

WITHDRAWN: Floral biology, pollinators and reproductive success of *Abutilon grandifolium* in urban green spaces of Ciudad Autónoma de Buenos Aires

Pablo Daniel Cáceres

caceres2294@gmail.com

Universidad de Buenos Aires

Cecilia Estravis-Barcala

Universidad de Buenos Aires

Leandro Laino

Universidad de Buenos Aires

Rocío Lajad

Universidad de Buenos Aires

María Teresa Amela García

Universidad de Buenos Aires

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Floral biology, pollinators and reproductive success of *Abutilon grandifolium* in urban green spaces of Ciudad Autónoma de Buenos Aires

Cáceres, Pablo Daniel; Estravis-Barcala, Cecilia; Laino, Leandro; Lajad, Rocío; Amela García, María Teresa.

Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Depto. de Biodiversidad y Biología Experimental. Buenos Aires, Argentina.

Cáceres, Pablo Daniel; caceres2294@gmail.com; ORCID: 0009-0007-8661-268X

Estravis-Barcala, Cecilia; ORCID: 0000-0001-7922-7723

Laino, Leandro; ORCID: 0000-0003-0800-4404

Lajad, Rocío; ORCID: 0000-0002-2847-9389

Amela García, María Teresa; ORCID: 0000-0002-3745-1361

Abstract

Urban green spaces have gained importance in view of growing urbanization; however, limited research exists on the interactions of native plants with the local fauna, particularly comparing protected natural areas vs. recreated ones. To contribute to filling this gap, the floral biology and pollination of *Abutilon grandifolium* (Malvaceae) were studied in an Ecological Reserve and in a square where the local environment was recreated with native plants, both located in Ciudad Autónoma de Buenos Aires, Argentina. *A. grandifolium* was self-compatible. The flowers presented typical characteristics of biotic pollination (ornamented pollen, conspicuous perianth and rewards), particularly melittophily (diurnal anthesis, nectar, yellow-orange coloration, UV spectrum reward guides and pleasant odour). At both sites, the most frequent visitors were *Apis mellifera* and halictid bees, collecting nectar or pollen. While visits from the former were predominantly illegitimate, those from the latter were mostly legitimate. Occasional visitors included the bees *Xylocopa* sp. and *Bombus pauloensis*, syrphid flies, plus rarely, beetles. In specimens of all the bees, great amounts of *A. grandifolium* pollen was found in positions compatible with an effective transfer to the stigmas, therefore, all of them would be pollinators (when they access legitimately). The flies and the beetles would be mainly pollen and nectar thieves. In general, the identity, frequency and activity of the floral visitors coincided in the two sites, being species richness even greater in the square; therefore, the conditions that allow *A. grandifolium* to reproduce by xenogamy would be recreated in the square, thus maintaining its genetic variability.

Key words: *Abutilon grandifolium*, Apoidea, floral traits, floral visitors, native insect-plant interactions, urban green spaces

Introduction

The role of urban green spaces has gained great importance in recent decades in the face of increasing urbanization, and, more recently, in the context of the global coronavirus pandemic of 2019 (Covid-19). These spaces (either reserves, parks, squares, gardens), despite occupying small, generally fragmented areas, can function as biodiversity refuges (Goddard et al. 2010; Hall et al. 2017), constituting nodes in a network of bio-corridors (Haene 2020). They can even host notorious richness and abundance of species through proper management (Baldock 2021). They also have a positive impact on the health and well-being of citizens by allowing their interaction with nature (World Health Organization 2016; Haene 2020). Along with the recognition of their importance, in recent years, the role of native species in the conservation and restoration of ecosystems has been revalued, and consequently they have been incorporated into the design of such spaces (Faggi and Ignatieva 2009; Burgueño and Nardini 2019). In particular, regarding floral visitors, it has been shown that native plant species in urban green spaces attract more taxa and receive more visits than exotic ones (Zaninotto et al. 2022). Such planning contemplates continuous resource availability for different floral visitors (Araújo et al. 2022).

