



## SHORT NOTE

### Pollen Resources Stored in Nests of Wild Bees *Xylocopa ciliata* Burmeister and *Megachile pusilla* Pérez (Hymenoptera: Anthophila) in a Temperate Grassland-Forest Matrix

FG VOSSLER

Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CONICET/UADER/PROV. ENTRE RÍOS), Entre Ríos, Argentina

#### Article History

##### Edited by

Solange Augusto, UFU, Brazil

Received	11 May 2018
Initial acceptance	12 August 2018
Final acceptance	23 August 2018
Publication date	11 October 2018

##### Keywords

*Adesmia*; entomopalynology; *Galega*; *Lotus*; pollen feces; pollen provisions.

##### Corresponding author

Favio Gerardo Vossler  
Laboratorio de Actuopalynología  
CICYTTP (CONICET/UADER/PROV. ENTRE RÍOS)  
Entre Ríos, Materi y España, E3105BWA  
Diamante, Entre Ríos, Argentina.  
E-Mail: favossler@yahoo.com.ar

#### Abstract

Pollen analysis was employed to study the diet composition of two wild bees in a patch of temperate grassland invaded by exotic plants. Thirty pollen types from 14 families and two unidentified types were present in the seven samples analyzed. The three samples from *Megachile pusilla* Pérez were composed of 100% *Lotus glaber* and the four samples from *Xylocopa ciliata* Burmeister of abundant pollen (>5%) of *Lotus glaber*, *Galega officinalis*, *Adesmia bicolor* and type *Senna-Chamaecrista* (all Fabaceae). The latter resource likely foraged out of the park, which could be an evidence to support its pollen preference for Fabaceae. However, a larger number of samples is necessary to identify the specialization status of *X. ciliata*. The preference for *Lotus* in this site was due to temporal specialization as *M. pusilla* was identified as *polylectic* in its origin area, and this could help to explain its effective naturalization in the New World and other areas of the Old World.

Pollen analysis of nest provisions is a useful tool to identify their botanical origin and to understand the pollen specialization in bees (Cane & Sipes, 2006; Müller & Kuhlmann, 2008). Even extreme pollen specialists can take nectar from many floral species but forage pollen on a reduced number of host-plants (Robertson, 1925; 1926; Cane & Sipes, 2006; Minckley & Roulston, 2006; Müller & Kuhlmann, 2008; Vossler, 2013; 2014). Threshold values of 10 and 5% total pollen counts have commonly been used to distinguish minor or potential contaminants from abundant pollen types (Ramalho et al., 1985; Kleinert-Giovannini & Imperatriz-Fonseca, 1987; Cane & Sipes, 2006; Müller & Kuhlmann, 2008) which is necessary to correctly identify pollen specialization even in polylectic bees (Vossler, 2018).

The aim of the present study was to identify the botanical origin of nest pollen provisions of two wild bees (the exotic *Megachile pusilla* Pérez and the neotropical *Xylocopa ciliata* Burmeister) and their pollen specialization at a same site during the period of nesting activity. It is hypothesized that both species are polylectic, similar to most species of *Megachile* and *Xylocopa*.

