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Original article

Assessment of the pollen diet in a wood-dwelling augochlorine bee (Halictidae) using different approaches

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Abstract – Most studies on bee pollen diet are based on the abundance of each pollen type composing honey or pollen stores of whole nests (pollen counts). In an effort to characterize the diet of the augochlorine bee *Augochlora amphitrite*, we employed nest and intra-nest pollen analyses and bee flower visitation. In addition, we tested a novel approach, the index of relative importance (IRI), a method that combines pollen counts, pollen volume, and frequency of pollen occurrence. Using nest pollen analysis, we found that the *Ludwigia* type dominated pollen counts, comprising 56 % of whole nests, and thus would have concluded that this is the only most important pollen source in the diet. Instead, we found seven other important sources including *Ipomoea alba*, *Ipomoea cairica*, *Gymnocoronis spilanthoides*, and *Pavonia* types as well as 26 new floral hosts using all four methods in combination. Thus, we recommend that other studies of bee diet include these other approaches when possible and, especially, the novel approach of IRI.

Augochlora amphitrite **/ floral visitation / index of relative importance (IRI) / intra-nest pollen analysis / *Ludwigia***

1. INTRODUCTION

Pollination is an ecosystem service performed mainly by bees (Buchmann and Nabhan 1996; Proctor et al. 1996; Costanza et al. 1997; Waser and Ollerton 2006; Peters et al. 2013). At present, bees are the most important group of pollinators considering the number of species (>16,000 species) (Danforth et al. 2006) in the different environments of the world (Michener 2007). Due to the key role of bees as pollinators, bee biologists should aim to determine best practices or methods for accurately characterizing the diet of bees and groups of bees.

Bee diet can be studied by means of field observation of floral visitation and pollen analysis of pollen resources foraged. Floral visitation allows the identification of nectar and pollen plants; however, it is sometimes difficult to discriminate between them. It is also difficult to distinguish between male and female bees using this method, as male does not contribute for pollen provisioning and only gathers nectar (Cane and Sipes 2006). On the other hand, pollen analysis of nest provisions yields direct evidence of the presence of pollen plant-hosts and allows the quantification of each pollen source (Ramalho et al. 1985; Kleinert-Giovannini and Imperatriz-Fonseca 1987; Vossler et al. 2010; Smith et al. 2012). Moreover, direct observation of flowers from some plant species can often be difficult if they are inaccessible such as climbers or canopy/

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emergent tree species, and examining nest provisions can reveal pollen from these species (Cane and Sipes 2006). To study the pollen of nest provisions, it is necessary to find nests, a task that might be quite difficult to accomplish for several bee groups (Cane and Sipes 2006; Tellería and Vossler 2007; Vossler et al. 2010). For that reason, some surveys analyzed pollen loads from bees that were caught in flight or from bees deposited in entomological collections (Cilla et al. 2012; Vossler 2014a). On the other hand, when plant groups whose members are characterized by little or no morphological variation in their pollen grains (stenopalynous taxa sensu Erdtman (1952)) are present in bee diet, to identify the pollen specialization category of a bee species, pollen analysis and floral visitation methods must be combined (Vossler 2013).

To assess bee diets, an alternative to the commonly used nest pollen analysis (the pollen types found in a nest) is the intra-nest pollen analysis, which implies the study of the variety of pollen types found in each provision or groups of provisions within a nest. Although this deeper analysis has only been carried out for a few bee species (Malagodi-Braga and Kleinert 2009; Vossler et al. 2010; Rech and Absy 2011a, b), its importance is highlighted in this study. On the other hand, a combination of three measures into a single estimation of the relative importance of food types has been comprehensively used in other organisms, mainly fish, but never in bees (Hart et al. 2002). This index of relative importance (IRI) was proposed by Pinkas et al. (1971) and combines the counting of particular food items (such as prey species), estimation of their volume, and frequency of their occurrence (Hart et al. 2002). Although IRI does not include nutritional values of food items, it reduces bias in cases when only one of the components is used. It is especially important when prey items ingested are of different sizes and represent many prey types, as stressed by Tavares-Cromar and Williams (1996). This point was also highlighted for pollen resources composing *Apis mellifera* L. diet (Biesmeijer et al. 1992).

