http://dx.doi.org/10.1590/1519-6984.01516

Reproductive biology of endemic *Solanum melissarum* Bohs (Solanaceae) and updating of its current geographic distribution as the basis for its conservation in the Brazilian Cerrado

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Received: January 29, 2016 – Accepted: June 5, 2016 – Distributed: November 31, 2017
(With 6 figures)

Abstract

The genus *Solanum* (family Solanaceae) includes more than 1400 species and has buzz-pollinated flowers with poricidal anthers. The present study aimed to describe the distribution, breeding system and pollination mechanism of *Solanum melissarum*, a species endemic to Brazil. The study of breeding system was conducted in an urban forest fragment in Jataí, GO. Distribution data were gathered from floristic surveys and digital plant databases. The floral morphology and the pollination mechanism were studied on through field observations and preserved flowers. The breeding system was determined through hand pollination treatments. The species has a distribution only in the Brazilian Atlantic forest coastal, and this study provides the first records of *S. melissarum* for the state of Goiás. The pendulous flowers have poricidal anthers close to the stigma, with membranous thecae joined by a connective bearing osmophores that attract males of *Euglossa cordata* bees. As they collect fragrances, the bees press the thecae and pollen is released through a bellows mechanism. Based on the hand-pollination treatments, this species is self-incompatible. Isolated forest fragments may not include enough pollinators to ensure the pollination of plants with specialized systems. However, they are essential for the conservation of species with interesting phytogeographic patterns, such as the vicariance observed in *S. melissarum*, and for the conservation of regional diversity.

Keywords: bellows mechanism, *Euglossa cordata,* osmophores, self-incompatibility, fragrance collection.

Biologia reprodutiva da endêmica *Solanum melissarum* Bohs (Solanaceae) e atualização da distribuição geográfica atual, como base para a sua conservação no Cerrado Brasileiro

Resumo

O gênero *Solanum* (família Solanaceae) inclui mais de 1400 espécies e apresenta polinização vibrátil e flores com anteras poricidas. O estudo tem o objetivo de descrever a distribuição, sistema reprodutivo e o mecanismo de polinização de *Solanum melissarum*, uma espécie endêmica do Brasil. O estudo da biologia reprodutiva foi conduzido em um fragmento florestal urbano em Jataí, GO. As informações de distribuição foram obtidas através de trabalhos florísticos e de banco de dados digitais de plantas. A morfologia floral e o mecanismo de polinização foram estudados através de observações diretas em campo e em flores preservadas. O sistema reprodutivo foi determinado a partir de tratamentos de polinização manual. A espécie apresentava distribuição somente na Mata Atlântica litorânea brasileira, e este estudo demonstrou o primeiro registro de *S. melissarum* para o estado de Goiás. As flores são pendentes e com anteras poricidas próximas ao estigma, com tecas membranosas envoltas por um conectivo giboso com osmóforos que atraem machos da abelha *Euglossa cordata*. Na coleta de fragrâncias, as abelhas pressionam as tecas e o polén é liberado por um mecanismo de fole. Baseado nos tratamentos de polinização manual, a espécie é autoincompatível. Fragmentos florestais isolados podem não incluir polinizadores suficientes para garantir a polinização das plantas com sistemas especializados, no entanto, eles são essenciais para a conservação de espécies com padrões fitogeográficos interessantes, como o vicariância observada em *S. melissarum*, e também para a conservação da diversidade regional.

Palavras-chave: mecanismo de fole, *Euglossa cordata,* osmóforos, autoincompatibilidade, coleta de fragrâncias.

1. Introduction

The family Solanaceae A. L. Jussieu includes roughly 106 genera (Olmstead et al., 1999) and 2,300 species and has a cosmopolitan distribution, with its center of diversity and endemism in the New World (Hunziker, 2001). In this family, the inflorescences are determinate and sometimes reduced to solitary terminal flowers. The flowers are bisexual and radial, normally with five connate persistent sepals that may expand during fruit development. The pentamerous petals form a disk-shaped, tubular, campanulate, or fusiform corolla. Fruits are usually berries, capsules, or a schizocarp of nutlets (Souza and Lorenzi, 2005).

The genus *Solanum* is the richest of this family, with approximately 1,500 species around the world (Bohs, 2007) including many economically relevant species, such as tomato, potato, scarlet eggplant, and various peppers (Olmstead et al., 2008). The genus includes herbs, shrubs, trees, lianas, or rarely epiphytes. Individuals may be prickly or unarmed, glabrous or pubescent with simple, branched, stellate, peltate, glandular, or sometimes setose trichomes (Agra et al., 2009). One of the typical attributes of this genus is the absence of nectar as a floral attractant, combined with the presence of anthers with poricidal dehiscence (Nunes-Silva et al., 2010). This type of dehiscence occurs through small pores at the apex of the anthers, constraining the number of potential pollinator visitors. Thus, in many species of *Solanum* a vibration mechanism is required for pollen release (Bezerra and Machado, 2003), reducing the competition for pollinators and increasing the degree of specialization of the pollination system.