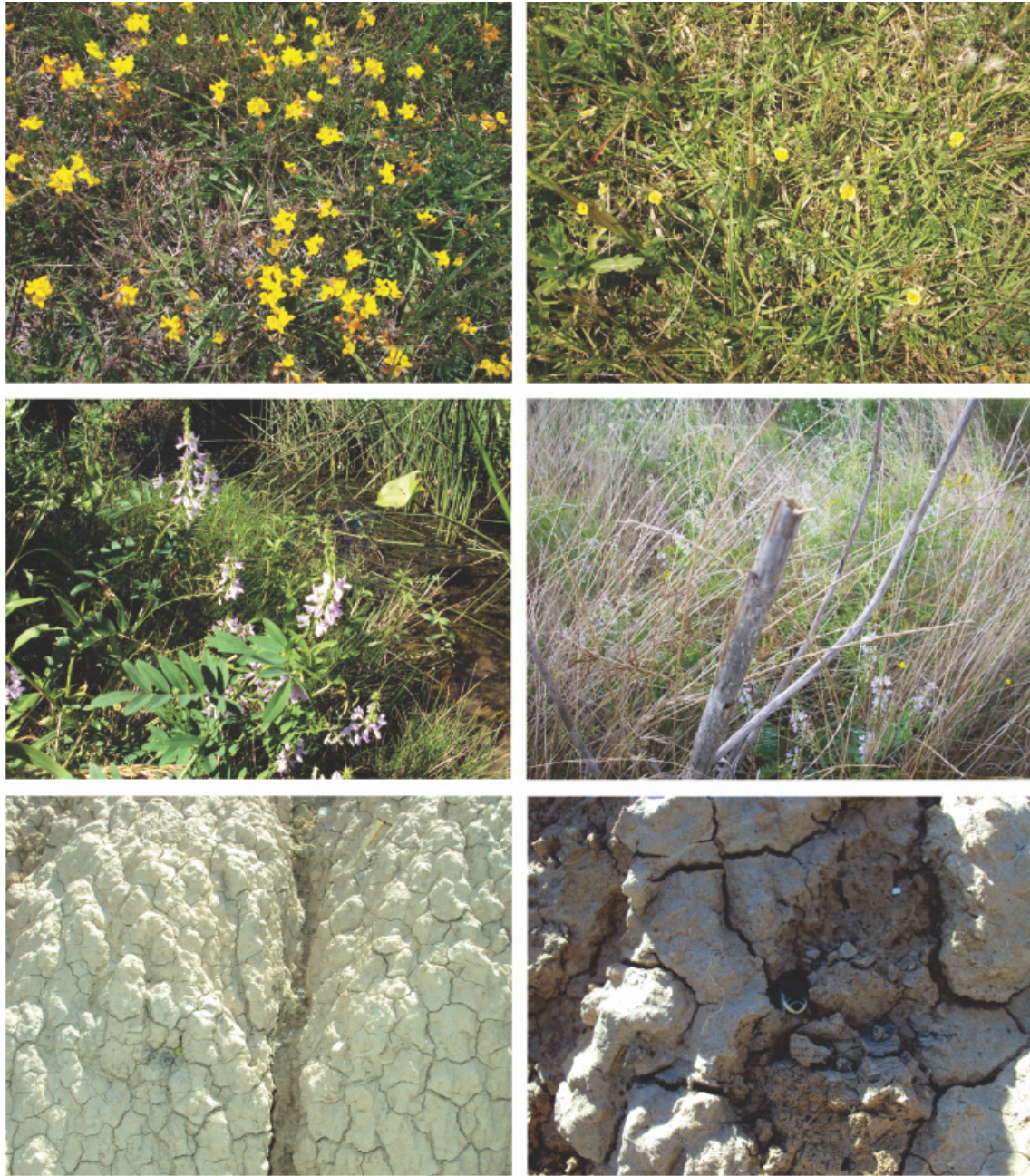
*M. pusilla* was found nesting in small soil cracks of a bank and *X. ciliata* in internodes of only large stems of the invasive *Dipsacus fullonum* L. (Fig 1). Their nests were hard to find in the site sampled and no studies on nest pollen provisions were performed for *M. pusilla* in areas where it was naturalized neither for *X. ciliata*. Nests were provisioned during summer and sampled during summer and fall (three nests of *M. pusilla* sampled on 12th February 2011 and two samples from nest 1 (nest 1A and B) of *X. ciliata* on 27th December 2008 and one sample (nest 1C) from 1st January 2009 and one sample from nest 2 in June 2012) (Table 1; Fig 1). The study was carried out at the Parque Ecológico Municipal de La Plata (34° 51'-52' S; 58° 03'-05' W), a 200 ha patch of temperate grassland composed of many melittophilous species native to the Oriental district of the Pampean region *sensu* Cabrera (1971) but heavily colonized by exotic plants (Vossler et al., unpublished data), at Villa Elisa city, Buenos Aires province, Argentina.