In Ciudad Autónoma de Buenos Aires (C.A.B.A.), Argentina, the cultivation of native vegetation in different green spaces has recently been prioritised. Between 2018 and 2019, the greatest increase in cultivation of native plants in public and private green spaces in the region was reported, accompanied by an increased richness of the species used (Haene 2020). However, there are few ecological studies about native plants and their interactions with fauna in green environments of this city, particularly floral visitors; those that exist were generally performed in botanical gardens or natural reserves with a diverse and extended floral offer through time (Suyama 1998; Mazzeo and Torretta 2015; Mosconi 2019; Gualdoni Becerra 2022).

The few ones that incorporated native flora in gardens and squares resulted in greater presence of insects and birds in few years (Haene 2018; Mesía Blanco 2019); this was also corroborated for bees in other cities of the world, presenting a major abundance of bees, in general, and of native bees, in particular, than in urban gardens without native plants (Pardee and Philpott 2014). In this sense, fast-growing native species, with few irrigation requirements, that adapt to local winds, are good candidates for planting in parks and urban gardens; for example, various Malvaceae shrubs.

These studies can also contribute to conservation and environmental education programs, making it possible to identify endangered or sensitive species and transfer successful practices to other green spaces in the city. In addition, the exchange of knowledge and experiences on native species with the community would facilitate the recognition of the value of this type of place. Urban green spaces and the living species that make them up have the potential not only to constitute biodiversity shelters but also cultural assets that underpin the local identity of their inhabitants and are a favourable setting for carrying out interdisciplinary projects with the participation of different actors from the community (neighbours, scientists, educators, plant growers, landscapers, NGOs, civil servants).

The study of the interactions between angiosperms and their pollinators is essential to understand the reproductive biology, evolution and distribution of flowering plants. One approach is the study of floral characters to determine pollination syndromes. The pollination syndromes hypothesis suggests that, by evolutionary convergence, unrelated plant species present common floral characters (e.g., architecture, shape, colour, reward, anthesis) associated with the habits, morphology and requirements of the pollinating agent (Faegri and van der Pijl 1979; Proctor et al. 1996; Fenster et al. 2004). Another approach to this interaction is to carry out faunal inventories that provide information on the composition, richness and abundance of floral visitors and their geographical and temporal variations (Abrahamovich and Díaz 2002); these surveys are important considering that the abundance of interacting parts regulates the frequency of interactions (Freitas et al. 2014). A third approach consists of recording floral visitors and their behaviour in flowers (Figueroa Fleming 2014; Inouye et al. 2015) and analysis pollen load or pollen in nests (Quiroz-García et al. 2001; Zych et al. 2013; Wilmer et al. 2017). These different approaches are not mutually exclusive and can be combined to gain a better understanding of biotic pollination.

The members of Malvaceae are mostly pollinated by insects (Knuth 1906), some being self-compatible (Parra-Tabla and Alonso 2021). Within this family, *Abutilon* comprises more than 150 tropical and subtropical species (Fryxell 2002), 11 of which inhabit the Southern Cone of South America (Zuloaga et al. 2020). Regarding pollination in this genus, there are mentions for some species: *A. pauciflorum* is pollinated by insects (De Marzi 2006), *Abutilon theophrasti* Medik. is visited by dipterans, butterflies and numerous species of bees (Lara Ruiz 2016), *Abutilon terminale* (Cav.) A. St.Hil. is visited by dipterans and bees (Sabatino et al. 2017), *A. indicum* (L.) Sweet is pollinated by honeybees (Abid et al. 2010). The pioneer *Abutilon grandifolium* (Willd.) Sweet, one of the recommended species for restoration purposes in Buenos Aires (Burgueño 2012a), is native from South America, particularly Peru, Bolivia, Chile, Paraguay, Uruguay and Argentina; it is widely distributed in the center and north of the last country, where it is encountered between 0 and 2000 m.a.s.l.. It is known by the common names of “malvavisco” (mallow) and “malva de monte” (woodland / forest mallow) in Spanish by “aguará po” (fox hand) in Mbya language (Martínez Crovetto 2012) and by “hairy Indian mallow” in English (Quiroz 2015). It grows in modified environments and disturbed soils. It has medicinal (Martínez Crovetto 2012), ornamental and textile uses, being cultivated for such purposes in some regions of Africa and China (Jaca et al. 2015). Although it is considered naturalized in certain regions of Australia, New Zealand and Pacific islands (GLOBAL COMPENDIUM OF WEEDS 2007), it was reported as invasive or potentially invasive in Hawaii and Portugal (Staples et al. 2000; Domingues de Almeida and Freitas 2012). It is a scantily branched shrub, generally with a single main stem. Each individual can reach 2.5 m in height. The leaves are wide, cordate. The stem is characterized by abundant simple trichomes. The flowers are solitary, axillary, complete, actinomorphic, pentamerous, with monadelphous androecium, multilocular ovary and axillary placentation. In the southern hemisphere, it blooms from spring to summer (Dimitri and Parodi 1972), from summer to middle autumn (Sersic et al. 2006), until late autumn (Jaca et al. 2015; Burgueño and Nardini 2019) or until winter (Burgueño 2012b), depending on the region where it grows. Its floral biology is almost unknown; only the conical epidermal cells of the petals were described, relating them with the interaction with pollinators and classifying the species as melittophilous (Costa et al. 2017); and the pollen has been characterised (Abdel Khalik et al. 2021) but not exhaustively.

