Gaertn.														0.1		
Amomyrtus luma (Molina) Legr. & Kaus.		6.7	0.7	0.6	0.9	1.2	1.5	0.3	0.6	0.3			0.7		0.9	
Anagallis alternifolia (*A. monelli* L.)														0.3		
Apiaceae													0.7			
Aristotelia chilensis (Molina) Stuntz	5.9	11.9	1.1	0.8	1.4	1.4	0.9	0.8	7.3	13.4	7.3	3.0	3.4	13.7	0.4	1.4
Azara serrata Ruiz & Pav.													1.1			
Azara sp.	0.4									*						
Baccharis linearis (Ruiz & Pav.) Pers.								*				0.1		0.1	0.4	0.3
Blepharocalyx cruckshanksii (Hook. & Arn.) Nied.	0.1	0.1	0.1	0.2	0.1	4.3	0.2	0.3	0.2							

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Species	Sample number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Brassica rapa L.											0.5	0.5				2.4
Buddleja globosa Hope						2.1							2.7	0.3		
Caldcluvia paniculata (Cav.) D. Don		0.3	4.1	4.5	2.4	0.6	1.2	2.7	2.8			2.6	1.8	0.7	0.4	1.0
Callitriche sp.														0.2		
Castanea sativa L.	0.8	0.4	2.7	11.2	1.7	7.7	2.6	0.5	1.0	1.4		0.4	1.8	1.7	1.7	1.4
Chacaya trinervis (Gillies ex Hook. & Arn.) Escal.														0.1		
Cissus striata Ruiz & Pav.		0.5			0.4	0.3	0.1		0.5	0.1	1.8	0.4	1.1	6.6		8.1
Coriaria ruscifolia L.											0.3					
Crassula moschata G. Forst.													1.1	0.2		
Cryptocarya alba (Molina) Looser														0.1		
Discaria serratifolia (Vent.) B. & H. ex Masters													1.1			0.3
Discaria trinervis (Gill. ex Hook. & Arn.) Reiche											0.5		3.4			
Echium vulgare L.						0.3						0.1				
Escallonia pulverulenta (Ruiz & Pav.) Pers.	3.0	1.4	2.7	0.2	1.0	1.3	0.2	0.1	4.3	1.9	2.5	0.6	0.5	12.6	0.9	0.3
Escallonia rubra (Ruiz & Pav.) Pers.	1.2	1.5			1.1	0.1	0.1	*	2.3	0.8				0.1		
Eschscholzia californica Cham.													0.7	0.6	0.9	
Eucalyptus globulus L'Her.		0.5					0.1				0.1	0.1				1.7
Eucryphia cordifolia Cav.	43.1	3.8	22.9	5.2	8.3	10.0	13.2	1.4	4.3	12.3	3.1	28.1	1.8	33.8	70.0	
Euphorbiaceae			*					1.1								
Fabiana imbricata Ruiz & Pav.						0.1					0.1					
Fuchsia magellanica Lam.												0.2	0.2			
Gaultheria mucronata (L. f.) Hook. & Arn.														0.1		0.3
Gaultheria myrtillioides Cham. & Schltdl.											0.2	0.1		0.2		
Gevuina avellana Molina						0.1						0.6	0.2	0.1	0.9	1.0
Gunnera tinctoria (Molina) Mirbel.												0.1				
Hybanthus parviflorus (Mutis ex L. f.) Baillon													0.2			
Hypericum perforatum L.													0.5			
Hypochaeris radicata L.	0.1	*	0.3	0.3	0.3	0.8	1.7	6.0	0.2	0.3	0.6	0.3		0.1	0.4	2.7
Lapageria rosea Ruiz & Pav.														0.1		
Lomatia ferruginea (Cav.) R. Br.							0.1									
Lomatia hirsuta (Lam.) Diels	0.2	0.1						0.2		0.3						
Lotus uliginosus Schkuhr	13.2	9.3	39.2	71.3	63.4	49.2	52.7	60.1	67.0	39.1	70.9	41.7	51.4	13.6	9.0	24.4
Luma apiculata (DC.) Burret	0.3	1.8	0.8	0.6	0.7	0.6	4.3	10.7	0.9	0.2		0.3	5.0	0.6	1.3	15.9
Luma chequen (Molina) A. Gray											3.1	4.1	5.6	1.1	3.4	4.1
Maytenus boaria Molina																0.3
Medicago sativa L.						1.1	0.2	0.5								