The whole pollen provisions from nest cells of *M. pusilla* and dry feces of post-defecating larvae from nest



entrances of *X. ciliata* were sampled and stored in plastic tubes at 5 °C. In the laboratory, they were dissolved in distilled water at 80-90 °C for 10-15 minutes, pressed when necessary using a glass rod, stirred by hand for a few minutes, and filtered. Finally, to obtain pollen sediment, samples were centrifuged at 472 x g for 5 minutes, and acetolized (Erdtman, 1960). Pollen types were identified at 400 and 1,000 x magnification and 500 grains per slide were counted (except in sample 1A as only 204 grains were found) using a light microscope Leitz Laborlux. Pollen grain identification was carried out comparing nest pollen grains with reference

pollen. The reference pollen collection was made from flowers of plant species mainly collected in Parque Ecológico Municipal de La Plata (Villa Elisa city) and La Plata city, in the northeastern of Buenos Aires province, Argentina. These plant specimens were pressed, dried, identified by the author and deposited in the Herbarium Lorentz (DTE) of Diamante, Entre Ríos, Argentina. The vegetation and flowering of the site was quantitatively recorded from August 2008 to August 2009 (Vossler et al., unpublished data). To identify pollen specialization categories, the lexica of Cane and Sipes (2006) and Müller and Kuhlmann (2008) were applied.



**Fig 1.** Pollen host blooming: A\_ *Lotus glaber*; B\_ *Adesmia bicolor*; C\_ *Galega officinalis*. Nesting sites of *Xylocopa ciliata* (D) and *Megachile pusilla* (E, F): D\_ Nest 1 of *Xylocopa ciliata* in a thick stem of *Dipsacus fullonum* surrounded by *Galega officinalis* and *Lotus glaber* during blooming; E\_ Leaf fragments in a completed nest cell (left) and transported by a female for nest building (right); F\_ Scopal pollen of *Megachile pusilla* during nest provisioning.

A total of 30 pollen types from 14 families and two unidentified types were present in the seven samples analyzed (Table 1). From them, most were minor pollen ( $\leq 5\%$ ) and only four types from two subfamilies of Fabaceae were abundant: *Lotus glaber*, *Galega officinalis*, *Adesmia bicolor* and type *Senna-Chamaecrista* (Table 1; Fig 2). The three samples of *M. pusilla* were composed of 100% *L. glaber*. The four samples of *X. ciliata* were composed of abundant pollen of the four Fabaceae in different representation and many minor pollen from many families (Table 1; Fig 2).

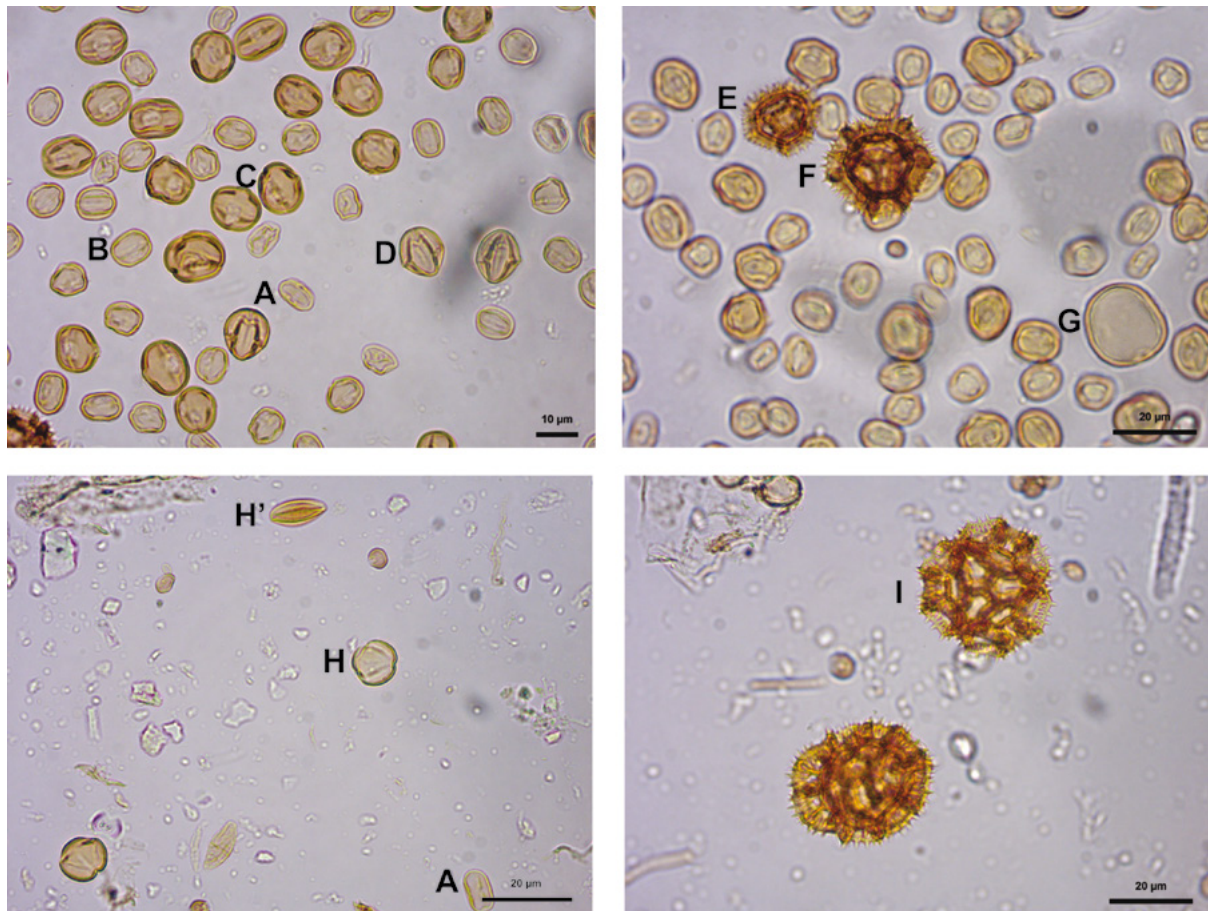
The fact that only *L. glaber* was identified composing the diet of *M. pusilla* would indicate strong specialization

