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Pollen content accumulated in nests of *Polybia scutellaris* (Hymenoptera, Vespidae)

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Abstract
Plant resources foraged by *Polybia scutellaris* were identified by analysing the pollen content of 14 nests in Magdalena (Buenos Aires Province, Argentina). Six nests were inhabited at the time of collection. The palynological analysis indicated that the wasps visited 33 different taxa of flowering plants during the active cycle of the colonies. Flowers visited by the wasps belonged to both native and exotic plants located close to the nests. The variety of resources foraged by *P. scutellaris* characterises this species as a generalist flower visitor.

Keywords: *Social wasp, generalist, flower visitor, pollen storage*

Polybia is a genus of social wasps belonging to the Vespidae, and is represented in Argentina by nine species. The wasp studied in this paper, *Polybia scutellaris* White, is widespread in Paraguay, Brazil, Uruguay and Argentina (mainly in eight provinces: Misiones, Corrientes, Santa Fe, Buenos Aires, Córdoba, Entre Ríos, San Juan and Mendoza; Willink, 1998).

Adult wasps of each colony share the nest and present a division of labour and a caste differentiation. Brood care is cooperative and larvae are fed by means of progressive provisioning (Hunt et al., 1987). According to Bruch (1936), the colony becomes active in spring (October) and the floral visits end in May. Wasps of the genus *Polybia* are predators of different arthropod orders (Bruch, 1936; Willink, 1998; Silva & Jaffe, 2002) and constitute potential biological control agents of plagues that affect human beings (Bertoni, 1911) and crops (Willink, 1998; De Moura et al., 2000; Reis et al., 2000). They forage flowers in search of nectar and produce small quantities of honey (Bertoni, 1911; Bruch, 1936; Tellería, 1996; Willink, 1998). *Polybia scutellaris* has been mentioned as a pollinator agent by Medan et al. (2006) and Basilio et al. (2006).

The nests are attached directly to the substrate, typically a twig (Figure 1A), and consist of a light cardboard resistant to climatic influence and formed by the wasps from cellulose pulp, their own secretions and water. External walls of the nest have conic, sharp-pointed protuberances, which are a characteristic of the species. The nest entrance is transversely narrow. Internally, nests have combs overlapping with several cells (Figure 1B). Different developmental stages of wasps can be found simultaneously in the cells of the same comb; i.e. eggs, larvae and pupae (Bruch, 1936). Predation on *Polybia* wasp nests by birds has been documented (Windsor, 1976). The palynological content of the honeys produced by *Polybia* wasps has been analysed by Costa de Bringas (1986), Tellería (1996) and Daners Chao (2003) in samples from the province of Córdoba, the Pampean region (Argentina) and Montevideo (Uruguay), respectively.

O'Rourke and Buchmann (1991) have suggested that studies of the diets of pollinators should include an analysis of the pollen stored in the nest, and propose the methodology applied in this study, which evaluates the floral resources foraged by *Polybia scutellaris*. The aims of this investigation were to determine: (1) the range of resources foraged by *P.*

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scutellaris; (2) the temporary pattern of visitation activity; (3) the relationship between the relative location of the nests and the type of floral resource used by the colonies; and (4) whether there are significant differences between the pollen richness in inhabited nests and that of uninhabited nests.