Most literature on bee diets based on palynological studies estimates the importance of each floral resource by counting the pollen grains of each pollen type composing honey or pollen stores of whole nests. Other fewer studies also included volume estimates of the pollen types foraged (da Silveira 1991; O'Rourke and Buchmann 1991; Biesmeijer et al. 1992; Vossler et al. 2010; Vossler 2014b). However, no studies have combined both in a single measurement and index. For this reason, the aims of this study were to identify the pollen resources foraged by using nest and intra-nest pollen analyses and quantify the relative importance of each pollen host in the diet of the native bee *Augochlora amphitrite* (Schrottky) using IRI. The results here obtained by pollen analysis and floral visitation were compared with those of other *Augochlora* species and with previous floral records for *A. amphitrite*. Some floral resources of *A. amphitrite* have been recorded from the Pampean region (Dalmazzo and Roig-Alsina 2011).

The family Halictidae is composed of four subfamilies with both polylectic and oligolectic bees (Michener 2007). Studies on diet of Augochlorini species are scarce, and there are no recorded cases of oligolecty. Most of the information available for these bees consists of floral visitation records compiled in bee catalogues (Hurd 1979; Moure 2007). The diet of most *Augochlora s. str*. species has mainly been known by means of floral visit records (Antonini and Martins 2003; Dalmazzo 2010; Gimenes 2002; Gonçalves and Melo 2005; Imperatriz-Fonseca et al. 2011; Krug et al. 2010; Lopes-Azambuja and Blochtein 2007; Minussi and Alves dos Santos 2007; Schlindwein 1998; Schlindwein and Wittmann 1997; Singer and Cocucci 1999; Steiner et al. 2010). However, pollen analysis was done for *Augochlora alexanderi* Engel, *Augochlora esox* (Vachal), and *Augochlora isthmii* Schwarz (Zillikens et al. 2001; Wcislo et al. 2003).

2. MATERIALS AND METHODS

2.1. Study site and nesting area

This study was carried out in the reserve Refugio Natural Educativo "Ribera Norte" (34° 28′ 10″ S, 58° 29′ 40″ W) located in the northeast of Buenos Aires province, Argentina. This reserve consists of a 10 Ha fragment of riparian forest located on the banks of the Río de la Plata. The soil is waterlogged during certain periods of the year. In this reserve, the riparian forest contains a tree stratum of 12 to 15 m in height, shrubby and herbaceous strata, and abundant lianas and

epiphytes (Cabrera and Zardini 1978). This forest has been invaded by several alien plant species, such as "ligustrina" (*Ligustrum sinense* Lour.) (Oleaceae) and "lirio amarillo" (*Iris pseudacorus* L.) (Iridaceae).

Two nest aggregations of the wood-dwelling native bee *A. amphitrite* were found 1 m apart from each other and 80 m away from the river. One of them was found in a fallen and decomposed tree trunk of *Salix humboldtiana* and the other in a decomposed railroad sleeper of "quebracho colorado" (*Schinopsis* sp.) (Anacardiaceae). One aggregation was composed of 14 inactive nests having only feces and the other of three nests having both feces and provisions (Online Resource 3). The structure of these nests was described by Dalmazzo and Roig-Alsina (2012).

2.2. Pollen analysis of nest provisions

Pollen analysis of each cell (intra-nest pollen analysis) was carried out to elucidate the importance of particular pollen types that might have been hidden during the averaging process of all cells of nests. During autumn (April 2008) and during summer (February 2009), 51 cells (nests 1–14) and 25 cells (nests 15–17) were, respectively, extracted and pollen masses or feces were analyzed. Although nests were sampled during February and April, their pollen reserves and/or feces could have been foraged at any time from September to March. To obtain pollen sediment from each cell, provisions and feces were dissolved in water at 80–90 °C for 10 min; this mixture was then hand-shaken and centrifuged at 472×*g* for 10 min. Pollen sediment was mounted on slides and dyed fuchsia following the Wodehouse (1935) technique. Pollen identification was carried out by comparing pollen provision slides with the pollen reference of plants that grow in the reserve under a Nikon Eclipse E200 light microscope. A total of 300 pollen grains per cell were counted (the number of cells per nest ranged from 1 to 11, see Figure 2). The minimum number of grains to be counted was established by means of a previous microscopic examination of the slides. No new pollen types were identified in the samples when 300 grains were counted. This procedure is also commonly used for vegetation sampling in phytosociological studies and it is known as "the minimal area" or "species-area curve" (Kent and Coker 1992, p. 41). Pollen types occurring in >10 % were considered as the most important pollen resources, following Ramalho et al. (1985) and Cortopassi-Laurino and Ramalho (1988). As the *Ludwigia* grains were dispersed as tetrahedral tetrad, they were counted as individual grains. Furthermore, as some individual grains from *Ludwigia* tetrads were detached, all grains were counted and then the number was divided by four. In the present study, the pollen type *Ludwigia* included all *Ludwigia* species that have their pollen grains aggregated as tetrads, the type *Baccharis* included *Baccharis* species and *Solidago chilensis*, the type Lamiaceae included four species in three genera of Lamiaceae, the type *Pavonia* included two *Pavonia* species, the type *Solanum*? included three *Solanum* species, and the type *Croton* included *Croton* species and *Manihot grahamii*. Pollen grains from provisions were examined and photographed using a PHILIPS XL30 scanning electron microscope (Figure 1).