on this species. However, as all the samples were from the same day, this result could be related to resources availability. Moreover, pollen analysis of 24 loads from 18 localities reported *Lotus* as abundant pollen and many families as hosts of *M. pusilla* in its area of origin, which is around the Mediterranean Sea in the Old World (Soltani et al., 2017), suggesting *polylecty*. Therefore, the preference for *Lotus* in this site was interpreted as temporal specialization. Its polylectism could help to explain its effective naturalization in the New World and other areas of the Old World.

An intensive usage of *Lotus* and other three genera in disparate tribes of Fabaceae was observed in *X. ciliata*.

**Table 1.** Abundance (%) of the pollen types found in *Xylocopa ciliata* and *Megachile pusilla* nest pollen samples, in alphabetic order of their families. The abundant resources ( $> 5\%$ ) are shown in bold. ‘+’ includes pollen types present in the slides but not recorded during the counting.

Plant family	Pollen type	<i>Xylocopa ciliata</i>				<i>Megachile pusilla</i>		
		nest 1 A	nest 1 B	nest 1 C	nest 2	nest 1	nest 2	nest 3
Amaryllidaceae	<i>Zephyranthes minima</i>			+				
Apiaceae	<i>Eryngium</i>				0.39			
Apiaceae	type <i>Ammi</i>		+					
Arecaceae	Arecaceae						+	
Asteraceae, Astereae	<i>Grindelia pulchella</i>		+		2.39			
Asteraceae, Astereae	type <i>Baccharis</i>		+		1.19			
Asteraceae, Cardueae	<i>Carduus acanthoides</i>				1.99			
Asteraceae, Cardueae	<i>Cirsium vulgare</i>			+	4.58			
Asteraceae, Eupatorieae	<i>Eupatorium buniifolium</i>				0.39			
Asteraceae, Heliantheae	<i>Ambrosia tenuifolia</i>						+	
Asteraceae, Lactuceae	<i>Picris echioides</i>			+			+	
Asteraceae, Lactuceae	type <i>Hypochaeris</i>		+	1.86				
Asteraceae, Vernonieae	<i>Vernonia</i>			+	1.39			
Brassicaceae	type <i>Nasturtium</i>						+	
Caprifoliaceae	<i>Lonicera japonica</i>		+				+	
Casuarinaceae	<i>Casuarina</i>				+			
Celtidaceae	<i>Celtis</i>						+	+
Dipsacaceae	<i>Dipsacus fullonum</i>			+			+	
Fabaceae, Caesalpinioideae	<i>Gleditsia triacanthos</i>		0.15				+	+
Fabaceae, Caesalpinioideae	<b>type <i>Senna-Chamaecrista</i></b>				<b>22.91</b>			
Fabaceae, Papilionoideae	<i>Adesmia bicolor</i>			<b>32.94</b>	0.79	+		
Fabaceae, Papilionoideae	<b><i>Galega officinalis</i></b>	<b>28.43</b>	<b>49.84</b>	<b>56.19</b>	<b>22.51</b>		+	
Fabaceae, Papilionoideae	<b><i>Lotus glaber</i></b>	<b>69.12</b>	<b>49.84</b>	<b>7.81</b>	<b>40.04</b>	<b>100</b>	<b>100</b>	<b>100</b>
Fabaceae, Papilionoideae	<i>Vicia sativa</i>	1.47	+	+				
Myrtaceae	<i>Eucalyptus</i>			+				
Poaceae	Poaceae type 1		+		0.79	+		+
Poaceae	Poaceae type 2	0.98	+					
Solanaceae	<i>Salpichroa origanifolia</i>				0.20			
Solanaceae	<i>Solanum</i>				1.18			
Verbenaceae	<i>Verbena</i>		0.15					
Unidentified	Unidentified 1 (4-colporate psilate)						+	
Unidentified	Unidentified 2 (monosulcate)				0.39			