Materials and methods

Sampling location and collection of nests

The nests studied were collected in Magdalena, a province of Buenos Aires, Argentina (35°04′S, 57°31′W), along the National Road 11, in March 2001 and January 2002. A total of 14 nests were collected. Six nests were inhabited by *Polybia scutellaris* and eight were uninhabited. Five of these nests were collected in the same place (Estancia 'Rocamo'). The area sampled is in a humid prairie of the Salado River Basin. The composition of the plant community is influenced by flooding in the lower fields and by intense cattle activity favoured by the native grassland. Several introduced species, such as *Salix babylonica* L., *Populus alba* L., *Eucalyptus* sp. and *Acacia* sp., and native trees, such as *Celtis ehrenbergiana* (Klotzsch) Liebm., *Scutia buxifolia* Hutch. & M. B. Moss, *Schinus longifolius* Speg., *Jodina rhombifolia* Hook. & Arn. ex Reissek, *Sambucus australis* Cham. & Schltdl. and *Phytolacca dioica* L. are cultivated in the area (Vervoorst, 1967). Adult wasps (Figure 1C) were collected from each inhabited nest and then identified in the laboratory (Willink, 1950). The reference specimens were deposited in the section of Entomology of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires.

Material processing and data analysis

Samples of 20 to 30 g of the material of the discs were taken from every nest (Figure 1B). The samples were embedded in 96% alcohol and macerated in a mortar. They were later acetolysed following the method of O'Rourke and Buchmann (1991). The