Molecular studies resulted in changes of infrageneric positions, with the inclusion of *Lycopersicon* and *Cyphomandra* in *Solanum* (Bohs, 1995), outlining broader phylogenetic hypotheses both for the family and for the genus (Agra et al., 2009). The old genus *Cyphomandra* Mart. ex Sendtn. (Solanaceae) includes about 35 species of neotropical shrubs and small trees (Bohs, 1995). Within the Solanaceae, *Cyphomandra* belongs to the subfamily Solanoideae and tribe Solaneae, characterized by flattened seeds with curved embryos and abundant endosperm, valvate, induplicate, or plicate corolla a estivation, and filaments inserted near the base of the anthers (Bohs 1995). *Solanum melissarum* Bohs, previously known as *Cyphomandra divaricata* (Mart.) Sendtn., is now part of genus *Solanum*. One of the main characteristics of the *Solanum* species that used to belong to *Cyphomandra* is the presence of large connectives with osmophores, which attract bees specialized in fragrance collection, in addition to the poricidal anthers (Sazima et al., 1993). *S. melissarum* is endemic to Brazil, and is typical of the Atlantic forest vegetation of the northeast, southeast and south regions of Brazil (Stehmann et al., 2014). Up until now, there have been no records of this species in the state of Goiás, the discover of a new reproductive population (producing viable offspring) is essential for the design of conservation strategies in the near future.

Mutualistic relationships between flowers and pollinators are frequently regarded as the result of interactions in which floral structures facilitate pollen transportation and mediate vector behavior to allow pollination (Faegri and Pijl, 1979). The animals involved in these pollination systems range from generalists to complete specialists, such as the bees in tribe Euglossini, which are attracted to the flowers of different families to collect the fragrances released by them. One hypothesis to explain this behavior is that the males of these species use the perfume collected to attract the females (Faegri and Pijl, 1979; Sazima et al., 1993), although this explanation is controversial (Holland, 2015). In these specialized interactions, there is only one floral resource, the fragrance, and the mechanism for pollen release is usually specific and restrictive. Few visitors are attracted and, therefore, they are essential for the pollination of these species. However, in terms of system stability, the absence of one of the species involved may result in reproductive failure. This caveat is exacerbated in heavily altered areas (Johnson and Steiner, 2000), like the Cerrado in Southeastern Goiás, Brazil, where large agricultural areas render the environment degraded and fragmented. This issue may directly affect the distribution of certain species, because in the absence of a connection between natural areas and consistent gene flow, populations become locally threatened and big gaps in distribution may be observed later.

An example of a more specialized system is the one of flowers with poricidal anthers. In these flowers, pollen is offered as a resource, and visitors display a typical behavior, grabbing the stamens, contracting their thoracic muscles and vibrating to release the pollen from the anthers, in a process known as buzz pollination (Nunes-Silva et al., 2010). There is no nectar and the pollen is only released through vibration, therefore constraining the number of potential pollinators, such as bees of the tribe Euglossini of the family Apidae (Nunes-Silva et al., 2010). There are five genera in this tribe: *Euglossa, Eufriesea, Eulaema, Aglae* and *Exaerete*. *Euglossa* is the genus with the largest number of species, and includes bees of medium to large body sizes (8-18 mm). These bees range in color from metallic green and blue to copper violet (Darrault et al., 2006), and have long tongues, up to 28 mm long in *Euglossa rugilabris* (Dressler, 1982). Species of this genus are found from Argentina to the United States (Silveira et al., 2002), and they are usually involved in specialized pollination systems (Sazima et al., 1993). The present study is the first report of the compatibilily system for this specie *S. melissarum.*

In addition to their buzzing ability, some bees of this tribe collect fragrances or scents, which are common in the former genus *Cyphomandra*. This behavior of males in the Euglossini tribe is well-known and studied (Bezerra and Martins, 2001; Rech and Brito, 2012), that way, the males are considered essential pollinators in tropical and subtropical forests (Darrault et al., 2006; Dressler, 1982).

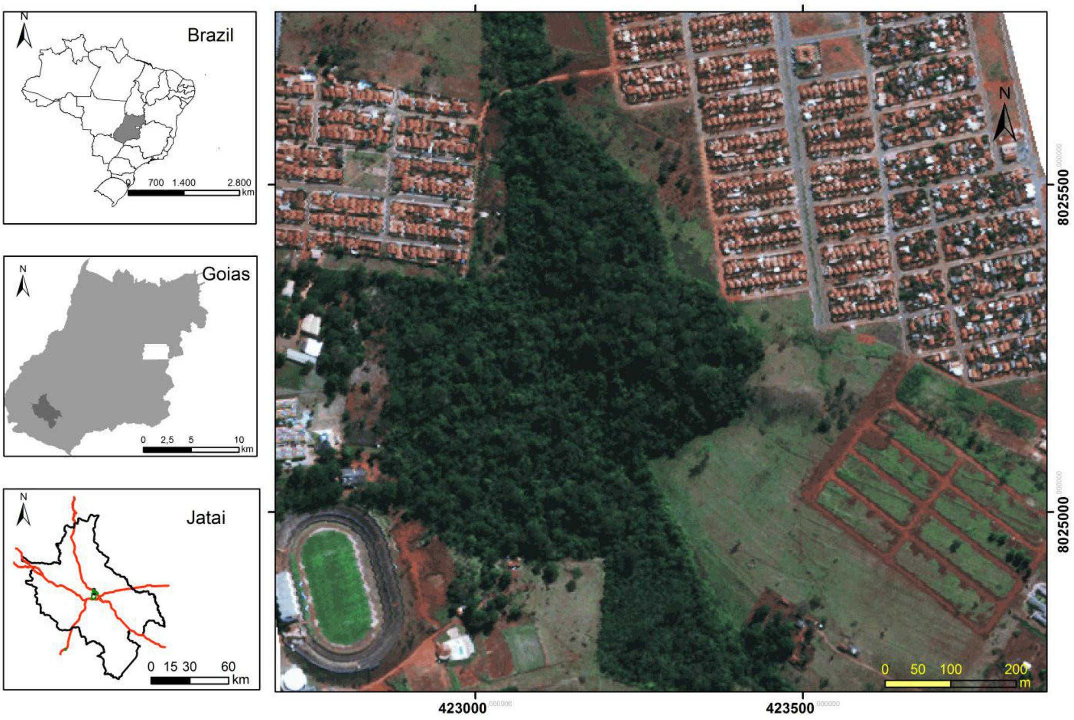
The present study aimed to expand the known geographic distribution of *S. melissarum* beyond the Atlantic Forest and to describe its floral morphology and compatibility system, focusing on the pollination mechanism.