**Fig 2.** Abundant (A, B, C, and H) and minor (D, E, F, G, I) pollen types identified from nests of *Xylocopa ciliata*, seen under light microscope. A\_ *Lotus glaber*; B\_ *Galega officinalis*; C\_ *Adesmia bicolor*; D\_ *Solanum*; E\_ type *Hypochaeris*; F\_ *Picris echinoides*; G\_ Poaceae type 1; H and H'\_ type *Senna-Chamaecrista* (H': colapsed grain); I\_ *Vernonia*.

The high abundance of Fabaceae in only four samples could be considered as a strong evidence of pollen specialization on the whole family: *oligolecty* (*sensu* Cane & Sipes, 2006) or *broad oligolecty* (*sensu* Müller & Kuhlmann, 2008). Furthermore, as vegetation was recorded in this site where nests were sampled, the pollen types foraged could be ascribed to the plant species found within this area. Moreover, this fact allowed for the identification of the abundantly foraged type *Senna-Chamaecrista* as not belonging to this natural plant community and likely belonging to an ornamental species cultivated in the urbanized landscape surrounding this park and therefore foraged out of the park. This fact could be a further evidence to support its pollen preference for Fabaceae.

Palynological studies have not yet been carried out in this *Xylocopa* species. Some of the records on floral resources seem to suggest *polylecty* (Sakagami et al., 1967; Sakagami & Laroca, 1971; Hurd, 1978; Schlindwein, 1998; Schlindwein et al., 2003; Gonçalves & Melo, 2005; Dalmazzo, 2010). However, the differentiation between males and females as well as nectar and pollen intake, necessary to identify the pollen specialization status (Cane & Sipes, 2006; Müller & Kuhlmann, 2008; Vossler, 2013; 2014; 2018), was not done in most of these studies, but the fact that *Solanum* has pollen-only flowers (Vogel, 1978; Buchmann, 1983) suggests that it

is a legitimate pollen host of *X. ciliata*. The study of a larger number of nest pollen samples is necessary to identify the specialization status of *X. ciliata* and would likely include *Solanum* as an abundant pollen host.

#### Acknowledgement

In memory of my dear friend Silvana Durante, specialist in Megachilidae, who encouraged me to start the study of the life history of bees. I also would like to thank Luciana Hiriart and Ivana Tapia for their help in the sampling of the vegetation of the Parque Ecológico Municipal de La Plata from August 2008 to August 2009 and the searching of bee nests.

#### References

- Buchmann, S.L. (1983). Buzz pollination in angiosperms. In C.E. Jones & R.J. Little (Eds.), *Handbook of Experimental Pollination Biology* (pp. 73–114). New York: Scientific and Academic Editions.
- Cabrera, A.L. (1971). Fitogeografía de la República Argentina. *Boletín de la Sociedad Argentina de Botánica* 14: 1–42.