In order to detect differences in pollen composition of nest provisions, a cluster analysis was performed. For cluster analysis of nests (matrix in Q-mode) and pollen types (R-mode), the Ward's method and Euclidean distance measure were applied to the abundance values of the data matrix. The *two-way clustering* option was applied to see simultaneous clustering in R-mode and Q-mode (Online Resource 1). For cluster analysis, the PAST statistic package version 1.81 (Hammer et al. 2008) was used.

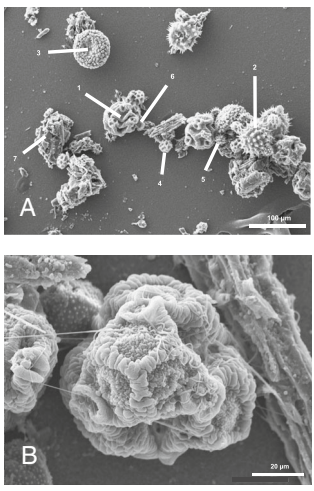

Figure 1. a Scanning photo-micrograph of pollen grains found in *A. amphitrite* provisions of the nest 16, showing pollen types of different volumes: *1* collapsed tetrads of *Ludwigia*, *2 Ipomoea cairica*, *3* type *Croton*, *4 Sonchus oleraceus*, *5 Smallanthus connatus*, *6 Gymnocoronis spilanthoides*, *7* sawdust fragments from cells. Scale bar 100 μm. **b** Scanning photo-micrograph of a tetrad of *Ludwigia*. Scale bar 20 μm.

2.3. IRI

For ranking the relative importance of the pollen types found in the provisions, the index of relative importance (IRI) was estimated using the formula: IRI=(N+V)F, where N is the numerical percentage, V is the volumetric percentage, and F is the frequency of occurrence percentage (Pinkas et al. 1971; Hart et al. 2002). The *numerical percentage* was calculated by counting the number of grains per pollen type (or tetrads for *Ludwigia*) and then they were added across the 17 nests. This value was divided by the total sum of all these values and multiplied by 100, obtaining the N value (the percentage value of counts) (Table II). To calculate the *volumetric percentage*, the mean absolute volume of each pollen type was divided by the sum of mean absolute volume of all types (15 types) and then multiplied by 100 (Table II). To measure absolute volumes of monads (14 pollen types) and individual grains of *Ludwigia*, the methodology of Buchmann and O'Rourke (1991), O'Rourke and Buchmann (1991), Vossler et al. (2010), and Vossler (2014b) was followed. For *Ludwigia* (tetrad), this value was multiplied by four. Finally, *frequency of occurrence percentage* is the percentage of samples containing each pollen type. In this study, the IRI was calculated considering each nest, not each cell. As example, the pollen type *Ipomoea alba* was found in 4 out of the 17 nests; therefore, 4 was divided by 17 and multiplied by 100 obtaining thus the F value (23.5 %).

According to IRI, the most important pollen hosts of a diet are those having a combination of maximum values of numeric percentage (N), large volumes (V), and/or high frequency of occurrence among samples (F). Because only a few grains of the types Lamiaceae, *Sagittaria montevidensis*, and *Solanum*? were available in the slides, their volume values were taken from the types *Salvia*, *S. montevidensis*-*Hydrocleys*, and *Solanum kurtzianum* from Roulston et al. (2000), Vossler (2014b), and Fernández et al. (2009), respectively.