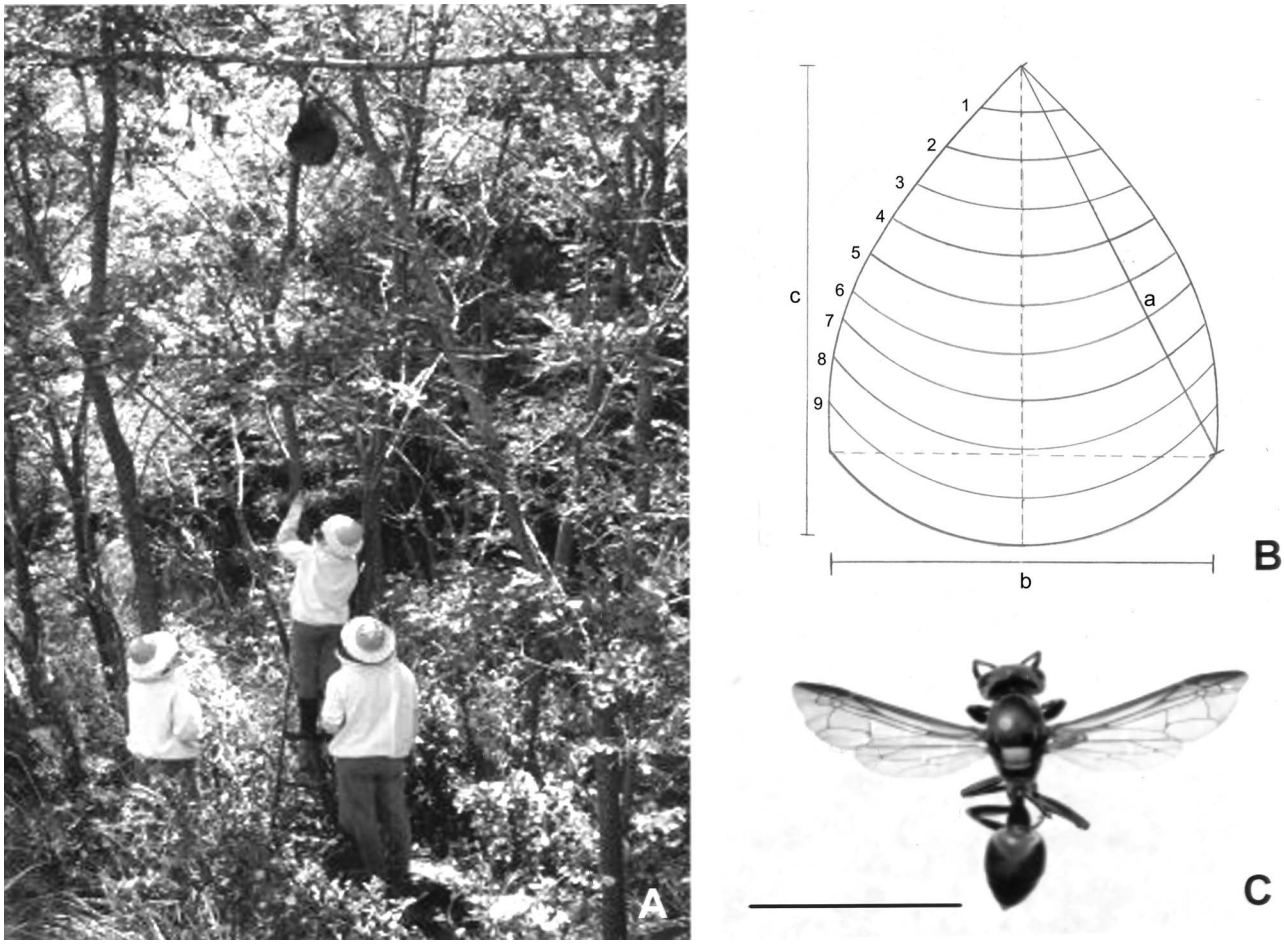

Figure 1. **A.** Collection of a nest. **B.** Scheme of a longitudinal section of a nest with nine combs overlapping (1–9); *a* = 16 cm; *b* = 24 cm; *c* = 18.5 cm. **C.** General aspect of *Polybia scutellaris*. Scale bar – 0.9 cm.

identification of the pollen types (an average of 500 grains per nest) was made with a light microscope using the reference collection of the Palaeobotany and Palynology Laboratory of the Facultad de Ciencias Exactas y Naturales (UBA) and available bibliographic sources (Markgraf & D'Antoni, 1978; Roubik & Moreno, 1991). The percentage of each morphological type was calculated from the data of the pollen content of the cells. Four categories were established to facilitate the interpretation of the percentage data: dominant 'D' (>45%), secondary 'S' (16–45%), minor importance 'M' (3–15%) and trace 'T' (<3%), following the methodology of Louveaux and colleagues (cf. Tellería, 1996). The richness in pollen types accumulated in the cells was determined for each nest in order to determine the total floral resources foraged by *Polybia scutellaris* after several seasons of activity. The constancy C of each identified pollen type 'i' in the nests studied was calculated according to the following formula (cf. Matteucci & Colma, 2002): C = (number of nests containing the pollen type 'i' in their cells/total number of nests) × 100.

The pollen data obtained were processed using Statistica (Version 5) for Windows. The Wilcoxon two-sample test was used to compare the mean of richness of pollen types between inhabited and uninhabited nests. A cluster analysis was performed, using the Pearson correlation coefficient to group the nests according to relative percentage of pollen types accumulated in the cell, and to evaluate the relationships between the vicinity of the nests and the floral resource type used by the colonies.

Results

A total of 33 pollen morphological types were identified: four at species level, fifteen at generic level, three at tribe level, five families in which the most likely genera types were determined, and six at family level. The pollen grains of Chenopodiaceae and Caryophyllaceae were grouped because both were observed as traces and they have a similar morphology. The other taxa represented only as traces were morphologically different; therefore they were not grouped. In the tribe Astereae, *Aster* sp., *Solidago* sp., *Conyza* sp. and *Baccharis* sp. were grouped, and in the tribe Lactuca, *Lactuca* sp., *Sonchus* sp., *Hypochoeris* sp. and *Taraxacum officinale* F. H. Wigg were also grouped by their similar morphology.