2. Material and Methods

The study was conducted in April and May of 2011 and 2013; but in 2012 there was a fire that killed a large number of the sampled individuals. The study was conducted in an urban forest fragment with an area of approximately 15 ha, commonly known as "Mata do Açude" (17º51'34"S and 51º43'33"W), in Jataí, southeastern Goiás (Figure 1). Most of the fragment is covered by gallery forest along the stream and semi-deciduous seasonal forest in well-drained areas, with patches of cerrado *sensu stricto* Coelho CP, personal comunication. The fragment is degraded by trash and fires, because it is surrounded by settlements and therefore has a higher propensity to burn, with vegetation suppression as a consequence.

The floral morphology and the pollination mechanism were studied on ten randomly selected individuals. Approximately seven flowers and inflorescences were collected per individual and preserved in 70% alcohol to count the number of flowers per inflorescence and to measure length and width of corolla, stamens and stigmas. Measurements were taken using a digital caliper. All morphological traits, such as shape, color and texture of perianth, androecium, and gynoecium, were described as the flowers were being measured. Data on floral longevity, time of opening and floral anthesis, odor presence, and stigmatic receptivity (using 3% H_2O_2) were obtained from direct focal observations in the field. Both fresh and preserved flowers were studied in order to describe the pollination mechanism and potential floral resources. The analysis protocol was adapted from Sazima et al. (1993).

Twelve adult individuals were used to determine the compatibility system. Flower buds were isolated in pre-anthesis using mesh bags, and the subsequent flowers underwent one of four treatments: (1) spontaneous self-pollination - previously bagged fruits were left intact to verify fruit formation (n=35); (2) manual self-pollination - hand-pollinated using the flower's own pollen (n=65); (3) cross-pollination - hand-pollinated using pollen from different individuals, at least 30 m apart (n=54); (4) open pollination (control) - flowers were marked and left exposed, and the number of fruits formed was recorded (n=145). Flowers were marked with threads of different colors to enable inspection at a later date (Coelho and Barbosa, 2003). The Index of Self-Incompatibility (ISI) was calculated as the ratio of the fruit set from self pollination to the fruit set from manual cross-pollination; values below 0.25 indicate self-incompatible species (Bullock, 1985). The Reproductive Efficacy (RE) of the pollinators was calculated as the ratio of the fruit set from open pollination to the fruit set from cross-pollination treatments (Ruiz and Arroyo, 1978). This index estimates the real contribution of pollinators



Source: Geoeye Image (1B2C3R), May 2010. Resolution 2 m.

Author: Geoinformation Laboratory - UFG / Jataí

Figure 1. Geographic location of the urban forest fragment "Mata do Açude", in Jataí-GO, with an area of 15 ha (17º51'34"S and 51º43'33"W).

to the reproductive success of the studied plant species. A subset of the hand-pollinated flowers (ten self-pollinated and ten cross-pollinated) were collected 24 h after pollination and observed under an epifluorescence microscope to observe pollen tube growth and identify possible sites of incompatibility (Martin, 1959).

Fragrance collection behavior by flower visitors was described based on direct focal observations. Both the amount of time spent in the flower and flying between flowers, and also the behavior of the visitor after leaving the flower were recorded. In addition, we manually removed pollen, mimicking the potential behavior of the bees, to improve the description of the pollination mechanism. Photographs and movies helped describe visitor behavior.

Marked individuals of *S. melissarum* were observed in 30 min cycles in the morning and afternoon for nine days, for a total of 50 h of observations, to identify and describe floral visitors and putative pollinators. We recorded the behavior, frequency of visits, type of visitor, visit length and time of visit. One visit was defined as the arrival of a visitor at the plant, regardless of the number of flowers visited. Visitors were collected, preserved and sent to specialists for identification.

The geographic distribution of this species was determined by reviewing published floristic surveys of Brazil, and by querying worldwide herbarium databases (SPLINK, 2017; GBIF 2017).