continue

continuation

Species	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Melilotus indicus (L.) All.						0.5										
Monimiaceae												0.1				
Myrceugenia exsucca (DC.) O. Berg											1.1	2.1	1.4	1.0	2.6	9.8
Myrceugenia planipes (Hook. & Arn.) O. Berg	0.8	0.5	1.8	1.8	0.4		6.7	13.1	0.8	0.4						
Noccaea magellanica (Comm. ex Poir.) Holub																0.7
Nothofagus obliqua (Mirb.) Oerst.													0.7		0.9	1.0
Otholobium glandulosum (L.) Grimes												0.1				0.3
Persea lingue (Ruiz & Pav.) Nees ex Kopp											0.1	0.8	0.2			
Plantago sp.	0.3	0.1				0.1		0.1		0.2	0.5		1.1	1.0		0.3
Poaceae											0.2				0.4	0.3
Pratia repens Gaud.														0.1		
Primula magellanica Lehm.														0.2		
Quinchamalium chilense Molina		*										0.2	0.2			
Raphanus sativus L.		0.1	0.4	1.9		0.9	0.4	1.0	*	0.3	0.8		0.7	0.1	2.6	
Rhamnaceae												0.7		0.2		0.7
Rhamnus diffusus Clos														0.1		
Rubus idaeus L.						0.1			0.1							
Rubus ulmifolius Schott		0.1	0.1		1.5						0.7	1.9	2.9	0.6		4.4
Schinus polygamus (Cav.) Cabr.												0.2	0.2	0.3	0.9	1.0
Sonchus sp.						0.1										
Sophora cassioides (F. Phil.) Sparre						0.2										
Tepualia stipularis Griseb.											0.4					
Thymus vulgaris L.												0.2				
Trevoa quinquenervia Gill. & Hook.													1.4			
Trifolium glomeratum L.													4.5	0.3		0.7
Trifolium pratense L.	0.3	0.1	0.3	0.3	2.8	3.1	0.1	0.5	0.3		0.9	8.7			0.9	11.5
Trifolium repens L.						1.4			*							
Tristerix corymbosus (L.) Kuij													0.5			
Tristerix verticillatus (Ruiz & Pav.) Barlow & Wiens						0.1		*								0.7
Tropaeolum azureum Miers ex Savi																
Tropaeolum speciosum Poepp. & Endl.											0.2	0.1	0.2	0.1		
Ugni molinae Turcz.						1.0										
Weinmannia trichosperma Cav.	30.3	60.8	22.9	1.1	12.0	11.3	14.0	0.4	7.3	27.5	1.7	1.4	1.6	8.3	1.3	1.0
Non identified					1.5					1.0	2.0	0.5		0.3		1.0
Total percentage	100	100	100.1	100	100	100	100.3	99.8	99.9	99.5	99.6	100.3	100.8	99.8	100.2	99.7

* = indicates pollen types appearing with percentages lower than 0.1%.

Table 2. Pollen types compositions of pollen spectrum of honey samples (seventeen to thirty one). Total percentages higher or lower than 100% are caused by approximation of some species percentages

	Sample number														
Species	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Acacia dealbata (Link.) F. J. Mull.					0.1	0.2						0.7			
Adenopeltis serrata (W. Aiton) I. M. Johnst.		0.3													
Adesmia confusa Ulib.									0.4	0.4					
Amaranthus sp.		0.1	0.1	0.4											
Anarthrophyllum cumingii (Hook. & Arn.) F. Phil.								1.8							
Apiaceae			0.1			1.1									
Aristotelia chilensis (Molina) Stuntz	16.7	23.9	15.9		6.6	19.3			83.3	93.9					
Asteranthera ovata (Cav.) Hanst.						1.3									
Astragalus sp.		0.1	0.2												
Azara serrata Ruiz & Pav.	7.4														
Baccharis linearis (Ruiz & Pav.) Pers.		*	0.2				0.4		0.1	*			2.4		
Blepharocalyx cruckshanksii (Hook. & Arn.) Nied.		0.4													
Boquila trifoliolata (DC.) Dcne		0.3													
Brassica rapa L.		1.6	1.9				21.2	10.5	1.0	0.1			3.6	3.5	18.2
Buddleja globosa Hope		0.1													
Calceolaria thyrsiflora Graham		3.0			6.8	5.6									
Carduus pycnocephalus L.											0.3	0.7			
Cestrum parqui L'Her.															2.9
Chacaya trinervis (Gillies ex Hook. & Arn.) Escal.									0.1	0.2					
Chenopodiaceae			0.1		0.1	0.5				*					
Cirsium vulgare (Savi) Ten.											0.3	0.7			
Cissus striata Ruiz & Pav.				1.6											
Colliguaja odorifera Molina		*			1.1	2.9				*					
Convolvulus sp.		0.1	0.3			0.5									
Corynabutilon ceratocarpum (Hook. & Arn.) Kearney								0.9							
Crinodendron patagua Molina									0.2	0.3					
Cucurbita pepo L.							0.4								
Cydonia oblonga Mill.															2.9
Datura stramonium L.									*						
Echinopsis chiloensis (Colla) Friedrich & G.D.Rowley									1.8	0.3					
Echium vulgare L.	1.9	0.9	9.3		0.3	1.8									
Escallonia pulverulenta (Ruiz & Pav.) Pers.	1.9	0.2	0.1	28.4	1.4	3.1	7.2	13.6	0.1	0.5	24.3	33.3	35.3	26.8	2.5
Escallonia rubra (Ruiz & Pav.) Pers.					1.3	7.7									4.5
Eschscholzia californica Cham.			0.3			0.2									
Eucalyptus globulus L'Her.		1.1		0.9	0.8	0.2			0.1	0.1	3.6			2.1	1.7