During its active period, *Polybia* foraged a total of 33 floral resources in the population studied, with an average of 12.5 (standard deviation: 3.59, n = 14) different taxa per nest. The most representative types of pollen grains found in the cells belonged to the families Myrtaceae, Alismataceae, Apiaceae, Asteraceae, Oleaceae and Poaceae (Table I). In 11 of the 14 nests studied, *Eucalyptus* sp. (Figure 2A) was the dominant type, whereas in the other 3 it constituted a secondary or less important component. Secondary pollen types were: *Sagittaria montevidensis* Cham & Schltdl (Figure 2B), *Conium-Ammi* sp. (Figure 2C), *Eryngium* sp. (Figure 2D), Astereae, *Ligustrum* sp. and Poaceae. Poaceae pollen was a minor component in most nests. Diverse pollen grains, such as *Nothoscordum* sp., *Alternanthera* sp. (Figure 2E), *Apium* sp., *Hydrocotyle* sp., Lactuceae, *Celtis ehrenbergiana*, Cyperaceae, *Sapium* sp., *Acacia caven* (Molina) Molina, *Lotus* sp., *Trifolium* sp., *Mentha* sp., Onagraceae and *Salix* sp., were present only as traces in some of the nests studied. The Wilcoxon two-sample test indicated a significant difference (n = 6, n = 8, W = 65.5, p = 0.0060) in mean richness of pollen types between inhabited (15.33) and uninhabited (10.38) nests.

Constancy of pollen types (Figure 3)

Pollen from Astereae, *Eucalyptus* sp. and Poaceae was found in cells of every nest analysed. Pollen grains of the *Conium-Ammi* complex and *Eryngium* sp., as well as of Anthemideae (Figure 2F), *Ambrosia* sp., *Ligustrum* sp., *Althernanthera* sp., *Echium* sp., *Sagittaria montevidensis* and the Chenopodiaceae-Caryophyllaceae complex were found in at least half of the nests studied.

Cluster analysis (Figure 4)

The five closest nests (1, 2, 8, 9 and 14) located in campo 'Rocamo' were grouped in the dendrogram according to the relative percentage of the pollen types accumulated in their cells. By means of this analysis, inhabited nests (1, 3, 5, 6, 10 and 14) were not separated from uninhabited nests (2, 4, 7, 8, 9, 11, 12 and 13).

Discussion

The analysis of the pollen accumulated in the cells of the 14 nests collected determined that the community of *Polybia scutellaris* studied visited 33 taxa during the whole active period. The number of resources foraged was similar to that found by Tellería (1996) when analysing the pollen content of the honeys produced in six *P. scutellaris* nests collected in the Argentine pampas. The author observed that wasps visited 34 taxa in a period of three months (January, February and March). Thus, the palynological analysis of the material accumulated in the cells resulted as comparable to that of the study of several honeys sampled in different stages of the season.

Table I. Pollen types identified in nest cells grouped by family.

	Nests													
Taxa	1[a]	2	3[a]	4	5[a]	6[a]	7	8	9	10[a]	11	12	13	14[a]
Alismataceae *Sagittaria montevidensis*	T		T		S		M			M		T		T
Alliaceae *Nothoscordum* type											T	T		
Amaranthaceae *Alternanthera* type			T	T	T		T			T	T		T	T
Anacardiaceae *Schinus* sp.		T	T										M	M
Apiaceae *Apium* sp.													T	
Apiaceae *Conium-Ammi* type	M	M	M	T	T	T	T	M	M	T	T			S
Apiaceae *Eryngium* sp.	T	T	M	T	M	S	S	T		M	T	T	T	T
Apiaceae *Hydrocotyle* sp.					T		T			T				
Asteraceae *Ambrosia* sp.	T	T			M	T	T	M	T	T				T
Asteraceae Anthemideae	T	T	T		M	M	T	T	T	T				T
Asteraceae Astereae	M	T	T	T	M	S	S	M	T	M	T	T	T	M
Asteraceae *Carduus* sp.					T	T								
Asteraceae Lactuceae		T				T	T	T		T				
Boraginaceae *Cordia* sp.						T						T	M	
Boraginaceae *Echium* sp.	T	M	M		T	T	T	M	T					
Brassicaceae							M							
Celtidaceae *Celtis ehrenbergiana*					T	T		T	T			T		
Chenopodiaceae-Caryophyllaceae	T		T		T	T	T		T	T				
Cyperaceae											T	T		
Euphorbiaceae *Sapium* sp.						T						T		
Fabaceae *Acacia caven*												T		
Fabaceae *Lotus* sp.														T
Fabaceae *Trifolium* type										T				
Iridaceae			T											
Lamiaceae *Mentha* sp.					T									
Lamiaceae Type 1										T				T
Myrtaceae *Eucalyptus* sp.	D	D	D	D	S	S	M	D	D	D	D	D	D	S
Oleaceae *Ligustrum* sp.	M	S	M		T	T		T	T	T				M
Onagraceae					T									
Poaceae	M	M	M	M	M	M	M	M	M	M	S	M	M	M
Polygonaceae *Polygonum* sp.												M		T
Rhamnaceae *Scutia buxifolia* Reissek													M	
Salicaceae *Salix* sp.			T		T									
Unknown			T		T	T		T		T		T		T
Richness in pollen types	11	11	15	6	19	16	14	12	10	16	8	13	9	15

