Functional specialization and phenotypic generalization in the pollination system of an epiphytic cactus

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ABSTRACT

Plant-pollinator interactions range from obligatory specialists to facultative generalists, and floral morphology and pollination system may not match completely. The floral biology, reproductive system and floral visitors of a species of the tribe Rhipsalideae were investigated with a focus on the consistency between the pollination system and the floral phenotype. *Rhipsalis neves-armondii* is an obligate xenogamous species, due to self-sterility. Its flowers are white, small and diurnal, and radially symmetrical. These features, along with their small amount of nectar, characterize the flowers as phenotypic generalists. The most frequent pollinators were a solitary oligolectic species of Andrenidae (*Rhophitulus solani*), two species of Meliponinae (*Trigona spinipes* and *T. braueri*) and *Apis mellifera*. Despite the generalist floral phenotype, the pollination system is functionally specialized, since only small bees performed effective visits. Flowers of *R. neves-armondii* may represent a case of cryptic floral specialization in which attributes other than morphology act as filters, restricting them to a single functional group of pollinators. Moreover, the four most frequent species of pollinators cover a spectrum ranging from solitary oligolectic to social polylectic bees, including an exotic species. These results illustrate the distinct dimensions of specialization-generalization that may occur in the pollination process of a single species.

Keywords: Cactaceae, floral biology, montane rain forest, oligolectic bees, pollinators, reproductive system, *Rhipsalis,* self-incompatibility

Introduction

The interactions between plants and pollinators are one of the most frequent associations in nature, and are highly important in structuring populations, communities and ecosystems (Ollerton 2006; Sargent & Ackerly 2008). Mutualism between these two groups of organisms is based on a condition of mutual exploitation, in which the animals acquire food or some other resource, while the plants obtain effective vectors for their sexual reproduction (Nicolson & Wright 2017). In this relationship, factors such as the efficacy and frequency of visitation of pollinators, together with the morphological attributes of both species influence the plant's reproductive success (Stebbins 1970; Ne'eman *et al*. 2010; Freitas 2013; King *et al*. 2013). Assessment of the morphological adjustment between flowers and pollinators is important in evaluating plant-pollinator interactions (Anderson *et al*. 2010). This perspective is related to the concept of pollination syndromes, which establishes that sets of floral attributes, such as shape, color, size, odor and resources, reflect the specific group of pollinators. Thus, certain flower characteristics are expected to be adaptive responses to the pressures exerted by the respective group

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of pollinators, leading to convergent floral evolution in unrelated species (Faegri & Pijl 1979).

The view that pollination systems tend toward specialization, which is implicit in the concept of pollination syndromes, has been challenged after some studies proposing that generalist systems are quite frequent (Waser *et al*. 1996). This debate has expanded past the initial polarization, to arrive at the understanding that plant-pollinator interactions are distributed on a gradient from one extreme of obligate specialists, involving interaction between only two species, to the other extreme of facultative generalists, in which flowers can be appropriately served by a wide range of pollinators (Johnson & Steiner 2000; Ollerton *et al*. 2007). Broadly viewed, this categorization refers to the plant niche width, whereby specialist plant species would have narrower pollinating niches than would generalist species (Grant & Grant 1965; Armbruster 1993; Thomson & Wilson 2008; Gómez *et al*. 2014). The concept of specialization of plant-pollinator interactions has been refined to include a variety of parameters from different perspectives (ecological, phenotypic and evolutionary) (e.g. Ollerton *et al*. 2007). All these proposals are valid, but a clear definition in the context of each study is necessary, because different interpretations of specialization have different evolutionary and ecological implications (Armbruster 2017).

Considering the wide spectrum of degrees of specialization or generalization in the relationships between plants and pollinators, Ollerton *et al*. (2007) proposed to categorize the specialization-generalization of a plant species in relation to its pollinators in three modalities: (1) *functional*, which means the diversity of pollinators of a plant at higher taxonomic levels or functional groups (e.g. plants pollinated by bats, resin-bees or hummingbirds); (2) *ecological*, which means the number of pollinator species that interact with the plant; and (3) *phenotypic*, which encompasses the floral characters that make the flower potentially more or less restrictive to a pollinator group (e.g. type and accessibility to the floral resource). From this perspective, it is possible to have opposing combinations of these categories, as in cases where phenotypically generalist flowers are functionally specialists and vice versa (Ollerton *et al*. 2007). Recently, Armbruster (2017) suggested changing the term "*functional*" to "*functional group*" to bring more clarity to the classification, since the former term is already used in other fields of study.