3. Results

Solanum melissarum studied individuals were self-incompatible, and natural pollinators (control) were less effective than hand-pollination (Table 1), since there were significant differences in fruit set between cross-pollination and pollination of open flowers by floral visitors. Pollen tube growth was not interrupted in the cross-pollination treatment, in contrast to self-pollinated flowers, where pollen tube growth stopped in the stigma or in the style, identifying possible sites of pre-zygotic self-incompatibility.

The flowers of *S. melissarum* are pendulous and arranged into simple cyme inflorescences. This flower position facilitates pollen release through a bellows mechanism (Figure 2), which is aided by gravity. The pores of the anthers are turned towards the pistil and are 1.5 mm, on average, below the stigma, allowing bees to touch both the gynoecium and the androecium during their visits (Figure 3). Anther thecae are membranous and very flexible, held together by a rigid gibbous purplish connective (Figure 4B and C) that produces a characteristic scent, which attracted male *Euglossa* bees.

Pollen grains accumulate by gravity inside thecae, and they are easily released as bees collect fragrance from the connective and press the thecae, which acts as a bellows to push the air out along with the pollen grains (Figure 2). Afterwards, the anther walls revert to their initial shape, allowing other bees to visit. This bellows mechanism was tested manually by applying lateral pressure on the anther thecae, which released a great amount of pollen, compared to simple vibration and gravity. Although this seems to provide effective pollination of the flowers, the fruit set from open pollinations was lower than the fruit set from hand pollinations (Table 1).

We observed 24 visits to *S. melissarum* individuals. Plants with a higher number of flowers seemed to attract more visitors. Three species of visitors were observed: two species of bees and one beetle. The main visitor was the bee *Euglossa cordata*, observed in 15 visits. This bee visited the flower for up to 30 s, inserting its head and thorax in the hypanthium (Figure 3). They visited all open flowers in the individual and were more frequent between 10:00 a.m. and 1:00 p.m. The other bee species (family Halictidae) was observed seven times between 10:00 a.m. and 12:00 p.m., and its behavior was similar to *E. cordatas*. However, its smaller body size did not allow it to press the anther thecae. The beetle was observed in one flower only, and it was considered an infrequent visitor unable to pollinate the species due to its observed behavior.

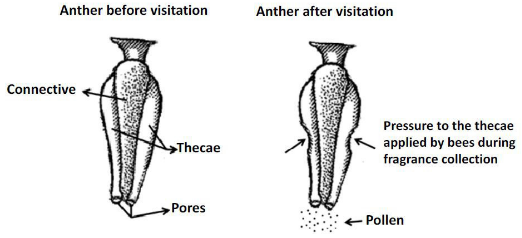


Figure 2. Pollen release mechanism from anthers of *Solanum melissarum* flowers (adapted from SAZIMA et al., 1993).

Table 1. Outcome of the reproductive system assessment for *Solanum melissarum* in an urban forest fragment in Jataí, GO.

Treatment	Number of flowers	Number of fruits formed	%
Hand cross-pollination	54	30	55.55 a
Hand self-pollination	65	2	3.27 c
Spontaneous self-pollination	35	0	0.00 d
Control – open pollination	145	46	31.50 b
Index of Self-Incompatibility (ISI)		0.06	
Reproductive Efficacy (RE) of Pollinators		0.57	

Different letters in the same column indicate significant differences at $P < 0.05$ – Fisher's exact test.

Figure 3. *Euglossa cordata* collecting fragrance and pollinating flowers of *Solanum melissarum* in an urban forest fragment in Jataí, GO.

Individuals of *E. cordata* land on the flower and insert their head, thorax and anterior legs in the hypanthium, where they scrape the gibbous connective with their front tarsi to collect fragrance (Figure 3). This scraping or scrubbing movement enhances aromatic substance removal from the connective. The length of time spent inside the flower varied considerably; on average it was 14 s, with a maximum of 30 s (Figure 3). After leaving the flower, the bees would hover next to the flowers, brushing the front tarsi onto the hind tibiae (Figure 5), which have a enlarged surface to store the collected substances (Bembé, 2004; Dodson, 1966). These movements outside the flower lasted 5-10 s, and immediately afterwards the bee would search for another open flower to reinitiate collection and potentially pollinate.

All the records and published literature on *S. melissarum* indicate a wide geographic distribution, but there are few records in each sampled area. This species has been found in the states of Bahia, Espírito Santo, Minas Gerais, Paraíba, Paraná, Santa Catarina, Rio de Janeiro and São Paulo, for a total of 152 records in Species Link, (SPLINK, 2017) (Figure 6). For the Midwest region (Centro-Oeste) of Brazil, there are only two records in the state of Goiás, collected by the authors of the present study, and one record from 1930 at an undefined location in Mato Grosso do Sul. The other collections of *S. melissarum* come from Paraná (38 records), Bahia (25), Espírito Santo (22), Minas Gerais (17), Rio de Janeiro (15), São Paulo (13), Paraíba (11) and Santa Catarina (6), in addition to two records with no locality data. In the Global Biodiversity Information Facility database (GBIF 2017), which aggregates survey and distribution data of organisms around the world, there are 16 records of *S. melissarum* from Brazil: from Mato Grosso do Sul (1 record), Mato Grosso (1), Paraná (1), Minas Gerais (1), Espírito Santo (1), Bahia (2), Rio de Janeiro (3), and Paraíba (5).