2.4. Floral visitation

Field observations of *A. amphitrite* on flowers were carried out between September 2008 and March 2010, which is the period of flight activity of *A. amphitrite*. Bees were caught and deposited in the entomology collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). Visited plants were collected, pressed, dried, identified, and deposited in the Herbarium of La Plata (LP), Buenos Aires, Argentina. The results of pollen analysis and floral visitation were compared with *A. alexanderi*, *A. esox*, and *A. isthmii* and with previous floral records for *A. amphitrite* (Online Resource 2).

3. RESULTS

3.1. Nest and intra-nest pollen analysis

A total of 15 pollen types belonging to 24 plant species and 10 plant families composed the diet of *A. amphitrite* (Table I, Figure 1). This bee species used significant amounts of pollen (>10 % per nest) from five species of five families: *Ludwigia* (Onagraceae), followed by *Gymnocoronis spilanthoides* (Asteraceae), *Ipomoea cairica* (Convolvulaceae), *Commelina diffusa* (Commelinaceae), and *Syagrus romanzoffiana* (Arecaceae).

All nests were composed of at least 56 % of *Ludwigia* (Onagraceae). However, the intra-nest analysis revealed that more than 40 % of 12 cells from seven nests were composed of seven pollen types other than *Ludwigia*: *Echinodorus grandiflorus* (1 cell, 49 %), *S. romanzoffiana* (1 cell, 62 %), *G. spilanthoides* (6 cells, 43–95 %), *Sonchus oleraceus* (1 cell, 41 %), *C. diffusa* (2 cells, 40–61 %), and *I. cairica* (1 cell, 45 %). The type *Baccharis* (41 %) was found in a cell together with *G. spilanthoides* (Figure 2).

Nests were clustered by similarity of composition and abundance of their pollen types, showing two clusters (Online Resource 1). These two groups of nests were different in the abundance of *Ludwigia* and the presence of *G. spilanthoides*. Thus, most of the nests studied (group B) presented extremely high abundance of *Ludwigia* (83.5 to 98.3 %) and absence of *G. spilanthoides* while five nests (Group A) from 55.9 to 72.4 % of *Ludwigia* and from 12.3 to 37.2 % of *G. spilanthoides* (see Online Resource 1). Each group was composed of nests belonging to the 2008 and 2009 sampling periods; as example, group B was composed of nest 7 from 2008 and nest 17 from 2009.

Table I. Absolute frequency (number of nests having each pollen type) and abundance of each pollen type per nest (minimum to maximum values).

Plant family	Pollen type	Absolute frequency	Abundance
Alismataceae	*Echinodorus grandiflorus*	5	0.01–9.80
	Sagittaria montevidensis	2	0.05–0.09
Arecaceae	*Syagrus romanzoffiana*	4	0.03–15.78
Asteraceae, Astereae	Type *Baccharis*	2	0.03–4.16
Asteraceae, Eupatorieae	*Gymnocoronis spilanthoides*	6	0.47–37.17
Asteraceae, Heliantheae	*Smallanthus connatus*	1	2.36
Asteraceae, Lactuceae	*Sonchus oleraceus*	3	0.04–3.70
Commelinaceae	*Commelina diffusa*	4	0.17–14.74
Convolvulaceae	*Ipomoea alba*	4	0.11–1.04
	Ipomoea cairica	16	0.23–16.04
Euphorbiaceae	Type *Croton*	1	0.10
Lamiaceae	Type Lamiaceae	1	0.43
Malvaceae	*Pavonia*	2	0.17–0.19
Onagraceae	*Ludwigia*	17	55.89–97.14
Solanaceae	Type *Solanum*?	1	0.10

Taking into consideration the similarity of frequency of occurrence and abundance in nests, pollen types were grouped in four clusters: group 1 (*Ludwigia*), group 2 (*G. spilanthoides*), group 3 (*C. diffusa* and *I. cairica*), and group 4 (the remaining pollen types) (Online Resource 1).