continue

continuation

Species	Sample number														
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Fabaceae			0.1		0.3										
Fabiana imbricata Ruiz & Pav.			0.7												
Foeniculum vulgare L.									0.1	*					
Fuchsia magellanica Lam.		*	0.2		8.2	14.7									
Galega officinalis L.									3.8	2.4					
Gaultheria mucronata (L. f.) Hook. & Arn.					0.1	0.3									
Gevuina avellana Molina						4.7									
Helenium aromaticum (Hook.) Bailey									0.1						
Hydrangea serratifolia (Hook. & Arn.) F. Phil.					0.1										
Hypochaeris radicata L.			0.2		0.3					*	1.3	1.4	1.8	0.3	
Kageneckia oblonga Ruiz & Pav.												2.2	3.6		
Lamiaceae					0.1										
Lauraceae	3.7														
Lithrea caustica (Molina) Hook. & Arn.		0.2	0.6	11.3	0.3	0.1			0.1						2.9
Lotus uliginosus Schkuhr	9.3	30.5	7.7		16.7		40.2	44.3	0.1	0.1					31.4
Luma apiculata (DC.) Burret	3.7			10.0	12.6		8.0	6.1			9.7				
Malva sp.						0.3			*						
Marrubium vulgare L.							1.1								5.4
Maytenus boaria Molina										*					
Medicago polymorpha L.							13.3	14.5							
Medicago sativa L.		2.2	1.9	1.6	9.8	11.3			0.7	*	13.9				
Melilotus indicus (L.) All.			0.2					4.4	0.1	0.1					7.9
Mentha pulegium L.				2.2					0.1						
Mitraria coccinea Cav.		0.7	0.4			0.8									
Mutisia sp.													1.8	7.6	
Myrceugenia exsucca (DC.) O. Berg									0.5	0.3					
Myrtaceae		1.6	1.1												
Nothofagus dombeyi (Mirb.) Oerst.							1.1								
Nothofagus obliqua (Mirb.) Oerst.					0.1			0.4							
Otholobium glandulosum (L.) Grimes		0.9								*					
Pelletiera verna A.St.-Hil.						0.6									
Peplis portula L.					0.3	0.8									
Phacelia secunda J. F. Gmel.						0.2									
Plantago lanceolata L.										0.1					
Plantago sp.					0.1										
Poaceae			0.2							0.1					
Polycarpon tetraphyllum (L.) L.															0.8

continue

continuation

Species	Sample number														
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Prunus domestica L.												2.9	0.6	4.4	
Prunus sp.	13.0									*					
Puya sp.			0.1		0.1										
Quillaja saponaria Molina	5.6	0.7	3.0	25.5	4.6	5.5			0.1	0.1	22.0	38.4	32.3	29.4	9.5
Ranunculus chilensis DC.										*					
Raphanus sativus L.					0.1										4.1
Relbunium hypocarpium (L.) Hemsl.		*	0.1												
Retanilla stricta Hook. & Arn.	1.9														
Retanilla trinervia (Gill. & Hook.) Hook. & Arn.											7.8	4.7	11.4	10.9	1.7
Rhamnaceae		0.5	0.2												
Ribes sp.									0.3	0.3					
Ricinus communis L.					0.9										
Robinia pseudoacacia L.		*													
Rubus idaeus L.		2.8	0.5												
Rubus ulmifolius Schott	13.0	25.2	51.4	16.2	24.6	14.5			5.6	0.2	16.8	14.0	7.2	9.7	2.1
Ruta chalenpensis L.										0.1					
Salix humboldtiana Willd.	1.9				0.6	0.5			0.4			0.4		1.2	
Sarmienta repens Ruiz & Pav.									0.2	0.1					
Schinus latifolius (Gill. ex Lindl.) Engler			0.7												
Senecio sp.									*						
Silene plutonica Naudin									*						
Stachys grandidentata Lindl.									*						
Teucrium bicolor Sm.									0.1						
Trevoa quinquenervia Gill. & Hook.					0.1					*					
Trifolium pratense L.							5.3	0.9	*						
Trifolium repens L.									0.3	0.1		0.4			
Trifolium sp		0.4	1.4												
Tristerix corymbosus (L.) Kuij		0.2				0.3									
Tristerix verticillatus (Ruiz & Pav.) Barlow & Wiens												0.4		0.3	
Tropaeolum azureum Miers ex Savi		0.1				0.3									
Ugni molinae Turcz.		0.2			1.0	0.3									
Urticaceae		*													
Vicia faba L.							1.9	2.6						3.8	1.7
Weinmannia trichosperma Cav.	5.6	0.9	1.2												
Non identified	15.0	0.1		1.5	0.4	0.5			0.3	0.1					
Total percentage	100.6	99.3	100.4	99.6	99.9	100.1	100.1	100	100	99.9	100	100.2	100	100	100.2