Solanum melissarum has simple extra-axillary scorpioid inflorescences 8.95 ± 1.73 cm long (Figure 4A), bearing 35.6 ± 8.6 flowers (n = 7). The flower buds are turbinate (Figure 4A). The calyx is campanulate and laciniate, with a 19.12 ± 1.77 mm long corolla (n = 62), white to vinaceous, campanulate-stellate, with reflexed lanceolate lobes (Figure 4B and C). Anthers are poricidal and 5.66 ± 0.57 mm long (n = 296) (Figure 4B and 4), and the filaments are much shorter than the subsessile anthers. The connective is vinaceous, gibbous, and more thickened on the basal region (Figure 4B and C). The slender pistil is 8.61 ± 0.56 mm long and the stigma extends a little above the stamens (Figure 4B). The flowers are pendulous (Figure 4D). One to three flowers per inflorescence per day opened acropetally early in the morning (around 7:30 a.m.), each lasting approximately four days. In the first morning, the corolla was wide open but some of the anther pores were closed; the stigma was receptive, as indicated by its shimmer (Figure 4C) and by the 3% H_2O_2 test. By the afternoon of the first day, all pores were open and the stigma remains receptive. On the second day the flowers were wilted and no longer receptive, and the reproductive parts, e.g. the stigma, became darker (Figure 4C). Floral life spam lasts up to 24 hours, as indicated by focal observations and stigmatic receptivity tests. Although not tested statistically, the distance of the anthers to the style was greater in second-day flowers, increasing herkogamy as the stigma and the anther pores become farther apart (Figure 4C).

By the third day the corolla was almost closed and started showing some signs of senescence. On the fourth day the corollas of unpollinated flowers fell, and only the pistils of pollinated flowers were left behind. The flowers have a noticeable scent that cannot be compared subjectively to any other known scent.

4. Discussion

The present study showed that *S. melissarum* is allogamous and depends on floral visitors for pollen dispersal and successful fruit formation. These results are consistent with studies on other Solanaceae species

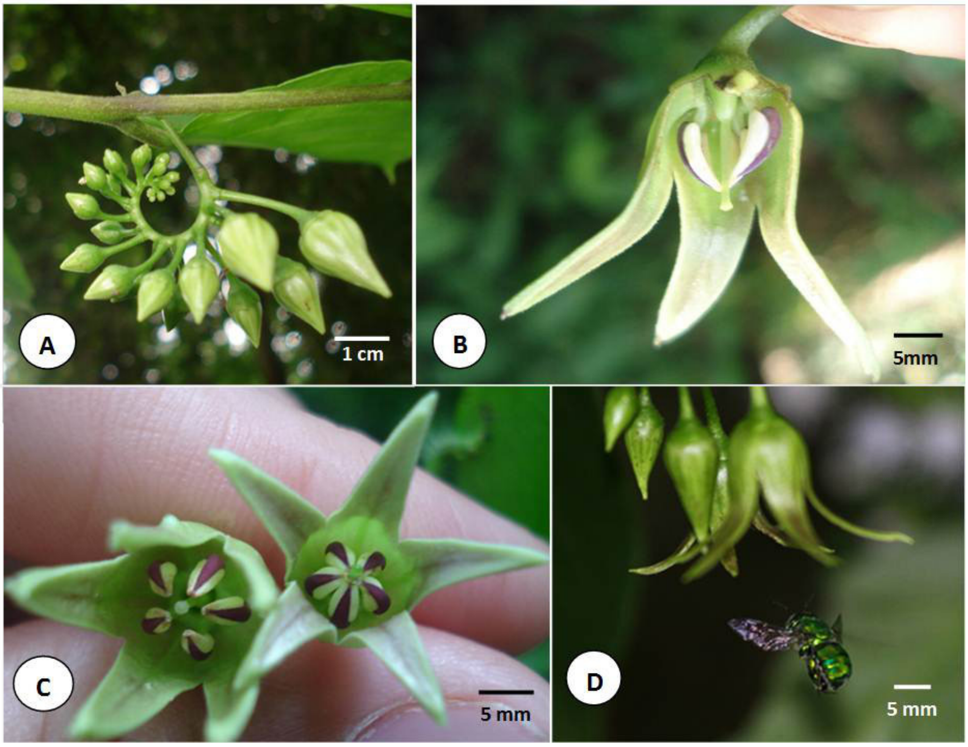


Figure 4. Details of the inflorescence and flowers of *Solanum melissarum*. A – Scorpioid inflorescence and flower buds. B – Lateral view with one petal removed, showing the gynoecium and the androecium. C – Bottom-up view of the flowers, showing the stigmas in the center of each flower surrounded by anthers with large purple connectives. First- (right) and second-day after anthesis flowers (left). D – Pendulous flower position, with an *Euglossa cordata* bee approaching for a visit.