The aim of this work was to study the floral biology of *A. grandifolium* in urban green spaces of the Ciudad Autónoma de Buenos Aires. In particular, to assess the reproductive system, floral traits and floral visitors (and among them, the pollinators), comparing the composition, richness and frequency of the latter in a protected natural area and in a public square with a sector with native plants recreating local natural habitats.

Materials and methods

Study sites

The study was carried out in two populations of *A. grandifolium* in the Ciudad Autónoma de Buenos Aires (C.A.B.A.), Argentina (Fig. 1). The first is a natural population located in the Ciudad Universitaria-Costanera Norte Ecological Reserve (RECU-CN) (34°32'23.5"S 58°26'30.9"W). This protected area (Fig. 1, A) covers an area of 18 hectares, bordering the Río de la Plata river. Its origin dates back to the 1960s as a result of filling with rubble. Since then, native and exotic plant species (144 registered / 238 estimated) have spontaneously established, forming three vegetation units: wetland, forest and grassland with groves (Melzi Fiorenza et al. 2020). Within this place, 10 specimens of *A. grandifolium* were selected.

The second population is located in Plaza Clemente, Colegiales neighbourhood of commune 13 of the Ciudad Autónoma de Buenos Aires, Argentina (34°34' 54.7"S, 58°26' 39.9"W), approximately 7 km away from the reserve. It is a 0.73 ha square (Fig. 1, B), inaugurated in 2019, where native species were planted, recreating three natural environments of the region: grassland, marginal forest, and logging. Within this place, 5 specimens of *A. grandifolium* were selected. Observations were made from September 23 to October 6, 2019 at the first site and from September 28 to November 6, 2020 at the second.

Reproductive system

To determine this, three sets of buds close to anthesis were selected from different individuals; sets 1 and 2 were bagged with organza bags; when the flowers opened they were submitted to one of the following treatments: 1) spontaneous self-pollination: the bags were left to exclude floral visitors during anthesis and let spontaneous deposition of own pollen, 2) induced self-pollination: the bags were removed, pollen was manually transferred to the stigma of the same flower and the bag was placed again, 3) free pollination: the flowers were tagged around the pedicel, allowing pollinators to visit and after anthesis they were bagged to prevent losses or damages by frugivores. The changes of each flower were monitored daily until fruit set. Immature fruits were harvested between 7 and 11 days after the abscission of the corolla, as it is possible to identify mature and immature or abortive seeds since then. The relative reproductive success (RRS) = (N° fruits/ N° flowers) X (N° seeds / N° ovules) according to Dafni (1992) was calculated for each treatment; for this, the number of ovules per ovary and per carpel was estimated from the total number of developed seeds plus the abortive seeds of ten immature fruits harvested from different specimens.

Flower traits, anthesis, flower phases, reward and attractants

To determine the floral phases, 51 buds about to open were tagged and the following floral characters were recorded during anthesis and analyzed all together afterwards: moment of opening and closing of the corolla, position and maturation of the reproductive cycles, floral functional structural type, color (compared to the chart of Kelly 1965). The flowers were observed every hour, between 10 a.m. and 5 p.m., for 6 consecutive days. Stigmatic receptivity was determined following the method of Osborn et al. (1988), considering the presence of bubbles after placing hydrogen peroxide on the stigmas as a positive result; to avoid a false positive (with pollen deposited on the stigmas), flowers with not yet dehiscent anthers, bagged at the bud stage were tested. The smell was detected and characterized from an olfactory test of floral pieces of each cycle in separate hermetic containers for a period of 15 minutes; for the location of osmophores, they were submerged in neutral red, and the stained areas were registered. The presence of nectar was explored in 10 flowers bagged since the bud stage. Flowers in the different phases of anthesis were photographed with a Pentax ME analogical camera with black-white photographic film and a Kodak 18A UV filter to record UV reflection/absorption patterns and detect reward guides in that spectrum.