* = indicates pollen types appearing with percentages lower than 0.1%.

Table 3. Pollen types compositions of pollen spectrum of honey samples thirty two to forty six. Total percentages higher or lower than 100% are caused by approximation of some pollen types percentages

	Sample number														
Species	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Acacia caven (Molina) Molina					4.7				1.1		0.1		0.4		
Acacia dealbata (Link.) F. J. Mull.											0.1		0.1		1.1
Adesmia confusa Ulib.				1.3											
Adesmia sp.										0.4	2.1	1.6	6.9		
Alnus glutinosa (L.) Gaertn.		0.4													
Alona sp.										*					
Amaranthus sp.											0.1			2.2	
Anthemis cotula L.															0.2
Apiaceae								0.3				2.7	0.9		0.7
Aristotelia chilensis (Molina) Stuntz		10.2		2.1				1.0	5.8						
Azara serrata Ruiz & Pav.	14.9				3.4						0.2		0.2		
Azara sp.										0.4		0.4			
Baccharis concava Pers.											0.6				
Baccharis linearis (Ruiz & Pav.) Pers.		0.4			0.6					0.5		*			
Baccharis marginalis DC.													0.5		
Bahia ambrosioides Lag.						1.7									
Beilschmiedia miersii (Gay.) Kosterm.									1.3						
Berberis empetrifolia Lam.												*			
Brassica rapa L.	6.5				17.8	6.7				8.9	7.6	55.2	4.3	15.2	2.7
Cactaceae										0.2					
Caesalpiniaceae										1.0	0.4				
Calceolaria sp.											0.5	0.2			
Chacaya trinervis (Gillies ex Hook. & Arn.) Escal.										0.5	2.3	1.1			
Chenopodiaceae		0.4		0.2								0.1	0.1		
Chenopodium ambrosioides L.								0.4							
Chorizanthe vaginata Benth.										0.2		0.1			
Cicendia quadrangularis Griseb.											0.3				
Citronella mucronata (Ruiz & Pav.) D. Don		2.3										*			
Colletia spinosa Lam.									1.7						
Colliguaja odorifera Molina										1.9	0.3			0	0.2
Convolvulus sp.					5.0								0.1		0.2
Cordia decandra Hook. & Arn.										5.3	7.6				
Cryptantha sp.										1.1					
Cryptocarya alba (Molina) Looser					0.3									4.0	
Cuscuta sp.										3.0	0.4				
Diplacus parviflorus (Green) A. L. Grant													0.1		