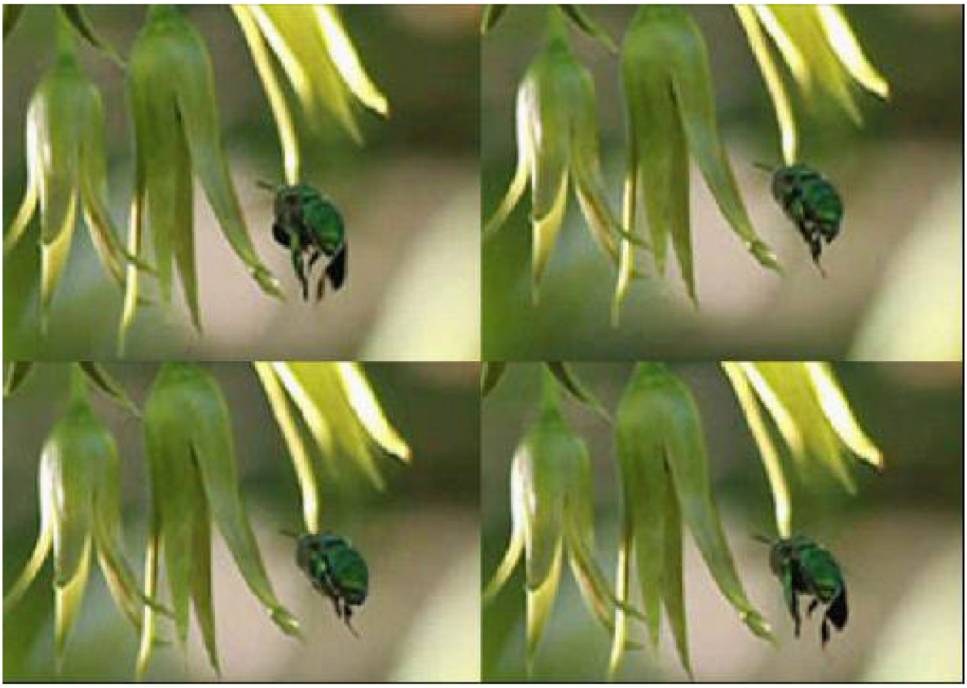


Figure 5. Sequence of movements by *Euglossa cordata* after visiting a *Solanum melissarum* flower in an urban forest fragment in Jataí, GO. The sequence of pictures shows the movements used to transfer collected substances from the front to the hind legs.