Pollenkitt, reserves, viability and pollen morphology

To detect the main type of storage products/reserves in pollen grains (starch or lipids, respectively), as well as the presence of pollenkitt, grains from fresh anthers were stained with iodine solution and sudan IV, respectively. After identifying the reserve, pollen viability was evaluated in flowers recently opened and in flowers at the end of anthesis; grains of normal size and content that stained were considered viable while smaller, collapsed, empty or not stained grains were considered non-viable (Kerns and Inouye 1993; Amela García 1999). To describe pollen morphology, acetolysed grains (sensu Erdtman 1952) and grains fixed in ethanol 70% and dehydrated in an ascending ethanol series (D'Ambrogio 1986), were observed and measured with optical and scanning electron microscopes (Zeiss Supra 40 FESEM) microscopes respectively; the terminology of Punt et al. (2007) was followed.

Floral visitors and pollen load

Censuses of floral visitors to specimens of *A. grandifolium* were carried out at the reserve (35) and the square (50), totaling 8 hours 45 minutes and 12 hr 30 minutes, respectively. Each census lasted 15 minutes and was conducted on each shrub every hour from 10 a.m. to 17 p.m., at the same moment when the floral phases were recorded. For each visiting taxon, its identity, number of visits, duration of the visit, floral phase visited, and reward collected were registered. Visits were categorized as legitimate (if the visitor contacted the reproductive parts of the flower) or illegitimate (if the visitor collected a reward without coming into contact with the stigmata). Specimens of each visiting species were captured for their definitive taxonomic determination and analysis of the pollen load. For this, permanent preparations of the pollen they carried were made by micro-acetolysis (Genise et al. 1990). For the identification of said pollen, an herbarium collection of sympatric plant species with biotic pollination syndrome in simultaneous flowering was gathered and pollen slides were made by acetolysis (Erdtman 1952). The pollen load carried by the visitors was recognized and quantified by comparison with these reference preparations using light microscopy (LM); in the cases of scarce load, it was analyzed using scanning electron microscopy (SEM), mounting the parts of the insects that carried pollen as well as pollen from *A. grandifolium* and other Malvaceae (*Sphaeralcea bonariensis*) in blossom in the study area with very similar palynological characteristics, for accurate identification.

Statistical analysis

Statistical analysis were performed with R v3.6.2 (R Core team 2019) and the *glmmTMB* (Brooks et al. 2017) and *emmeans* (Lenth 2020) packages were used. Models in general were simplified as follows: significance of the different terms was tested starting from the higher-order terms model using likelihood ratio tests (LRT) to compare between models (Chambers and Hastie 1992) and non-significant terms ($p > 0.05$) were removed. *Post hoc* comparisons using contrast matrixes were performed with the *emmeans* function (Lenth 2020).

The differences in the number of mature seeds per fruit of the different reproductive treatments were evaluated using the Kruskal-Wallis test. To compare the visitation rate of the most frequent flower visitors in the square, a generalized linear mixed model (GLMM) was used with a negative binomial distribution to account for the overdispersion of the data. The model included the number of visits as a response variable, the type of visitor and their activity (honey bees exclusively collecting nectar in the successive visits, honey bees collecting pollen and/or nectar in the successive visits, and halictid bees) and the time of day (morning, from 10 a.m. to 1 p.m.; afternoon, from 2 to 5 p.m.) as fixed factors, the shrub and the date as random factors and the number of flowers recorded as offset, to correct the number of events for an estimate of population size.