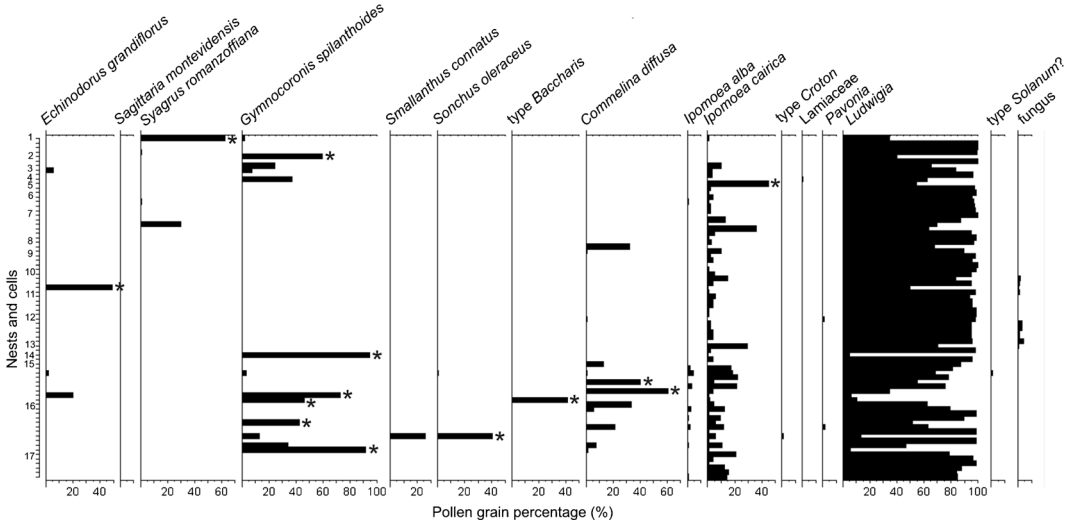

Figure 2. Composition of the 76 cells analyzed. *Bars* indicate the percentage of the pollen types of each cell. Some cells were composed of more than 40 % by other seven pollen types (*asterisk*) apart from *Ludwigia*. Nest *1* was composed of 4 cells, nest *2* of 4 cells, nest *3* of 1 cell, nest *4* of 1 cell, nest *5* of 3 cells, nest *6* of 4 cells, nest *7* of 6 cells, nest *8* of 3 cells, nest *9* of 4 cells, nest *10* of 5 cells, nest *11* of 5 cells, nest *12* of 6 cells, nest *13* of 3 cells, nest *14* of 1 cell, nest *15* of 10 cells, nest *16* of 11 cells, and nest *17* of 5 cells.

3.2. Absolute volume of pollen grains

The type *I. alba* had the highest value of absolute volume (1,105,322±200,296 μm^3) followed by the type *Croton* (246,214±79,057 μm^3), *Ludwigia* tetrads (222,824±82,846 μm^3), *Pavonia* (214,158±112,536 μm^3), and *I. cairica* (62,522±3557 μm^3); the remaining types had volume values less than 20,000 μm^3.

3.3. The IRI

The IRI values of the 15 pollen types foraged by *A. amphitrite* ranged from 0.45 for the type *Solanum*? to 9463.82 for *Ludwigia* (Table II). The extremely high IRI value of *Ludwigia* is due to both its intensive foraging by bees (N=82.93 %) and its constancy across all nests (F=100 %). Its V value was low (V=11.70 %), despite that it included the four grains of the tetrad. The high IRI value of *I. alba* was mainly due to the V value (58.06 %). This pollen type was found in nearly a quarter (F=23.53 %) of the nests and was hardly foraged (N=0.11 %). On the other hand, the IRI value of *I. cairica* was mainly due to the F value (94.12 %) because this pollen type was found in 16 out of the 17 nests and by N value of 5.40 %. The same occurred for *G. spilanthoides* (F=35.29 % and N=7.13 %). The fifth place of IRI value was for *Pavonia* due to both frequency of occurrence and volume (11.76 and 11.25 %, respectively) (Table II).

3.4. Floral visitation

In the site studied, *A. amphitrite* was observed visiting flowers of 19 plant species of nine families (Online Resource 2). Six out of these 19 species were also found in the provisions by the pollen analysis method, belonging to six families (Alismataceae, Asteraceae, Commelinaceae, Convolvulaceae, Malvaceae, and Onagraceae). The families Apocynaceae, Bignoniaceae, and Fabaceae were recorded only by floral visitation, and Arecaceae, Euphorbiaceae, Lamiaceae, and the type *Solanum*? possibly from Solanaceae only by pollen analysis. A total of 31 species (from both pollen analysis and floral visitation) were

Table II. Pollen types arranged in decreasing order of their index of relative importance (IRI) to bee diet. Values of N (numerical percentage), V (volumetric percentage), and F (frequency of occurrence percentage) are also provided.