continue

continuation

Species	Sample number														
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Dysopsis glechomoides Müll.-Arg.										0.3	1.0				
Eccremocarpus scaber Ruiz & Pav.											0.1				
Elytropus chilensis (DC.) Muell. Arg.		0.4													
Erythraea chilensis Pers.											0.1				
Escallonia pulverulenta (Ruiz & Pav.) Pers.	4.3	19.6	12.8	17.8	15.6	4.2	78.5	74.3	14.0	42.8	20.9	0.5	1.9	0.9	2.6
Escallonia rubra (Ruiz & Pav.) Pers.				46.9				3.8	17.7	1.1	1.2	*	5.7		
Eschscholzia californica Cham.									0.4	0.1	0.3	0.8	1.5		0.7
Eucalyptus globulus L'Her.	1.2							4.4	2.2	1.7					27.8
Fabiana imbricata Ruiz & Pav.													0.5		
Foeniculum vulgare L.		1.1								0.1	0.1				
Fuchsia lycioides Andr.				0.2						0.4	2.3		0.2	24.1	5.7
Galega officinalis L.										0.7	0.8	0.6	0.2		
Gamocarpha poeppigii DC.											0.2				
Gunnera tinctoria (Molina) Mirbel.										*					
Gymnophyton sp.												1.2			
Heliotropium stenophyllum Hook. & Arn.													37.6		
Hosackia subpinnata G.Don										0.1					
Hybanthus parviflorus (Mutis ex L. f.) Baillon										0.2	0.5		0.6		
Hydrangea serratifolia (Hook. & Arn.) F. Phil.									1.1						
Hypericum perforatum L.											0.4				
Hypochaeris radicata L.	0.2											*	0.1	2.2	
Jubaea chilensis (Molina) Baillon					21.9		1.9								
Kageneckia oblonga Ruiz & Pav.										0.2	0.1				
Lamiaceae								0.3	3.0					3.1	
Lamium amplexicaule L.										1.4		0.2			
Larrea nitida Cav.										0.7					
Lauraceae		0.8													
Lithrea caustica (Molina) Hook.& Arn.		3.0		0.9				0.4	0.4				0.6		
Lobelia polyphylla Hook.& Arn.															0.7
Lonicera caprifolium L.										0.1					
Lotus corniculatus Schkuhr														7.6	0.2
Lotus uliginosus Schkuhr	36.2		19.2	0.9											
Luma apiculata (DC.) Burret		0.8		1.3											
Lycopersicon esculentum Miller		0.4													
Maytenus boaria Molina													0.9		
Medicago sativa L.			4.9					4.0	7.3				0.1		
Melilotus indicus (L.) All.	17.7									2.1	0.2	1.0			
Menonvillea sp.							0.2					0.9			

continue

continuation

Species	Sample number														
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Mentha pulegium L.	1.8				2.2										
Microseris pigmaea D. Don													0.2		
Mitraria coccinea Cav.		0.4											0.1		0.2
Muhlenbeckia hastulata (J. E. Sm.) Johnst.													0.4		
Myoschilos oblonga Ruiz & Pav.													0.2		
Myrceugenia corraeifolia (Hook. & Arn.) O. Berg										5.5	17.5	3.7			
Myrceugenia exsucca (DC.) O. Berg		2.6												11.2	
Myrceugenia obtusa (DC.) O. Berg								1.4							
Myrceugenia sp.									1.5						
Myrcianthes coquimbensis (Barneoud) Landrum & Griffo															25.3
Myrica pavonis C.DC.											0.1				
Nolana paradoxa Lindl.							0.2								
Onagraceae												0.1	0.1		
Otholobium glandulosum (L.) Grimes							11.0								
Oxalidaceae												0.1			
Persea americana Miller			1.0		15.0	19.3	0.2	0.3					0.1		
Pinus radiata D. Don							0.2							0.4	
Plagiobotrys sp.										2.4		0.4			
Plantago sp.										0.1	1.5	3.7			
Pleurophora pungens D. Don												0.7			
Polycarpon tetraphyllum (L.) L.										0.3	0.7	0.1			
Polygonum sp.											0.1	*			
Prunus persica (L.) Batsch			2.0												
Prunus sp.		4.5			7.5								0.1		
Puya sp.										0.1			0.1		
Quillaja saponaria Molina		28.7	17.2	5.4		42.0	7.7		6.7					17.4	
Quinchamalium chilense Molina											0.4		0.1		
Raphanus sativus L.								5.6	31.5			5.4	1.4	6.3	
Retanilla stricta Hook. & Arn.										0.7	4.5				
Retanilla trinervia (Gill. & Hook.) Hook. & Arn.		1.5	21.7			26.1									20.5
Rubus ulmifolius Schott	7.1	19.6	19.2	9.0	5.0					0.1		0.5	14.6		0.7
Rumex acetosella L.													0.4		
Salix humboldtiana Willd.		1.9	2.0												
Salix sp.											2.0	0.1	1.0		
Sarmienta repens Ruiz & Pav.											0.3	*			
Schinus latifolius (Gill. ex Lindl.) Engler										11.5	13.3	15.5	16.5		9.5
Schizopetalum gayanum Barn.										0.3					