[a]Inhabited nests. Occurrence: T, traces; M, minor importance; S, secondary; D, dominant.

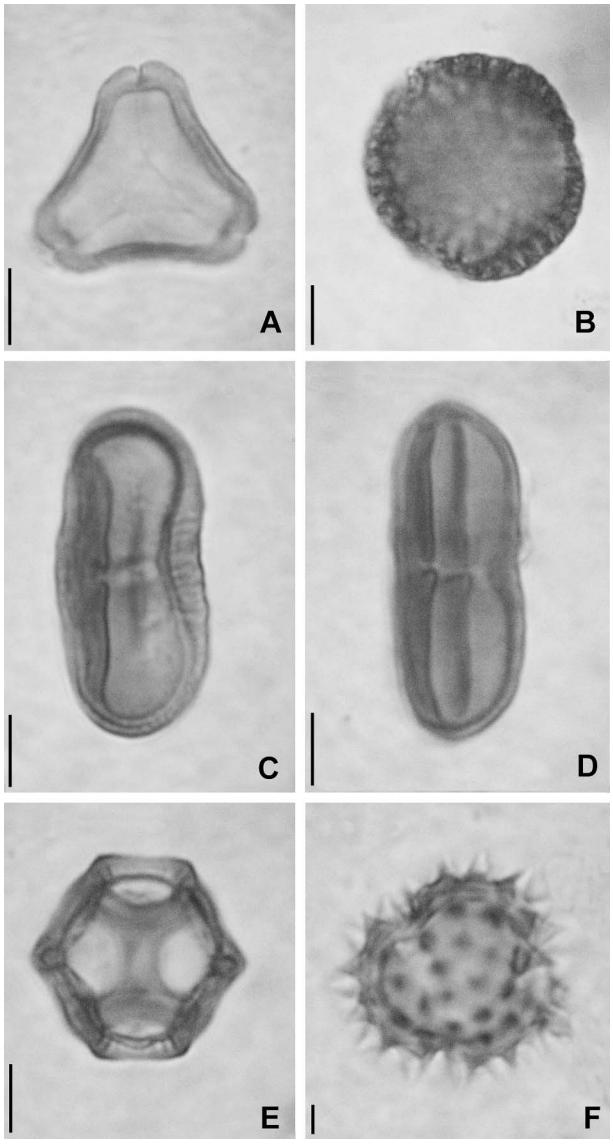

Figure 2. Pollen types identified in nest cells. **A.** *Eucalyptus* sp. **B.** *Sagittaria montevidensis*. **C.** *Conium-Ammi* spp. **D.** *Eryngium* sp. **E.** *Alternanthera* sp. **F.** Anthemideae. Scale bars – 5 μm.