Pollen type	N	V	F	IRI=(N+V)F
Ludwigia	82.93	11.70	100.00	9463.82
Ipomoea alba	0.11	58.06	23.53	1368.83
Ipomoea cairica	5.40	3.28	94.12	817.15
Gymnocoronis spilanthoides	7.13	0.09	35.29	254.74
Pavonia	0.02	11.25	11.76	132.59
Type *Croton*	0.006	12.93	5.88	76.12
Commelina diffusa	1.71	0.99	23.53	63.43
Syagrus romanzoffiana	1.24	0.28	23.53	35.80
Echinodorus grandiflorus	0.80	0.10	29.41	26.57
Sonchus oleraceus	0.22	0.34	17.65	9.98
Sagittaria montevidensis	0.009	0.31	11.76	3.70
Type *Baccharis*	0.25	0.06	11.76	3.56
Type Lamiaceae	0.03	0.33	5.88	2.07
Smallanthus connatus	0.14	0.21	5.88	2.03
Type *Solanum*?	0.006	0.07	5.88	0.45
Total	100	100	405.88	12,260.82

identified in this study, 26 of them are new floral records for *A. amphitrite* (Online Resource 2).

4. DISCUSSION

4.1. Nest and intra-nest pollen analyses and its importance in assessing bee diets

Nest pollen analysis allowed the identification of the pollen hosts foraged by this augochlorine bee. Cluster analysis revealed that nest provisions sampled during two different years (April 2008 and February 2009) had similar composition since *Ludwigia* was at flowering during summer-autumn when nest pollen was sampled (Online Resource 1 and 3).

According to mean values of pollen percentage per nest (nest pollen analysis), *Ludwigia* was the most important pollen resource in the diet of *A. amphitrite*. However, the intra-nest pollen analysis showed that some cells were composed of more than 40 % of other seven pollen hosts. If only the average values had been analyzed, these seven pollen hosts would have been overlapped by *Ludwigia* and misinterpreted as contaminant (pollen types <5 % in average values). This issue has already been discussed when assessing the diet of other bees such as *Diadasia diminuta* by Cane and Sipes (2006). Even more, the intra-nest pollen analysis was important in the detection of different pollen hosts composing the diet of other bees (Malagodi-Braga and Kleinert 2009; Vossler et al. 2010; Rech and Absy 2011a, b).

4.2. The importance of IRI to assess bee diets

According to IRI, the most important pollen hosts for the diet of *A. amphitrite* were those having a combination of high values of numeric percentage, large volumes, and/or high frequency among the samples; they were *Ludwigia*, *I. alba*, and *I. cairica*, followed by *G. spilanthoides* and *Pavonia*. If only *N* values were used, the importance of *Pavonia* and *I. alba* would be underestimated and *C. diffusa* and *S. romanzoffiana* overrated in the diet of *A. amphitrite*. If only *V* values were used, the importance of *G. spilanthoides* would be underestimated and type *Croton* overrated. If only *F* values were used, the importance of type *Croton* would be underestimated and *Echinodorus grandiflorus* overrated. The advantage of IRI is that it combines number, frequency, and volume values avoiding the underestimation of food items (i.e., pollen types) with large volume but scarcely foraged, or reverse, which happens when these values are considered separately. Furthermore, food eaten by many individuals but in small numbers or small volumes will have a high frequency of occurrence, but may not be highly important in the diet (Pinkas et al. 1971; Hart et al. 2002).

The index of relative importance has mostly been applied to fish diets, but some authors have suggested that it could be successfully applied to dietary studies in other animal groups (Hart et al. 2002). The present study constitutes the first application of IRI to the study of bee diets. It was a very useful indicator of the relative importance of the several pollen types that composed the diet of *A. amphitrite*, and it can be used in the diet characterization of other bee groups.

4.3. Methodological aspects in the use of IRI

To assess the diet from pollen masses of *A. amphitrite*, a total of 300 grains per cell proved to be enough as all pollen types found in each slide were present in that counting. Moreover, as all cells of each nest were analyzed (from 1 to 11 cells), the total number counted ranged from 300 to 3300 grains per nest. A total of 1000 grains per nest (counted on three slides) was used by Ramalho and Kleinert-Giovannini (1986) as representative to study stingless bee diet. On the other hand, the counting of a higher number of grains is necessary to analyze honey composition (Vergeron 1964) due to the presence of many pollen types from nectar plants, which are commonly underrepresented. The minimum number of grains considered as representative of a sample (either cell or nest) must be in accordance to the sample nature (pollen vs honey, number of pollen subsamples considered as a sample, etc.) and must be previously clarified by the researcher. Vergeron (1964) established that the minimum number of grains to be counted for honey samples will be

different according to the richness of pollen types found in a sample.