continue

continuation

Species	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
	Sample number														
Sisymbrium sagittatum Hook. & Arn.										0.6	1.0	1.1	0.2		
Solanum ligustrinum Lodd.								1.2	1.9						
Solanum tuberosum L.										0.2					
Sonchus oleraceus L.															0.2
Stachys bridgesii Bentham								0.4	0.9						
Stachys grandidentata Lindl.														5.4	0.9
Stellaria cuspidata Willd.ex Schlecht.													0.1		
Trevoa quinquenervia Gill. & Hook.											1.1		0.1		
Trifolium glomeratum L.								0.4							
Trifolium repens L.	9.2	0.8		9.0						1.9	5.5	0.6	0.1		
Tristerix corymbosus (L.) Kuij					0.9										
Urticaceae								0.2	0.7						
Vicia faba L.	0.8							0.4							
Non identified		0.4		5.1				1.2	0.9	0.9	1.2	1.3	1.5		
Total percentage	99.9	100.2	100	100.1	99.9	100	99.9	100	100.1	100	100	99.9	100.8	100	100.1

* = indicates pollen types appearing with percentages lower than 0.1%.

Results

The botanical origins of each of the 46 honey samples are shown in tables 1 to 3. Unifloral honey is considered if contains at least 45% from a single pollen types (Montenegro *et al*. 2005a), 17 of the 46 samples analyzed were identified as unifloral. Of these, eight were of *Lotus uliginosus* Schkuhr (*lotera* or *alfalfa chilota*), two of *Aristotelia chilensis* (Molina) Stuntz (*maqui*), two of *E. pulverulenta* (*barraco*), one of *E. rubra* (siete camisas), one of *E. cordifolia* (*ulmo* or *muemo*), one of *Weinmannia trichosperma* Cav. (*tineo*), one of *Rubus ulmifolius* Schott (*mora*) and one of *Brassica rapa* L. (*yuyo*).

Forty six botanical families were identified in 46 honey samples with pollen grains of *E. pulverulenta* present in them. These include Fabaceae and Myrtaceae (represented in 14 samples); Rhamnaceae (in 8 samples); Rosaceae (in 4 samples); Anacardiaceae, Boraginaceae, Brassicaceae, Lamiaceae, Lauraceae and Solanaceae (in 3 samples each); and Asteraceae, Cunoniaceae, Euphorbiaceae, Gesneriaceae, Proteaceae and Vitaceae (in 2 samples each).

The correspondence analysis permitted the identification of three types and geographical associations of honeys (figure 2). The first group (geographic type 1) includes the six honey samples harvested in the extreme north of the study area (IV Region) in the Mediterranean climate zone with arid trends. The second group (geographic type 2) was composed of 24 honey samples produced between the V and VIII regions in the Mediterranean zone with semiarid, subhumid and humid trends. The third group (geographic type 3) corresponded

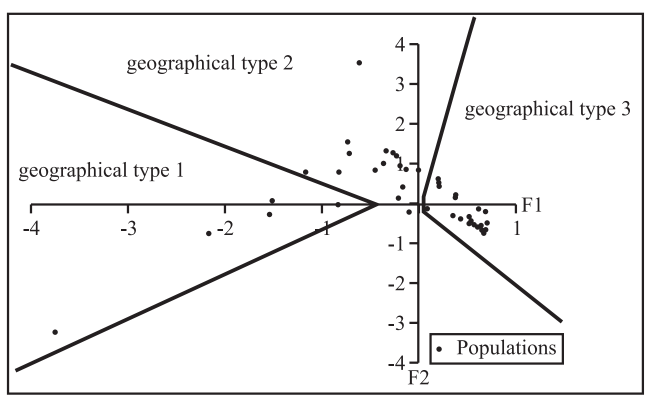

Figure 2. Correspondence analysis F1 x F2. The diagram shows Geographical Type 1 formed by 6 samples of honeys all of them harvested at the IV Region (samples 41-46). Geographic Type 2 formed by 24 samples of honey between V Region and VIII Region (samples 17-40). Geographical type 3 formed by 16 samples from IX Region (samples 1-16)

to the sixteen honey samples obtained in the extreme south of the study area (IX Region), in the Mediterranean climate zone with perhumid trends.

By applying a correspondence analysis (figure 3) the dominant genera in each geographic type could be determined. The singular values, eigen values, inertia percentage, cumulative percentages and chi squared are shown in table 4. The cumulative percentages of the two first axis comprised 16.6 of the variability. The main inertias were exhibited by *Heliotropium stenophyllum* Hook. & Arn. for the samples of geographic type 1, *Quillaja saponaria*, *Retanilla trinervia* Hook. & Arn. and *Raphanus sativus* L. for geographic type 2, and *Weimannia trichosperma*, *Aristotelia chilensis* and *Lotus uliginosus* for samples of geographic type 3. The unifloral honeys of *Escallonia pulverulenta* appeared in the type which we define as geographic type 2, originated from the vegetation of the V region, and corresponding to the typical sclerophyllous matorral of Central Chile. The pollen fractions of these honeys contain 78.5% (sample 38) and 74.3% (sample 39) of *E. pulverulenta* pollen. An exploratory classification using the cladistics software "Hennig86" (figure 4) indicated that the species found in the honey samples form three clearly delimited groups. These groups are 1. *Aristotelia*-*Amomyrtus*. 2. *Weinmannia*-*Eucryphia*-*Lotus*, 3. *Escallonia*-*Lithrea*, *Brassica*-*Raphanus*-*Schinus*-*Castanea*-*Cordia*, *Persea, Prunus* and *Retanilla.*