Results

Reproductive system

In all the treatments fruits with seeds were produced, developing 8-10 mericarps per fruit (Table 1). Each ovary contained 58 ± 2 ovules (5-7 ovules/carpel). Relative reproductive success was high in all treatments (values close to 1), due to the high fruit/flower ratio together with the high seeds/ovules ratio (Table 1). Seed set was higher in fruits obtained by free pollination than in the rest of the treatments, but the differences were not significant ($\chi^2 = 2.9465$, $p = 0.2292$).

Anthesis and floral phases

Most of the monitored buds opened around 10 hr ($n=36$) and only 15 opened after noon; 11 of these last ones, remained open until the afternoon of the following day, averaging 17 hr of anthesis (range: 4-25 hr), while most of the ones that opened in the morning closed in the afternoon, averaging 6 hr (range: 4-7 hr) of anthesis.

Four phases were recognized from the opening to the closing of the corolla (Fig. 2), defined by the following characteristics: Bud about to open (Fig. 2, A): intense orange corolla, with non-displayed petals, visible through the slightly separated sepals. Light yellow non-dehiscent anthers. Stigmas hidden between the anthers, receptive.

Phase 1 (Fig. 2, B): deep orange corolla, petals beginning to separate. Some dehiscent anthers. Stigmas visible between the anthers and receptive.

Phase 2 (Fig. 2, C): corolla open, yellow-orange, bell-shaped (diameter of opening 2-3cm). The coloration is more intense at the edges of the petals, becoming lighter towards their base. All anthers dehiscent, pollen is found dropped at the base of the corolla. The stigmas are located between the anthers, rarely exceeding them.

Stage 3 (Fig. 2, D): yellow-orange corolla, 1 or more petals recurved backwards, sometimes taking altogether the shape of a bowl (diameter of opening 3-4 cm). Anthers dehiscent, some stigmas exceed the height of the anthers.

Phase 4 (Fig. 2, E): corolla closing, whitish and progressively dehydrating (especially from the distal edges of the petals).

After phase 4, the corolla fell (Fig. 2, F) and after a few days, if fertilisation had occurred, the growth of the ovary and the development of the fruit were evident, which reached maturity after 11 days (Fig. 2, G).

Attractants and rewards

In the UV spectrum, (Fig. 3 B) a contrasting pattern was observed in all the floral stages, with an absorbent ring around the reproductive cycles and at the base of the petals, while the rest of the floral pieces were reflective. According to the olfactory test, the odour was faint and emitted by the androecium, whose anthers were the unique floral pieces that were stained with neutral red, thus revealing the location of the osmophores (Fig. 4 A). The nectaries, made up of glandular trichomes, were located on the basal margins of the unguiculate petals (Fig. 4 B); nectar was present in phases 2 and 3.

Pollen: pollenkitt, reserves, viability and morphology

The pollen grains stained positively both with sudan IV and iodine solution, so they had lipids and starch as reserves (Fig. 3, C). Pollenkitt was present among the grains (Fig. 3, C). The percentage of viability was 95 % in phase 1 (n = 447 grains from 3 flowers), decreasing to 89 % in phase 3 (n = 872 grains from 3 flowers).

A. grandifolium presented a spherical grain (Fig. 5, A), with an equinated (Fig. 5, B), papillate and perforated (Fig. 5, C) exine. The spines were conical, with concave sides, situated on a circular base (Fig. 5, D), rarely in pairs (Fig. 5, B, arrow), generally straight or slightly curved at the apex (Fig. 5, E). Seen from above, the ornamentation of the tectum resembled granules (Fig. 5, C) but seen from a side they resembled papillae (Fig. 5, D, white arrows). The diameter of the pollen grains was $51.58 \mu\text{m} \pm 3.12$ (n=6) (Table 2); it should be noted that the pollen grain of *Sphaeralcea bonariensis*, another Malvaceae in blossom in the study area, has very similar morphology but is 39% smaller than *A. grandifolium*, with a diameter of $31.65 \mu\text{m} \pm 0.68$ (n= 3), so they could be distinguished in the visitor pollen load. The diameter of the papillae was $0.20 \mu\text{m} \pm 0.02$ (n=4), while that of the perforations was $0.28 \mu\text{m} \pm 0.05$ (n=3) (Table 2). Spines had a length of $2.96 \mu\text{m} \pm 0.41$ (n=10), while their diameter was $2.37 \mu\text{m} \pm 0.45$ (n=2) at the base, $1.10 \mu\text{m} \pm 0.07$ (n=2) in the middle part and $0.31 \mu\text{m} \pm 0.02$ (n=2) at the apex (Table 2).