The range of resources foraged by *Polybia scutellaris* characterise this species as a generalist flower visitor. Although *P. scutellaris* is a native wasp, it often visited *Eucalyptus* sp., a dominant tree among the exotic forest taxa that grow in the region (Vervoorst, 1967). *Eucalyptus* sp. flowers provide large quantities of nectar, and, in different regions of Argentina, *Apis mellifera* produces monofloral honeys of them (Naab & Torroba, 1993; Forcone, 2003). The same predominance of *Eucalyptus* sp. was found by Tellería (1996), whereas *Polybia* honeys analysed by Costa de Bringas (1986) and Daners Chao (2003) in Córdoba (Argentina) and Montevideo (Uruguay), respectively, showed *Eucalyptus* sp. pollen grains only as traces.

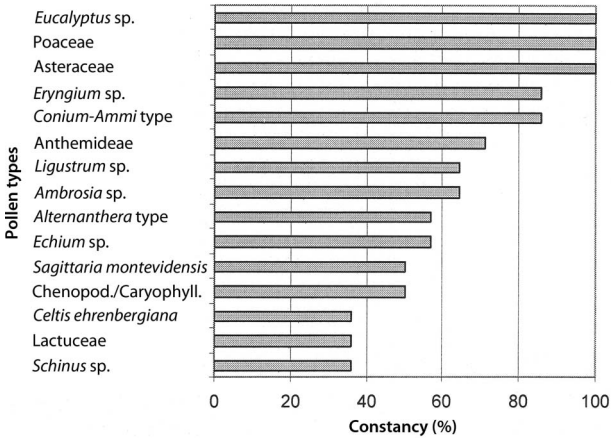

Figure 3. Constancy of pollen types in cells of every nest analysed. The graph shows consistency data >25%.

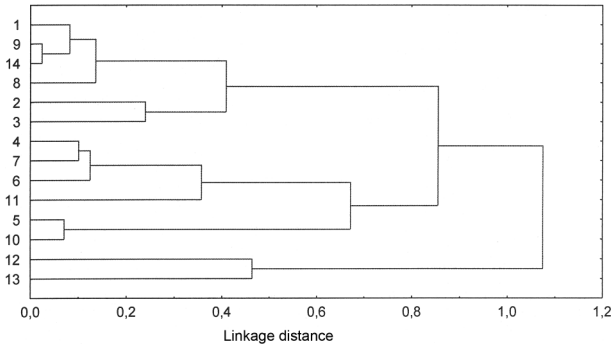

Figure 4. Dendrogram obtained from 14 nests by cluster analysis based on pollen types accumulated in their cells. Tree diagram for 14 variables, weighted pair-group average, 1-Pearson r.

Every colony studied used several aquatic plants as floral resources: *Polygonum* sp., *Althernanthera* sp., *Hydrocotyle* sp. and *Sagittaria montevidensis*, which are abundant in lentic aquatic habitats. The most visited flowers by *Polybia scutellaris* (*Conium-Ammi* sp., Asteraceae, *Eryngium* sp. and *Eucalyptus* sp.) have dense inflorescences. Since the pollen types found in the nests belonged to taxa flowering in spring–summer (*Eucalyptus* sp., *Conium-Ammi* sp.; Basilio & Torretta, 2006), it is evident that *Polybia scutellaris* visits flowers mainly during those seasons.

The pollen types and the relative percentage of them found in the cells were very similar in the nests located in the same place. These observations were corroborated by the cluster analysis, where the nearest nests are observed in the same cluster. This difference in the pollen types of the nests located in different places could be related with the plant community heterogeneity of each collecting place. This result seems to support the observations by Machado and Parra (1984) about the scarce capability of *Polybia*

scutellaris to return to the nests (about 150 m). This explains why wasps forage in the vicinity of their nests.

The mean samples showed a higher richness in pollen types in inhabited nests. This is probably due to the presence of rare pollen grains from the honey deposited in the cells in use during the last season. The pollen grains found in unhabitated nests represent the residue of the activity of the colonies. Therefore, carrying out the study during a long period of time and considering the pollen accumulated in cells did not result in an increase in the variety of flower resources foraged by wasps.

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