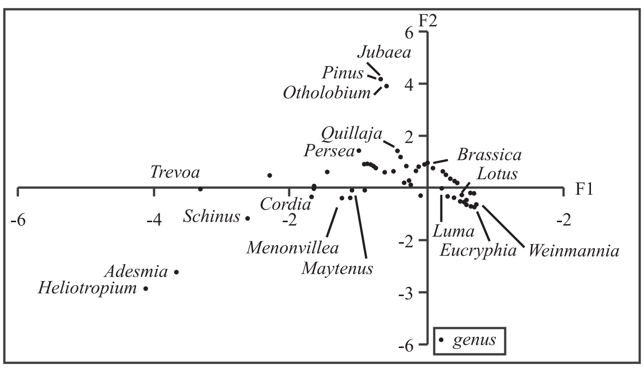

Figure 3. Dominant genera for any of three geographical types as depicted in F1 × F2 diagram of Correspondence Analysis. For species names see text

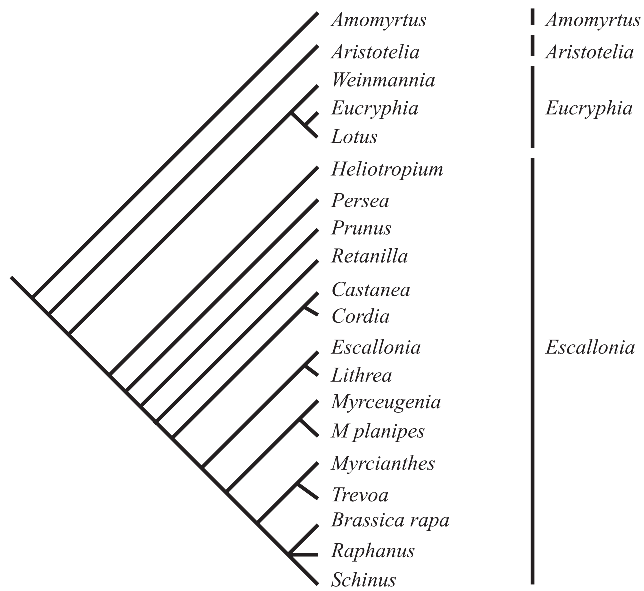

Figure 4. Cladogram showing *Escallonia* pollen-based clusters, identifying 4 major group of endemic honeys, *i.e. Amomyrtus*, *Aristotelia*, *Eucryphia* and *Escallonia* sorts.

Table 4. Singular values, eigen values, percentage of inertia, cumulative percentage and chi squares values for twelve components

	Singular values	Eigen values	Percent. of inertia	Cumulative percent	Chi-squares
1	0.90907971	0.82642591	8.92433679	8.92433679	2982.15917
2	0.8431511	0.71090377	7.6768463	16.6011831	2565.29736
3	0.80807533	0.65298575	7.0514061	23.6525892	2356.30006
4	0.7864535	0.61850912	6.67910284	30.331692	2231.89109
5	0.76900131	0.59136301	6.38595979	36.7176518	2133.93432
6	0.75402846	0.56855893	6.13970505	42.8573569	2051.64576
7	0.72304025	0.52278721	5.64542935	48.5027862	1886.47843
8	0.64976152	0.42219004	4.55910935	53.0618956	1523.4734
9	0.64296782	0.41340761	4.46427045	57.526166	1491.78201
10	0.60408885	0.36492334	3.94070266	61.4668687	1316.82643
11	0.58959502	0.34762229	3.75387353	65.2207422	1254.39555
12	0.57135119	0.32644218	3.52515565	68.7458979	1177.96711