Species bearing phenotypically generalist flowers generally have pollination systems with ecological and functional generalization, although they may be specialized in some dimension (e.g. McIntosh 2005; Ollerton *et al*. 2007; Shuttleworth & Johnson 2009; Narbona & Dirzo 2010; Niemirski & Zych 2011; Bartoš *et al*. 2015). Little is known about the mechanisms by which phenotypically generalist-plants filter their pollinators (Johnson & Steiner 2000), and a first step toward understanding these mechanisms is the description of the pollination system and its characterization in terms of phenotypic, ecological and functional group specialization-generalization (Ollerton *et al*. 2007; Armbruster 2017).

Cactaceae comprises about 1,500 species (Hunt *et al*. 2006) with a wide range of floral syndromes and pollination systems (Pimienta-Barrios & Castillo 2002; Mandujano *et al*. 2010; Schlumpberger 2012). Cacti are zoophilic but are associated with diverse animal groups, such as bees, butterflies, moths, bats, hummingbirds and other birds. Species may be pollinated exclusively or not by each animal group, leading to pollination systems with different degrees of functional-group specialization (Pimienta-Barrios & Castillo 2002; Mandujano *et al*. 2010; Schlumpberger 2012). Moreover, most species of Cactaceae are xenogamous, which makes them dependent on pollinators for sexual reproduction (Ross 1981; Boyle 1997; Mandujano *et al*. 2010).

"Cactus" usually evokes an image of large columnar shrubs with succulent spiny stalks, living in deserts or semi-arid environments. However, epiphytic cacti are a prominent component in the physiognomy of certain humid forests in the Neotropics. Epiphytism occurrs in ca. 10 % of the species of Cactaceae and has a strong phylogenetic signal, as it is restricted to Hylocereeae and Rhipsalideae (Barthlott 1983). Knowledge of the pollination biology of epiphytic cacti is sparse, being restricted to anecdotal reports (e.g. Fleming *et al*. 2009) and to a study on pollination by bats in *Weberocereus tunilla* (Tschapka *et al*. 1999). *Rhipsalis*, the largest epiphytic genus of Cactaceae, has flowers that suggest a generalist pollinating system, since they are small and radial, and their resources (nectar and pollen) are apparently accessible to many types of visitors.

We here described the pollination biology of *Rhipsalis neves-armondii* and discussed the results from the perspective of the degree of phenotypic, functional and ecological specialization-generalization. Our expectation was that pollination system of *R. neves-armondii* would be functionally and ecologically generalized because of its generalist floral phenotype.