Floral visitors and pollen load

Both in the reserve and in the square, the assemblages of floral visitors were mainly composed of hymenopterans belonging to Apoidea, represented by two families: Apidae and Halictidae; other less represented visitors were dipterans from Syrphidae and, the fewest, coleopterans (Table 3). Species richness was lower in the reserve than in the square, with 6 vs. 9 species, respectively (Table 3). In the reserve, a total of 124 floral visitors were registered, who performed 682 visits during the 35 censuses, giving a rate of 14.17 visits per hour (Table 3). *A. mellifera* performed most of the visits (81.1%), accumulating 73.39% of the relative abundance of visitors, followed by halictids who performed 7,6% of the visits with an abundance of 16.13%. The bees *Xylocopa* sp. and *Bombus pauloensis*, together with the syrphid flies and curculionid beetles showed the lowest abundances (4.03%, 2.42%, 3.23% and 0.81%, respectively). In the square, a total of 370 floral visitors were registered, who performed 1505 visits during the 50 censuses, giving a rate of 29.60 visits per hour. *A. mellifera* represented 88.6% of the abundance and 95.8% of the visits, followed by halictids, accounting for 8.4% of the abundance and 2.2% of the visits. Rarer visitors were two species of syrphids (0.8%) and one coccinellid beetle (0.3%) (Table 3).

In both environments, the two most frequent groups of visitors (honey bees and halictid bees) visited the flowers in different ways. Halictid visits lasted considerably longer than honey bee visits and the vast majority were legitimate (Table 3). On the other hand, honey bees made a greater number of illegitimate visits, in which they exclusively collected nectar, reaching 90.8% of the total visits in the square. Only when collecting pollen did they consistently perform legitimate visits. The rest of the visitors made almost exclusively legitimate visits. *Xylocopa* sp. and *B. pauloensis* mostly collected nectar; the syrphids mainly ingested pollen (Table 3); the coccinellid seemed to eat pollen and the curculionids seemed to lick nectar.

Honey bees exhibited different movements on the flowers according to the floral reward exploited. Individuals that illegitimately collected nectar (Fig. 8, A) landed on the pedicel or on the abaxial face of the calix and corolla, and inserted their proboscis between the petals, repeating the action in different pairs of them. Illegitimate visits were usual to flowers in phases 1, 2 and 3. These visits were generally brief and frequently the bees moved on to another flower of the same plant. On several occasions, while a bee was collecting nectar, a second bee approached and contacted the former, which flew to another nearby flower. On the other hand, honey bees that collected alternatively nectar or pollen on successive visits generally accessed each of these rewards legitimately. To collect nectar, they landed on the adaxial face of the petals, with the head towards the center of the flower and extended the proboscis towards the base of the petals, repeating this in a circular fashion (Fig. 8, B). After these visits, pollen was observed deposited on the head and thorax dorsum (Fig. 8, B). To collect pollen,

they manipulated the anthers with their legs, traversing different anthers and contacting the stigmas with the ventral region of the body (Fig. 8, C); then they hovered in front of the recently visited flower, forming the mass of corbicular pollen with movements of the legs and they returned to the same flower or visit a nearby one, continuing with pollen collection. On occasions, both nectar and pollen were collected in the same visit, licking nectar first and climbing on the anthers afterwards. In contrast, halictids mostly performed legitimate visits, both collecting nectar and pollen, with nectar theft being very rare (3 visits). They mainly visited flowers in phase 2. When collecting pollen, these bees moved through the anthers moving their legs and jaws, contacting the stigmas with the ventral surface of the body. When collecting nectar, they usually licked between contiguous petals (Fig. 8, D) and walked around the staminal column to the following nectariferous trichome bundle, thus widely contacting the reproductive organs (Fig. 8, E). Due to their smaller body, as compared to the honey bee's, they could fully enter the flower, so after one visit pollen was deposited on almost the entire body dorsum.