- Cane, J.H. & Sipes, S. (2006). Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In N.M. Waser & J. Ollerton (Eds.), *Plant-Pollinator Interactions. From specialization to generalization* (pp. 99–122). Chicago and London: The University of Chicago Press.
- Dalmazzo, M. (2010). Diversidad y aspectos biológicos de abejas silvestres de un ambiente urbano y otro natural de la región central de Santa Fe, Argentina. *Revista de la Sociedad Entomológica Argentina* 69: 33–44.
- Erdtman, G. (1960). The acetolysis method, a revised description. *Svensk Botanisk Tidskrift* 54: 561–564.
- Gonçalves, R.B. & Melo, G.A.R. (2005). A comunidade de abelhas (Hymenoptera, Apidae s.l.) em uma área restrita de campo natural no Parque Estadual de Vila Velha, Paraná: diversidade, fenologia e fontes florais de alimento. *Revista Brasileira de Entomologia* 49: 557–571. doi: 10.1590/S0085-56262005000400017
- Hurd, P.D. (1978). An annotated catalog of the carpenter bees (genus *Xylocopa* Latreille) of the Western Hemisphere (Hymenoptera: Anthophoridae). Washington: Smithsonian Institution Press, 106 p.
- Kleinert-Giovannini, A. & Imperatriz-Fonseca, V.L. (1987). Aspects of the trophic niche of *Melipona marginata marginata* Lepeletier (Apidae, Meliponinae). *Apidologie*, 18: 69–100.
- Minckley, R.L. & Roulston, T.H. (2006). Incidental mutualisms and pollen specialization among bees. In N.M. Waser & J. Ollerton (Eds.), *Plant-Pollinator Interactions. From specialization to generalization* (pp. 69–98). Chicago: The University of Chicago Press.
- Müller, A. & Kuhlmann, M. (2008). Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox. *Biological Journal of the Linnean Society* 95: 719–733. doi: 10.1111/j.1095-8312.2008.01113.x
- Ramalho, M., Imperatriz-Fonseca, V.L., Kleinert-Giovannini, A. & Cortopassi-Laurino, M. (1985). Exploitation of floral resources by *Plebeia remota* Holmberg (Apidae, Meliponinae). *Apidologie*, 16: 307–330.
- Robertson, C.H. (1925). Heterotropic bees. *Ecology* 6: 412–436.
- Robertson, C.H. (1926). Revised list of oligolectic bees. *Ecology* 7: 378–380.
- Sakagami, S.F., Laroca, S. & Moure, J.S. (1967). Wild bee biocoenotics in São José dos Pinhais (PR), south Brazil. *Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology*, 16: 253–291.
- Sakagami, S.F. & Laroca, S. (1971). Observations on the bionomics of some Neotropical xylocopine bees, with comparative biofaunistic notes (Hymenoptera: Anthophoridae). *Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology*, 18: 57–127.
- Schlindwein, C. (1998). Frequent oligolecty characterizing a diverse bee-plant community in a xerophytic bushland of subtropical Brazil. *Studies on Neotropical Fauna and Environment*, 33: 46–59.
- Schlindwein, C., Schlumpberger, B., Wittmann, D. & Moure, J.S. (2003). O gênero *Xylocopa* Latreille no Rio Grande do Sul, Brasil (Hymenoptera, Anthophoridae). *Revista Brasileira de Entomologia*, 47: 107–118. doi: 10.1590/S0085-56262003000100016
- Soltani, G.G., Bénon, D., Alvarez, N. & Praz, C.J. (2017). When different contact zones tell different stories: putative ring species in the *Megachile concinna* species complex (Hymenoptera: Megachilidae) *Biological Journal of the Linnean Society*, 20: 1–18. doi: 10.1093/biolinnean/blx023
- Vogel, S. (1978). Evolutionary shifts from reward to deception in pollen flowers. In A.J. Richards (Ed.), *The pollination of flowers by insects* (pp. 89–96). London: Academic Press.
- Vossler, F.G. (2013). The oligolecty status of a specialist bee of South American *Prosopis* (Fabaceae) supported by pollen analysis and floral visitation methods. *Organisms Diversity and Evolution*, 13: 513–519. doi: 10.1007/s13127-013-0134-6
- Vossler, F.G. (2014). A tight relationship between the solitary bee *Calliopsis (Ceroliopoeum) laeta* (Andrenidae, Panurginae) and *Prosopis* pollen hosts (Fabaceae, Mimosoideae) in xeric South American woodlands. *Journal of Pollination Ecology*, 14: 270–277.
- Vossler, F.G. (2018). Are stingless bees a broadly polylectic group? An empirical study of the adjustments required for an improved assessment of pollen diet in bees. In P. Vit, S.R.M. Pedro & D.W. Roubik (Eds.), *Pot-Pollen in Stingless Bee Melittology* (pp. 17–28). Cham: Springer. doi: 10.1007/978-3-319-61839-5