Materials and methods

Species and area of study

Rhipsalis is composed of 37 species, of which 86 % are endemic to Brazil (Taylor *et al*. 2015). The genus includes three phylogenetically well-supported subgenera: *Rhipsalis*, *Erytrorhipsalis* and *Calamorhipsalis*, this last having flowers with the pericarpel visibly immersed in the areola (Calvente *et al*. 2011b; Calvente 2012). *Rhipsalis neves-armondii* K.Schum. is an epiphytic (Fig. 1A), occasionally rupiculous species of the subgenus *Calamorhipsalis* from the Brazilian Atlantic Forest (Taylor *et al*. 2015). The flowers, although they are the largest of the genus, are less than 2 cm long (Fig. 1B, C). The fruits are violaceous berries (Fig. 1D),
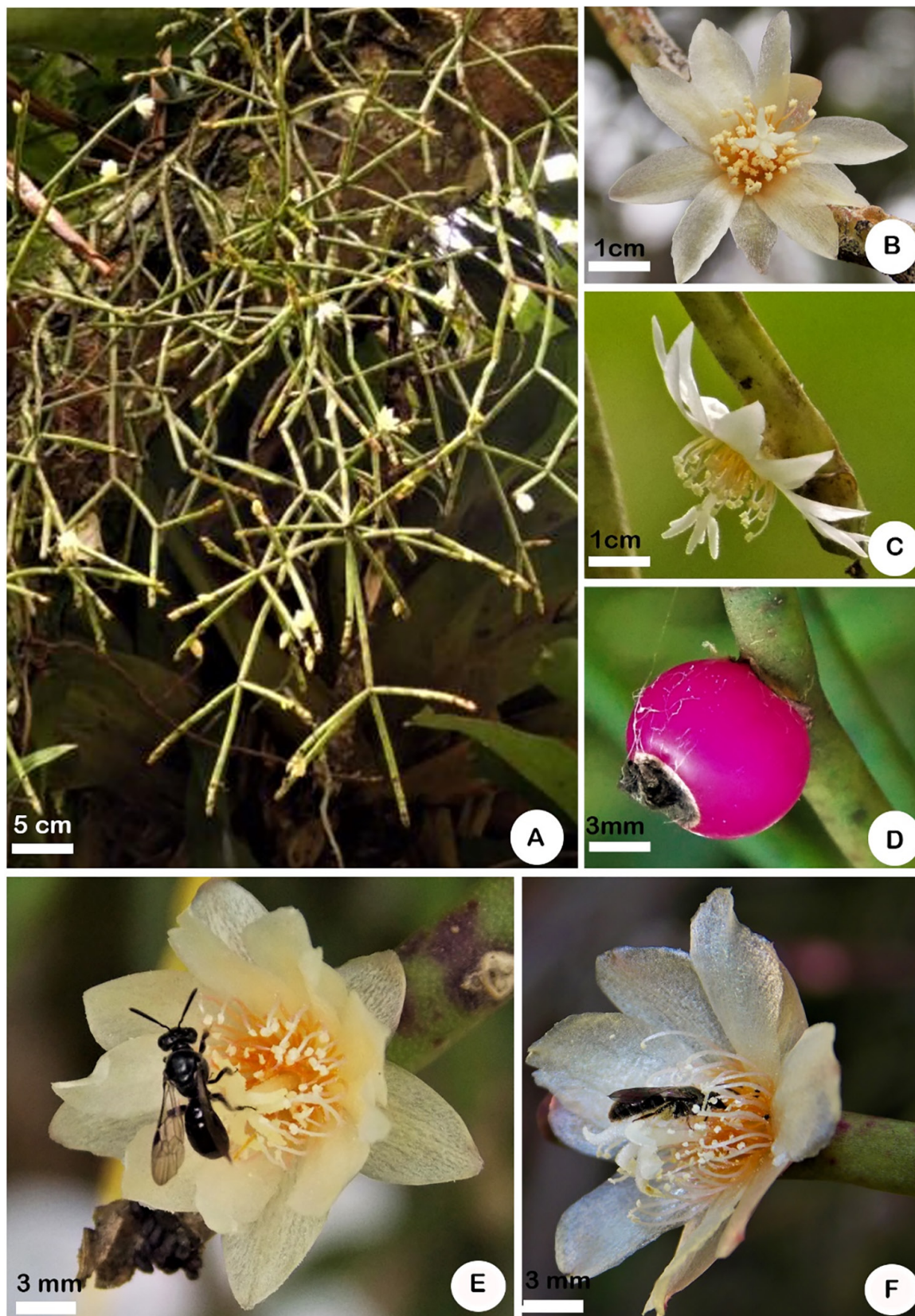
Figure 1. Flowering individual of *Rhipsalis neves-armondii* in the National Park of Serra dos Órgãos, southeastern Brazil (**A**). Flower in frontal (**B**) and side view (**C**), showing approach herkogamy due to taller stigma. Mature berry fruit (**D**). Visits to the flower by *Rhophitulus solani* (Andrenidae), with the bee using the stigma as a landing platform (**E**) and directing its head toward the base of the flower to take nectar (**F**).

which are attractive to birds of various groups that are the likely seed dispersers (Anderson 2001; Schlumpberger *et al.* 2006). A voucher of *R. neves-armondii* was deposited in the herbarium RB (632076).

The study was carried out in March and April 2014, in a remnant of the Atlantic Forest in the Serra dos Órgãos National Park (PARNASO), southeast Brazil (22°25'-22°32'S 42°59'-43°07'W, ca. 1000 m a.s.l.). PARNASO lies within the area of the Atlantic Forest where the highest species richness of *Rhipsalis* has been recorded (at least 18 species) (Calvente *et al.* 2005). The climate is tropical mesothermic with mild summers and a short dry season in the winter months, reflecting the effects of the altitude (Köppen 1936). Flowering occurred at the end of the rainy season, in March and April, and among the ten sympatric species of *Rhipsalis* in this area, only *R. neves-armondii* was flowering during this period in 2014.