Discussion

The analysis of 46 honey samples with presence of *E. pulverulenta* pollen collected in Chilean Mediterranean climate zones with different trends of aridity suggests that they can be subdivided into three geographic types, determined by their association with three different classes of botanical composition in their pollen spectra: 1. *Aristotelia-Amomyrtus*, 2. *Escallonia-Lithrea*, *Brassica-Raphanus-Schinus, Castanea-Cordia*, *Persea, Prunus, Retanilla* and 3. *Weinmannia-Eucryphia-Lotus*. *Escallonia pulverulenta* appears to be most strongly associated with plants of the second group (geographic type 2) (figure 3). This correlates well with its greater abundance, within its area of distribution, in the area of the Mediterranean climate zone with semiarid to humid trends (figure 1). In general the geographical types corresponded well with the different climate zones within the region with Mediterranean climate. The honey samples associated with geographic type 1, derived from the Mediterranean climate zone with arid trends; the honeys which determine the geographic type 2, derive from zones with semi arid, subhumid and humid trends; while geographic type 3 corresponded to honeys produced in the perhumid Mediterranean zone (di Castri & Hajek 1976).

Escallonia belongs to a monogeneric family (Escalloniaceae) or to Saxifragaceae. Little information exists about the participation of species of the family Saxifragaceae in honey production in other regions with Mediterranean climate zones (Andrade *et al.* 1999), but most of the literature related to the botanical origin of honey from Mediterranean climate regions has not reported the presence of pollen types associated with Saxifragaceae in the honeys analysed (Serra-Bonvehi & Ventura-Coll 1995, Mateo & Bosch-Reig 1998, Mendes *et al*. 1998, Tsigouri & Passaloglou-Katrali 2000, Herrero *et al*. 2001, Serrano *et al*. 2004, Soria *et al*. 2004). Chile is a country where it is possible to produce unifloral honey of *E. pulverulenta*, and the most promising location for its production is the Mediterranean climate zone with semiarid trends (the fifth and the metropolitan administrative regions, located between 32° and 33° of South latitude, approximately). The promotion of certification of unifloral *Escallonia* honey, and thereby of its origin, is highly recommendable, as currently only unifloral honeys of *Q. saponaria* (*quillay*) and *E. cordifolia* (*ulmo*) are recognised in the international market. The next step towards obtaining unifloral honey of *E. pulverulenta* or of other native and endemic plants is to ascertain the optimal season for harvest. This could promote the conservation of determined plant species with apicultural utility, and under some degree of threat, by encouraging their economic exploitation in a sustainable way with minimal ecological impact through apicultural activity (Butz 1997). This is particularly valid for those species with narrow geographic distributions concentrated in areas with dense human population, the presence of which implies permanent habitat impacts and a degree of vulnerability; such is the case for the Mediterranean climate zone of Central Chile (Cincotta *et al*. 2000).

Through statistical analysis which included the whole spectrum of pollen types present in the samples, we obtained only one type of unifloral honey, compared with the three or four classes obtained by melissopalynological analysis, which only considers the percentage of the dominant pollen type. Generally, honey is characterized by the abundance of some pollen types and the infrequency of others. Although the honey samples were derived from a large area (North, Center and South of the country), representing Chile's Mediterranean climate zone, many pollen morphs from the total of 105 pollen types found in the pollen spectrum of all samples were shared among several of them. In turn, much of the inertia was also shared, requiring eight to twelve components in the correspondence analysis which made it difficult to define clusters. Different climatic trends are described within the study area: arid, semiarid, humid, and perhumid or very humid (di Castri & Hajek 1976), yet only the last of these was easily identified; in the factorial analysis by the grouping of components, and in the cladistic analysis by the allocation of associated species (*Aristotelia* and *Amomyrtus*, both typical of the southern zone) to the first two branches which separate from the main group of the cladogram (see figures 3 and 4). However, by associating them with the melissopalynological analyses these methods may be useful for identifying the biogeographical origin of a certain honey. Because the discriminative power of these methods is not based on dominant pollen type alone, which can be adjusted by the addition of pollen to the honey, but rather includes the total of all pollen types which appear in the pollen spectra, this could thereby help to prevent adulteration. Today the prediction of zones with apicultural potential is fundamental to the commercialisation of products with added value. Chile could be converted in a country unique in the production of endemic honeys such as *E. pulverulenta* honey, an attribute for which it could become recognised worldwide.

Lotus uliginosus "bird trefoil" a dominant pasture species in the South geographic zone requires further comments: first the sampling take into account *Escallonia* containing profiles, nevertheless in eight cases appear consistently *Lotus*-frequencies. Many of them may be typified as multifloral no-native in the Chilean Norm (NCh 2981. Of 2005) terms, or still as a multifloral mixture (Ramirez & Montenegro 2004, Montenegro *et al*. 2008). In this study pollen of this species appears consistently associated with *Weinmannia* and *Eucryphia* pollen grains as can see on cladistic analysis.

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