Another source of error includes the estimation of the volume of pollen grains. We consider that volume formulae are helpful and reliable to estimate the volume of pollen grains and therefore to the understanding of bee diet. Although pollen grains are not geometric figures, the calculation of volume correction coefficient (Q) from mean diameters to the third power (d^3), as proposed by Silveira (1991), also brings about an error due to the fact that d^3 is the formula of a cube, and grains identified in *A. amphitrite* pollen diet were ellipsoidal and spherical but not cubic-shaped. Moreover, the volumetric correction coefficient (Q) suggested by Silveira (1991) and the volume values estimated by geometric formulae of sphere and ellipsoid are statistically similar (as stated by da Silveira 1991, p. 498). For all the above stated, we consider that the usage of spherical and ellipsoidal geometric formulae for volume calculation is adequate. As e and p axes are easy to distinguish between them and many articles provide pollen volume measurements (O'Rourke and Buchmann 1991; Buchmann and O'Rourke 1991; Biesmeijer et al. 1992; Roulston et al. 2000; Torres 2000; Fernández et al. 2009; Vossler 2014b), the usage of volume formulae is preferred.

4.4. Advantages and limitations of IRI

Advantages of IRI This index is a combination of three measurements into a single estimation of the relative importance of food types. Thus, this measurement gives us an idea about which resources are most important to a particular bee species (one IRI value is given for each plant resource). Therefore, they are ranked according to their importance for a bee in a given period and site of sampling.

Limitations of IRI The calculation of IRI implies a previous pollen volume calculation. To do this, it is necessary to measure the diameter of several pollen grains, which is time-consuming and it varies according to the number of pollen types involved in the sample and in the environment where samples were taken. However, it can be done at the beginning of the research together with the identification of pollen grains.

4.5. Pollen analysis and floral visitation as complementary methods

Pollen analysis of nest provisions allowed the identification of pollen plant-hosts foraged as well as the quantification of each of them including those with flowers difficult and/or inaccessible for observation (such as the palm tree *Syagrus romanzoffiana*). However, two aspects of the diet of *A. amphitrite* could be elucidated by floral visitation. Firstly, certain nectar hosts (such as *Asclepias curassavica* L.) (Apocynaceae) have pollen grains packed in pollinia and are not stored in nests. Secondly, the identification of the species foraged by bees that are constrained when pollen types were stenopalynous (such as *Ludwigia* and *Pavonia*) (Online Resource 2).

As nine floral hosts were only recorded by pollen analysis and 13 only by floral visitation, the use of both methods together allowed the identification of 31 species belonging to 16 genera and 13 families. The comparison between foraged and available floral resources suggested that the bias in pollen choice in this polylectic bee could be time-specific (Dalmazzo and Vossler, unpublished data). However, as floral resources identified by pollen analysis were foraged presumably at summer, others could had been intensively gathered at spring (September-November) as recorded by floral visits. For this reason, this study shows the importance for using these two complementary methods in a same environment to assess bee diets. A total of 68 plant species had been recorded by means of floral visitation along the geographical range of *A. amphitrite* (five of them were also recorded in the present study). By means of floral visits together with pollen analysis, 26 new floral hosts were recorded in the present study reaching a total of 94 hosts from 25 families for *A. amphitrite* (Online Resource 2). For *Augochlora s. str.* species in which both methods have been used, a broader spectrum of floral hosts was found (94 for *A. amphitrite*, 59 for *A. esox*, 14 for *A. isthmii*, and 2 for *A. alexanderi*).

In order to understand wild bees in their natural environment, a greater knowledge of the bee diet can be achieved using different approaches than only one.

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Evaluation du régime pollinique d'une abeille augochlorine (Halictidae) via différentes approches

Augochlora amphitrite* / visite des fleurs / index d'importance relative / IRI / analyse du pollen / intérieur du nid / *Ludwigia

Untersuchung der Pollendiät einer holznistenden augochlorinen Biene (Halictidae) mittels unterschiedlicher Ansätze

Augochlora amphitrite* / Blütenbesuch / Index der relativen Wichtigkeit (IRI) / intranidale Pollenanalyse / *Ludwigia

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