Floral biology

Corolla diameter and pistil length were measured in 25 flowers from five individuals, in which the number of tepals, stamens and ovules were also counted. The anthesis time and floral longevity were recorded by direct observation of 15 flowers of three individuals. We measured the spectral reflectance of floral structures using a USB2000+UV-VIS spectrometer with a Balanced Deuterium Tungsten Source (DH-2000-BAL, Ocean Optics Inc., Dunedin, FL, USA), calibrated with a standard white (BaSO4). The readouts were limited into wavelengths from 300 to 700 nm. The concentration and volume of nectar were measured with a pocket refractometer and microcapillaries (1 μL), respectively, in previously bagged flowers in pre-anthesis (N = 15) of five individuals. Nectar was measured at three different times in the same flowers, on the first day of anthesis at 9.00 AM (1) and 3.00 PM (2), and on the second day of anthesis at 9.00 PM (3). Flowers were rebagged after performing each measurement.

Reproductive system

Four pollination treatments were performed, to identify the reproductive system of *R. neves-armondii* as well as its dependence on animal vectors for seed production. The treatments were: (a) spontaneous self-pollination (n = 30), in which flowers in pre-anthesis were bagged to exclude visitors; (b) manual self-pollination (n = 25), in which flowers were bagged in pre-anthesis, and were then hand-pollinated, using pollen grains from their own anthers; (c) manual cross-pollination (n = 14), in which previously bagged flowers were pollinated with pollen grains from flowers of other individuals located at least 10 m distant; (D) open pollination (natural condition) (n = 28), in which flowers were kept open for natural pollination throughout anthesis. Treatments encompassed seven individuals and

were conducted between 9.00 and 11.00 AM on the first day of anthesis. After all treatments, flowers were kept bagged until the fruits were ripe. Fruits were collected 45-60 d after the treatment. We constructed general linear mixed models (GLMM), with binomial error distribution, to evaluate differences in fruit set among pollination treatments. Number of fruits was included as dependent variable, pollination treatments as fixed factor, and mother plant as a random factor. The occurrence of pollen limitation (PL) was evaluated by the index of pollen limitation, expressed by IPL = 1 – (Fcn / Fpc), where Fcn is the percentage of fruiting under natural conditions and Fpc is the percentage of fruiting after manual cross-pollination (Larson & Barrett 2000). Negative or near-zero values indicate absence of PL. In addition, the differences in the number of seeds per fruit between the cross-pollination and natural-pollination treatments were analyzed through the general linear mixed models (GLMM), with Poisson error distribution. Number of seeds was included as dependent variable, pollination treatments as fixed factor, and mother plant as a random factor. The statistical analyses were performed in the R environment.

Flower visitors

To catalog floral visitors and identify pollinators, focal observations were performed on four individuals at 20-min intervals, between 8.00 AM and 4.00 PM, totaling 26 h of sampling. We recorded information on how the insects approached the flowers, their frequency of visits, which resource was sought, and if they contacted the anthers and stigma. The absolute frequency and the relative frequency of visits were calculated, respectively, by the number of flowers visited / total observation time (26 h) and by the absolute frequency of the species / total absolute frequency x 100 (Freitas & Andrich 2013). Floral visitors were classified as pollinators if they contacted the stamens and stigma during visits. Pollinators were captured directly from the flower and preserved for later identification, except *Apis mellifera* individuals.

Results

Floral biology

Flowers of *R. neves-armondii* are lateral to subterminal, with radial symmetry and measured 1.98 ± 0.18 cm in diameter (mean ± SD throughout the text). The number of perianth elements (tepals) per flower was 12.12 ± 1.56, and they are patent, free and white. Approximately 100 stamens (83.88 ± 12.85) with different heights are placed around the style and the annular nectary located at its base. The flowers did not reflect UV and all their parts are white, except for the orange base of the filaments, which contrasts with the tepals (Fig. 1B-C). The length of the style-stigma was 0.79

± 0.07 cm, so that the lobed stigma was positioned above the stamens (i.e., approach herkogamy, Fig. 1C). Each flower produced 71.08 ± 13.79 ovules. Anthesis started at about 8.00 AM, with the outer tepals opening first, followed by the inner tepals. The stamens were initially bent toward the center, so the introrse anthers leaned on the style. With the advance of anthesis they acquired the erect position. The opening of the flowers was completed around 9.00 AM, and they closed at around 4.00 PM. Flowers are nyctinastic and the dynamics of opening and closing occurred in the same way on the second day of anthesis, which marked the end of anthesis.