The rest of the occasional flower visitors also differed in their behaviour in the flowers. The large *B. pauloensis* and *Xylocopa* sp. landed on the petals, contacting the reproductive cycles with the ventral surface of the abdomen (Fig. 8, F) and accessing the nectar with their proboscis. Of these bumblebees, visits of *Xylocopa* sp. were shorter but more frequent. On the other hand, a very small unidentified hymenopteran recorded in the square, sucked nectar at the base of the staminal column in longer visits than those in which it collected pollen from the anthers (Fig. 8, G). Regarding hoverflies, those of sp. 2 made long-term visits during which they collected pollen, landing on the anthers, moving limitedly, even not through the entire androecium; while one of the individuals of sp. 3 collected nectar by landing on the petals but, due to its body size, it did not contact the anthers or stigmas (Fig. 8, H). Lastly, a coccinellid was observed emerging from a flower in phase 1 carrying pollen on its mouthparts, legs and probably ventral surface of the body and curculionids were inside the flowers with their rostrum pointing the nectaries (Fig. 8, I).

The visitation rates of the most frequent visitors differed significantly between honey bees and halictids, even between visit type among honey bees (Fig. 6, A). The rate of honey bees exclusively collecting nectar was significantly higher (more than three times) than honey bees collecting pollen and halictids (*post hoc* comparison: HB.nectar vs HAL, t-ratio = 10.76 df= 73 $p < 0.0001$; HB.pollen vs HB.nectar, t-ratio = -5.36 df= 73 $p < 0.0001$). Also, significant differences were found between honey bees collecting pollen and halictids (*post hoc* comparison: HB.pollen vs HAL, t-ratio = 4.56 df= 73 $p = 0.0001$). On the other hand, the visitation rates were not affected by the time of day (Fig. 6, B): no differences were found between visits occurring in the morning and the afternoon (in *logit* scale, $\beta = 0.19$, SE = 0.19, $z = 0.99$, $p = 0.32$). Finally, considering floral phases, flowers in phase 2 received the highest number of visits either in the morning or in the afternoon (Fig. 7).

Most of the pollen carried by *A. mellifera* consisted of *A. grandifolium* on the dorsum of the head and thorax as well as *Rapistrum* sp. and *Raphanus* sp. on the corbiculae, while *Xylocopa* sp., *B. pauloensis*, Halictidae and Syrphidae carried large amounts of *A. grandifolium* in the ventral zone and of plant species not found in blossom in the surroundings of the studied plants (Fig. 9). Complementary SEM analysis showed that *A. mellifera* carried *A. grandifolium* pollen mainly between the antennae (Fig. 10, A) and also on the corbicles, in addition to *Conium* sp., *Sphaeralcea bonariensis* (Fig. 10, B-C) and another unidentified species (Fig. 10, D); in the halictids, *A. grandifolium* and *Sphaeralcea bonariensis* pollen was found on the ventral area of the head (Fig. 10, E-F), thorax and abdomen (Fig. 10, G), as well as on the hind legs (Fig. 10, H); the syrphids presented *A. grandifolium* pollen on head dorsum and eye margins (Fig. 10, I), on the ventral area of the abdomen (Fig. 10, J), as well as a large number of *Conium* sp. grains, mainly on the abdomen and on the hind legs (Fig. 10, K-L).

Discussion

The fruits and seeds obtained by induced and spontaneous self-pollination indicate that *A. grandifolium* is self-compatible and that its reproduction does not depend on a pollen vector. Since fruit production was >30%, it would be highly self-compatible, according to the classification proposed by Dafni (1992). Seed set was higher (though not significantly) through free pollination with respect to self-pollination treatments. The location of the stigmas slightly above the level of the anthers in some flowers (herkogamy) would facilitate xenogamy (Sargent et al. 2006; Waites and Agren 2006). Under natural conditions, the seeds could be produced by autogamy or xenogamy. In most of the buds, the stigmas were receptive before the dehiscence of the anthers (protogyny) and, once the anthesis had started, all the anthers were dehiscent; thus, allowing self-pollination. Facultative autogamy has been reported in other species of the genus (Abid 2006; Abid et al. 2010). It is probable that this condition favors the invasiveness of this species and even its naturalisation.

The floral traits (yellow-orange and bell to bowl-shaped corolla, nectar and pollen supply, diurnal anthesis, existence of a UV reward guide plus mild and pleasant aroma) are associated to the melittophilous pollination syndrome (Faegri and van der Pijl 1979). The nectar is available in several points, circularly situated, constituting a round-about flower (Endress 1996). The UV

absorbing circle (in contrast with the rest of the floral pieces) coincides with the location of the nectaries and where visitors legitimately suck; in turn, it frames the other reward, pollen.