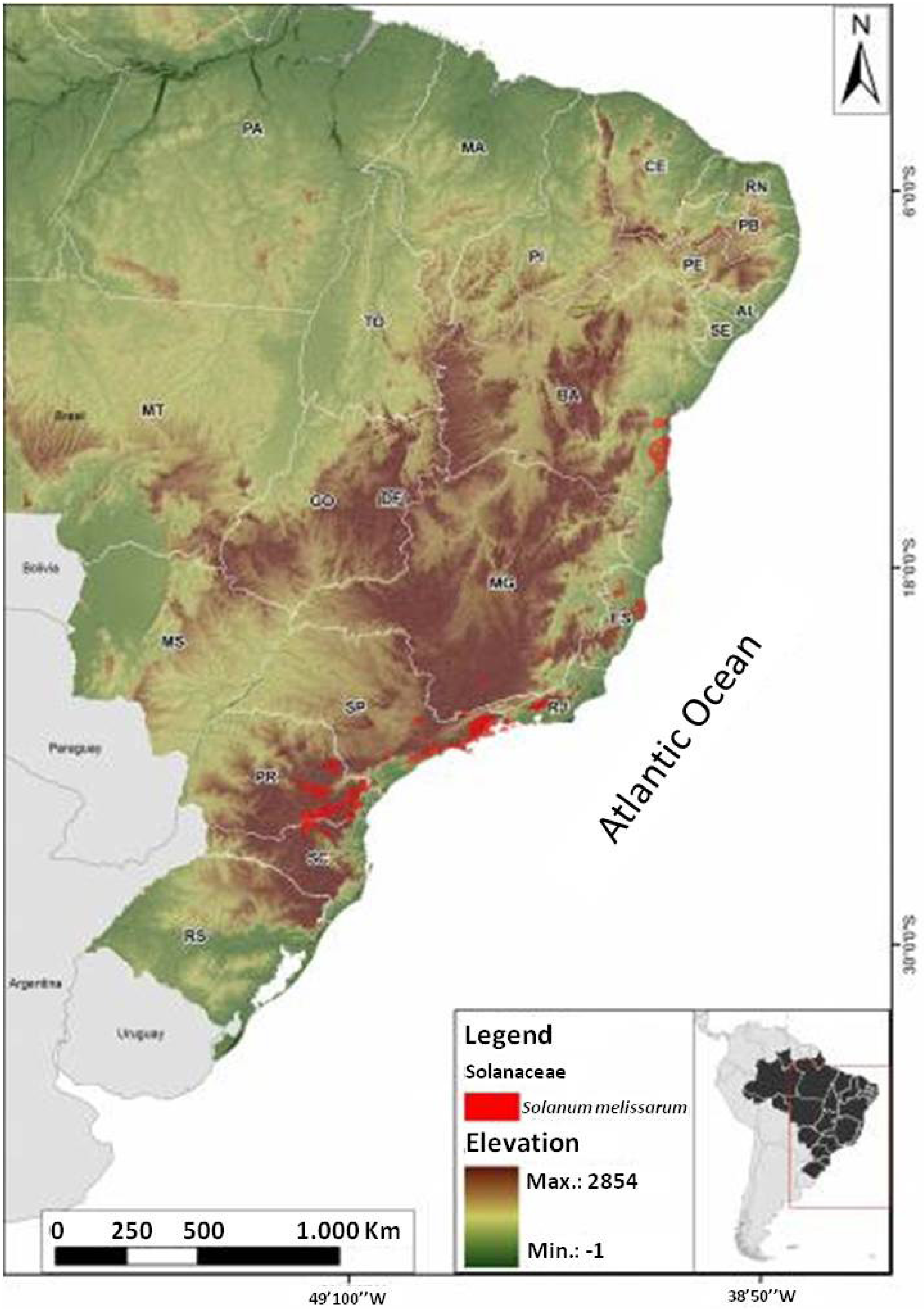


Figure 6. Geographic distribution of *Solanum melissarum* in Brazil, based on collections around the country. Source: (CNCFLORA 2017).

(Forni-Martins et al., 1998; Oliveira-Filho and Oliveira, 1988; Stehmann and Semir, 2001; Storti, 1988). The low number of open flowers per inflorescence per day and per individual increases visitor movement and optimizes pollen flow, something that is often observed in other studies (Bawa and Beach, 1983; Coelho and Barbosa, 2004). Based on field observations, the flowering period lasts over three months, with at least two flowering peaks during the year. This pattern of low resource presentation for long periods of time is well-described in the literature (Bawa and Beach, 1983; Coelho and Barbosa, 2004; Longo and Fischer, 2006), and reinforces the behavior to optimize floral visits and make visitors faithful. Despite these strategies to optimize visitor movement and pollen flow, the studied population of *S. melissarum* presented relatively low fruit set and reproductive efficacy, which may be directly linked to its complex pollination system.

The bellows pollination mechanism of *Cyphomandra* species (Sazima et al., 1993) may prevent visitors from being effective, as suggested in the present study by the low fruit set in open flowers compared to hand-pollinated ones. This may be explained by the specific relationship between *Euglossa* and these flowers, which produce perfume through osmophores in the connectives (Sazima et al., 1993; Soares et al., 1989), restricting the number of visitors and, therefore, pollen flow. The first descriptions of the bellows mechanism came from Delpino (1873), Harris (1905) and Dressler (1979) apud (Sazima et al., 1993). These reports were neglected at the time because there was little understanding of different floral resources. Later works described these reward syndromes in Solanaceae species, such as *Cyphomandra calcina*, *C. sciadostylis* and *C. hartwegii* (Sazima et al., 1993). The bellows mechanism works best for pendulous flowers, as is the case for *S. melissarum*. Pollen grains accumulate by gravity and are released when pollinators press the membranous anther thecae, pushing out the air along with the pollen. Buzato (1990) also showed the occurrence of this mechanism in species of *Mendoncia* (Acanthaceae), which are visited by bees, including *E. cordata*, and hummingbirds, who press the ventral region of thecae as they search for nectar and cause passive pollen release. However, the low fruit set in our control treatment may indicate that either the number of pollinators or their effectiveness is low, or that the bellows mechanism is not efficient in *S. melissarum*. This may be a direct result of human impacts on the study site, which is a small forest fragment under strong urban pressure. In addition, the small isolated populations in this fragment may be undergoing high levels of endogamy, resulting in genetic erosion and lower reproductive fitness, as observed for *S. lycocarpum* in fragmented areas (Moura et al., 2011). Specialized pollination systems are the first to be affected by fragmentation processes, because the reliance of a plant on one or two pollinators renders the system highly fragile. At first, the interaction becomes extinct, followed by the extinction of the species involved (Kearns and Inouye, 1997). On the other hand, in an interesting review of the effects of fragmentation on the pollination process, Aguilar et al. (2006) noted that more than specialized systems, such as *Solanum melissarum*, are affected by fragmentation, since the compatibility expressing their degree of dependence on pollination mutualism and consequently its effect fragmentation.

The bees in this study had a similar behavior to those observed visiting *Cyphomandra* flowers by Sazima et al. (1993) and Soares et al. (1989). Here, upon reaching an individual, the *E. cordata* bees searched for first-day flowers, hovering for some time before entering the flower. A similar behavior was observed in visits by *E. cordata* to other plant species (Sazima et al., 1993). During each visit, the bees touched the anthers and the connective and, since they are large-bodied, they also touched the pistil as they moved. After 14 s, on average, the bees left the flower to search for other flowers in the same condition, resulting in pollen flow. Other bees in this genus spend a similar amount of time collecting fragrances (Braga and Garófalo, 2003). The flower does not produce nectar, therefore visitors are attracted to this plant species primarily by the characteristic scent of the anther connectives (Sazima et al., 1993). The behavior of the bees in the family Halictidae was similar to the behavior of the *Euglossa* bees, but due to their small size and the low number of visits, they were not effective pollinators of *S. melissarum*. The green beetle was observed in only one flower and it was not considered a potential pollinator. Bezerra and Martins (2001) found eight species of Euglossini in a study using artificial fragrances in urban fragments, and *E. cordata* was the most frequent, especially when using eucalyptol. This shows that these bees are important to maintain specialized interactions in forest fragments, even if their body size is small, and highlights the importance of preserving these areas for species maintenance.