The flowers already contained nectar at the beginning of anthesis (14 of 15 flowers sampled), although the production was small. In the first measurement at 9.00 AM, 0.67 ± 0.33 µL of nectar had accumulated, with a concentration of 26.82 ± 14.11%. Nectar secretion ceased after the first withdrawal in 87% of the samples (13 flowers). The two flowers that secreted after withdrawal accumulated 0.8 µL and 0.1 µL of nectar (second measurement at 3.00 PM). No flower secreted nectar on the second day of anthesis.

Reproductive system

Rhipsalis neves-armondii is self-sterile and obligate xenogamous since fruit set and seed number were negligible after self-pollinations. The only fruit that was set after spontaneous self-pollination did not have seeds, and the three fruits that were set after the manual self-pollinations produced only a single seed among them (Tab. 1). The number of fruits differed significantly among treatments (X^2 = 87.31; df = 3; N = 97; p < 0.001; variance random effect = 7.62, SD = 2.76). The number of seeds per fruit did not differ between the cross-pollination treatments and the natural conditions (X^2 = 0.0002; df = 1; N = 42; p > 0.05; variance random effect = 1.71. SD = 1.31) so no pollen limitation was detected (ILP = −0.22) (Tab. 1).

Flower visitors

Female bees belonging to 14 species of three families (Andrenidae, Apidae and Halictidae) were responsible for almost all visits to flowers (98.2 %). In addition to the bees, only one unidentified species of wasp and one fly visited the flowers, each of them once and without making contact with the stigma (i.e., ineffective visitors). A half of bee species did not contact the stigma region during their visits either, and some of other species performed both efficacious and inefficacious visits (Tab. 2). *Rhophitulus solani* (24 %), *Apis mellifera* (23 %), *Trigona braueri* (15 %) and *T. spinipes* (14 %) were the most frequent visitors (Tab. 2) and possibly the most effective pollinators (sensu

Table 1. Fruit set and mean number of seeds per fruit for hand pollination treatments in flowers of *Rhipsalis neves-armondii*.

Treatments	Flowers	Fruit set (%)	Seed number (mean ± SE)
Spontaneous self-pollination	30	1 (3.33)	0
Hand self-pollination	25	3 (12.00)	1
Hand cross-pollination	14	11 (78.57)	40.12 ± 19.98
Natural conditions	28	27 (96.43)	35.70 ± 19.94

Table 2. Species of floral visitors of *Rhipsalis neves-armondii*, their absolute and relative frequency of visits (respectively the number of visited flowers / total observation time (26 h), and the number of visits by the species / total number of visits × 100). The last column shows the proportion of visits that resulted in contact of the visitor with the anthers and the stigma (efficacious visits). Frequency values represent pooled efficacious and inefficacious visits by floral visitors.

Species	Absolute frequency	Relative frequency (%)	Stigma contact rate
Rhophitulus solani Ducke, 1912	1.03	23.95	0.96
Apis mellifera Linnaeus, 1758	1.00	23.26	1.00
Trigona braueri Friese, 1900	0.65	15.12	1.00
Trigona spinipes Fabricius, 1793	0.61	14.19	1.00
Dialictus sp. 1	0.38	8.84	1.00
Ceratina sp. 1	0.19	4.42	0.40
Plebeia droryana Friese, 1900	0.08	1.86	0.00
Partamona helleri Friese, 1900	0.04	0.93	1.00
Augochlorini	0.04	0.93	1.00
Augochlorella sp. 1	0.04	0.93	1.00
Augochlorella sp. 2	0.04	0.93	0.00
Dialictus sp. 2	0.04	0.93	0.00
Ceratina sp. 2	0.04	0.93	0.00
Osirini	0.04	0.93	1.00
Diptera	0.04	0.93	0.00
Vespidae	0.04	0.93	0.00
Total	4.3	100	

Freitas 2013). Individuals of *R. solani* searched for pollen and nectar, and the large majority (96%) of their visits were efficacious (Fig. 1E-F, Tab. 2). Those bees frequently used the stigma as a landing platform, and then moved among the stamens looking for pollen on the anthers (Fig. 1E). They also used the style to move toward the base of the flower where nectar accumulated (Fig. 1F). Individuals of *A. mellifera* harvested only pollen. They used different parts of the flower for landing and holding (tepals, stamens and stigma) and contacted the stigma in all visits, due to their size. *Trigona braueri* and *T. spinipes* were highly effective visitors and behaved similarly to *A. mellifera*, although they also searched for nectar. The other species made less than 10% of the visits, most of them inefficacious (Tab. 2). Inefficacious visits mostly reflected "lateral approaches" by small visitors, when they used tepals for landing and hence made no contact with the stigma above.

Discussion

Rhipsalis neves-armondii was visited by species of different functional groups, belonging to three orders of insects. This broad spectrum of floral visitors suggests a functional generalist pollination system (*sensu* Ollerton *et al*. 2007). However, the visiting frequencies of visitors do not support this idea. Only three species of small native bees and *A. mellifera* touch stigma/anthers and showed high frequencies of visits to the flowers, an indicative of pollination effectiveness (*sensu* Freitas 2013). Thus, pollination of this species of cactus actually relied on a few bee species, indicating a pollination system with both ecological and functional-group specialization (Ollerton *et al*. 2007; Armbruster 2017). These finds did not corroborate the generalist pollination system we predicted based on its small open radial flowers that offer exposed nectar and pollen. Flowers with this phenotype are typically classified as generalists, based on the assumption that their pollen and nectar are easily accessible and can be collected by a wide range of flower visitors (e.g. Frame 2003). However, easy access to resources does not necessarily correspond to the occurrence of pollination by diverse groups. In polystemous flowers with radial symmetry, in which the anthers are positioned below the stigma (similarly to "papaver-type pollen flowers" sensu Vogel 1978), the pollination effectiveness of floral visitors depends on their size and their foraging behavior. Accordingly, degrees of specialization and mechanism of pollination (specifically the use of the stigma as a landing platform) equivalent to those observed in *R. neves-armondii* have been recorded for other species with similar flowers (e.g. *Hypericum roeperianum* and *H. revolutum*, Bartoš *et al*. 2015). These results indicate that the absence of clear morphological barriers to access floral resources may not be a universal indicator of phenotypic generalization (Ramirez 1989; Freitas & Sazima 2006), particularly in flowers in which the pollination mechanism requires specific behaviors of floral visitors.

Functionally or even ecologically specialized pollination systems involving flowers considered as "typically generalist" have been previously recorded in species of several families (e.g. Lindsey & Bell 1985; Ramirez 1989; Freitas & Sazima 2006; Ollerton *et al*. 2007; Shuttleworth & Johnson 2009; Narbona & Dirzo 2010; Niemirski & Zych 2011; Bartoš *et al*. 2015), including Cactaceae (species of *Ferocactos*, McIntosh 2005; as well as others *Rhipsalis*, C Martins unpubl. res.). In the case of *Ferocactus*, pollination is restricted to a small number of oligolectic bees that are closely associated with Cactaceae (*Diadasia rinconis*, *Svastra duplocincta* and *Ashmeadiella opuntiae*). In this system, more than 90% of the seeds produced by two species (*F. cylindraceus* and *F. wislizeni*) resulted from visits by those bees (McIntosh 2005). Other well-documented cases deal with species of Apiaceae (Lindsey & Bell 1985; Niemirski & Zych 2011). In *Angelica sylvestris* only a small group of flies contributed to pollination, although more than 70 species visited the flowers (Niemirski & Zych 2011). Similarly, *Thaspium* and *Zizia* species were pollinated only by an oligolectic solitary bee (*Andrena ziziae*), which led to the proposal of the concept of a pollination system with "cryptic specialization" (Lindsey & Bell 1985). This term refers to systems in which small changes in floral structure and physiology have the result that only a few among the broad spectrum of floral visitors are effective in pollination (Lindsey & Bell 1985). Similar reasoning could be applied to *R. neves-armondii*, in which non-specific attributes of floral morphology may be filtering the interaction with pollinators. For instance, characteristics of nectar such as volume and concentration are often correlated with certain pollinator groups, and this relationship is based mainly on their body size and energy requirements (Brown *et al*. 1978). The small amount of nectar in *R. neves-armondii* may therefore constitute a filter for certain pollinators-floral visitors with a high energy demand (Ollerton *et al*. 2007). However, this would not explain the small number of visitors, considering the diverse local fauna of small insects with supposed low energy and/ or pollen demands, so that other attributes involved with floral attraction and repulsion, such as odors, may be acting. In short, the flowers of *R. neves-armondii* seem to have non-morphological filters (e.g. Shuttleworth & Johnson 2009) that restrict the access of certain visitors to their resources, although the present data do not allow us to explore this question more deeply.