Despite focal observations of visitors and the bellows mechanism, this study showed that *S. melissarum* has low levels of natural pollination, given its low fruit set. This may be a direct result of having a specialized pollination system that excludes some visitors, as described by Sazima et al. (1993). The low fruit set from self-pollination also indicates that this species is self-incompatible, which is common in other Solanaceae (Forni-Martins et al., 1998; Oliveira-Filho and Oliveira, 1988; Stehmann and Semir, 2001; Storti, 1988). No fruits were formed through spontaneous self-pollination, reinforcing the existence of self-incompatibility and the need for an efficient pollinator of *S. melissarum*. Similar observations were made for *S. lycocarpum* (Oliveira-Filho and Oliveira, 1988).

The first records of *Solanum melissarum* in the state of Goiás are from semi-deciduous seasonal forest between 700 and 800 m a.s.l. Although it may be found at 1000 m a.s.l. in Brazil (Feliciano and Salimena, 2011), this species usually occurs between 50 and 800 m a.s.l. It is a typical understory species, growing in shaded areas of tropical forests, especially in the Atlantic forest of the states of Bahia, Espírito Santo, Minas Gerais, Paraíba, Paraná, Santa Catarina, Rio de Janeiro and São Paulo (Feliciano and Salimena, 2011). In Minas Gerais, this species is considered rare, with a few isolated records from low- and high-montane semi-deciduous seasonal forest (Oliveira-Filho, 2006). This species is also considered rare in the state of Paraíba, where it is associated to moist forests (Agra et al., 2009). Stehmann et al. (2014) report the occurrence of the species in the states of Alagoas, Pernambuco and Sergipe. These records for the Midwest region were not included in the Flora of Brazil (Stehmann et al. 2014). Therefore, the present study provides the first records of this species for the state of Goiás. However, no records from these states were found in the databases or in the published literature. The species is a terricolous sciophilous treelet (Agra et al., 2009; Feliciano and Salimena, 2011) found in semi-deciduous seasonal forest (Stehmann et al., 2009), moist upland forest ("brejos de altitude", (Agra et al., 2009)), and shaded areas of coastal forests in the Atlantic forest domain (Feliciano & Salimena, 2011; Stehmann et al., 2014).

These new records from southeastern Goiás, in the heart of the Brazilian Cerrado, may support the idea the an evidence of Atlantic forest intrusion, revealing its floristic influence on the Central Plateau of Brazil (Stehmann and Semir, 2001), and the discover of a new reproductive population (producing viable offspring) is essential for the design of conservation strategies in the near future. The wide geographic distribution of this species, albeit disjunct, with records from the Northeast, Southeast, South and now Midwest regions of Brazil, might be explained by a past forest connection, through the Central Plateau, between the tropical forests in the Amazon and in the Atlantic coast (Mori et al., 1981), characterized by relict dendritic networks of forest formations within the cerrado (Ledru, 1993; Ledru et al., 1994; Oliveira-Filho and Ratter, 2002; Rizzini, 1979).

In addition, these Cerrado forest formations may be explained by the great influence of the Atlantic vegetation on the origin of the Cerrado (Méio et al., 2003), which is mostly linked to the basin of Paraná river. Thus this species may be relict, explaining their low frequency in this biome. According to Meave & Kellman (1994), there is a great, although restricted, potential for riparian vegetation and associated seasonal forests to have sustained a large number of moist forest species during the dry episodes of the Quaternary period. This is supported by pollen studies that demonstrated the occurrence of large forest expanses in the Cerrado a few thousand years ago (Ledru, 1993; Ledru et al., 1994).

The present study provides the second record of this species for the Midwest region and for the state of Goiás. The first record was from 1930, but it had no exact locality description and could not be confirmed by specialists (Stehmann et al., 2014). Although this species is considered to have a low extinction risk (IUCN, 2014), the combination of its incompatibility system and complex pollination mechanism mean that *S. melissarum* is vulnerable, to a certain degree, when exposed to habitat fragmentation. These data demonstrate the importance of these records for the knowledge of the geographic distribution of the species, and especially as a way to provide input for the maintenance and conservation of the species. All of the records are associated to moist forest vegetation, highlighting the importance of preserving fragments of moist forests in general, and of semi-deciduous seasonal forest in the Brazilian cerrado in particular. These forests may contain relict species from a past of extensive forest formations in the cerrado, which served as refuge areas during the dry spells in the Quaternary period. They are essential to understand biogeographic questions and to maintain a very unique regional diversity (Oliveira-Filho and Ratter, 2001).

Acknowledgements

To Dr. Solange Cristina Augusto (UFU) and Dr. Gabriel Augusto Rodrigues de Melo (UFPR) for identifying the Euglossini bee. To Dr. João Renato Stehmann (UFMG) and Dr. Lynn Bohs (University of Utah) for identifying the plant species. To the biologists Juliano Moraes, Gabriel Eliseu and Paulo Victor Kanashiro for help during field work and photographs. To Dr. André Rodrigo Rech for suggestions on the manuscript. To FAPEG - Fundação de Amparo a Pesquisa do Estado de Goiás for financial support. To Projeto PELD Jataí - Pesquisas Ecológicas de Longa Duração (CNPq/FAPEG – 2012-10267001108) for partial financial support.

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