The bee *R. solani* was the most frequent pollinator in *R. neves-armondii*. Species of this genus, as well as members of Andrenidae as a whole, are usually oligolectic, i.e., bees that collect pollen in a small number of often related plants (Linsley 1958; Cane & Sipes 2006). This seems to be the case of *Rhophitulus*, since species of this genus were the most frequent visitors of other Cactaceae species in the PARNASO (*R. teres* and *Hatiora salicornioides*) and in other areas of the Atlantic Forest (*R. pentaptera*) but were not prominent in a community-level inventory in the PARNASO (L Freitas *et al*. unpubl. res.). This bee species uses the stigma of *R. neves-*

armondii as a landing platform and then moves toward the anthers and nectary. The approach herkogamy of *R. neves-armondii* flowers, together with the behavior of *R. solani* during the visits to the flowers possibly favor the deposition of cross-pollen on the stigma, as it is the first structure touched by the bee. Moreover, oligolectic bees transport less-heterogeneous pollen loads (Linsley 1958; Cane & Sipes 2006), which should lead to low deposition of interspecific pollen on the stigma, and the occurrence of oligolectic bees with high pollination effectiveness is common in Cactaceae (e.g. Mandujano *et al.* 1996; Schlindwein & Wittmann 1997). Mechanisms of pollination that favors cross-pollination are usually invoked as the result of selection to reduce self-pollination, due to the deleterious effects of inbreeding (see Busch 2005). However, since *R. neves-armondii* is highly self-sterile, probably due to self-incompatibility mechanism yet to be studied in detail, inbreeding is mostly restricted and pollination mechanism probably is related to the avoidance of interference between pollen presentation and stigma receptivity (Webb & Lloyd 1986; Narbona *et al.* 2011).

The other most-frequent bee species in the flowers of *R. neves-armondii* were *A. mellifera*, *T. spinipes* and *T. braueri*, which are highly generalist species, with numerous records reporting visits to a large number of species belonging to several pollination syndromes (e.g. Linsley 1958; Cortopassi-Laurino & Ramalho 1988). These bees collect pollen with high efficiency, storing large amounts in their corbicula. Visits by those three species to *R. neves-armondii* flowers always resulted in contact with the stigma and the anthers, reflecting the size of the bees and their movements within the flowers during pollen collection.

Rhipsalis neves-armondii is a mandatory xenogamous species, since self crosses result in a high abortion rate of fruits and seeds. Many species of Cactaceae with different pollination systems are dependent on pollinators for sexual reproduction (reviewed by Ross 1981; Boyle 1997; 2003; Pimienta-Barrios & Castillo 2002; Mandujano *et al.* 2010). However, a few studies have examined self-sterility mechanisms in this family, so failures of fruit and seed production after selfing could reflect either strong inbreeding depression or self-incompatibility (Mandujano *et al.* 2010). Some species of *Schlumbergera* and *Hatiora* are among the taxa with a genetically controlled self-incompatibility mechanism, specifically a gametophytic system controlled by a multi-allelic locus (Boyle 1997; 2003). These two genera belong to Rhipsalideae, together with *Rhipsalis* and *Lespismium* (Calvente *et al.* 2011a), so futher studies may define if the self-sterility in *Rhipsalis neves-armondii* is due to a similar self-incompatibility system.

In conclusion, the pollination system of *R. neves-armondii* population in the PARNASO can be characterized as specialized from the plant's perspective, because only a few small bees among dozens of anthophilous insects in the area visited its flowers effectively. However, from a zoocentric perspective these bees belong to distinct functional groups, from possibly oligolectic *R. solani* to super-generalist *A.*

mellifera. Behavioral differences, such as those involving the use of floral resources by bees, can lead to distinct selective pressures on flowers (see Armbruster 2017), even among taxonomically related species of pollinators and/or among species that belong to the same classical functional groups.

Acknowledgements

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