The pollen grains, greater than 50µm, are large, according to Erdtman's (1952) size categories; however, the measurements disagree with the description of *A. molle* (syn. *grandifolium*), reported by Shaheen et al. (2009). The exine presents perforations and spines, as in *Abutilon pictum* (Del Pino and Díez 1990). The shape of the spines and their cushion base is typical of the Abutilieae tribe, in contrast to representatives of other Malvaceae tribes (Christensen 1986; Naggar & Sawady 2008; Abdel Khalik et al. 2021).

The anthesis of *A. grandifolium* was entirely diurnal for most of the flowers. During it, the four phases were mainly distinguished by the degree of opening of the corolla (both in the most abundant bell-shaped or in the less abundant bowl-shaped), and, consequently, the exposure of the reproductive cycles, as well as by changes in its coloration.

Due to the closeness of the stigmas and the anthers, almost all the recorded visitor taxa are capable of transferring pollen (autogamous or allogamous) to the stigmas, either when they legitimately sip nectar or when they land on the anthers to collect (bees) or ingest (flies) pollen. Though, due to differences in their visit frequency, size and behaviour in the flowers, the following analysis must be considered. Pollen deposition of *A. grandifolium* was nototribic in *A. mellifera* and halictids, while sternotribic in the rest of the visitors. *A. mellifera*, though the most frequent visitor and its ability to pollinate during legitimate visits, almost half of the visits in the reserve and almost all of the visits in the square were illegitimate; so, in this type of visits, due to their way of accessing the nectar without contacting anthers or stigmas, they would act as nectar thieves (Inouye 1980). Nectar theft can negatively affect the reproductive success of plants, since nectar production has an energy cost, and the scarcity of nectar can reduce potential legitimate visits (Irwin et al. 2010). The absence of mechanisms that prevent illegitimate visits by *A. mellifera* to *A. grandifolium* flowers could be related to the fact that this interaction is relatively recent in evolutionary terms (Hanna et al. 2014), since these bees were introduced to America by humans for honey production a few hundred years ago (Whitfield et al. 2006). Only the pollen-collecting honey bees could be considered occasional pollinators of this species. Besides, regardless of the type of resource collected, *A. mellifera* visits were usually brief, and many times even abbreviated by the arrival of a second individual to the same flower. In contrast, the second most frequent visitors, the halictids, rarely visited *A. grandifolium* flowers illegitimately. Because their visits lasted longer than those of *A. mellifera* and, due to their smaller bodies, moved easier within the corolla getting in touch with a major number of anthers and stigmas, they could be considered the main pollinators of *A. grandifolium*. Among the less frequent visitors, the large bees, *Bombus pauloensis* and *Xylocopa* sp., given their robust bodies and abundant hairiness, they could transfer large amounts of pollen during their visits, which were all legitimate. The syrphids would be pollen (and occasionally nectar) thieves. Among the beetles, the coccinellid would be a pollen thief while the curculionid, who could ingest nectar through the typical rostrum of this family, would be a nectar thief. Most Coccinellidae are carnivorous, but some can feed on pollen (González 2009). Curculionids that visit flowers can get different rewards, including nectar (Bernhardt 2000; Lehnebach 2002). No visits by Lepidoptera were recorded, though they were present in both sites and have been reported as frequent visitors to *Abutilon indicum* and *Sida ovata*, both belonging to the Malvae and with similar floral morphology, attractants and rewards (Dawar et al. 1994; Abid et al. 2010).

The effectiveness of pollination is determined both by the efficiency of the pollinating agent in transferring pollen to the stigma and by the intensity of visits (Freitas 2013). The analysis of the pollen load (taxa, relative abundance and distribution on the body) confirmed the observations in the field of the activity of the most frequent visitors and provided information on those which performed few visits. All carried large quantities of *A. grandifolium* pollen in areas of the body capable of contacting the stigmas. In the case of honey bees and halictids, pollen deposited on their heads or body dorsum while sucking nectar may be transferred to the stigmas efficiently; on the contrary, pollen collected, i.e., carried on the corbiculae, would be taken to the nests; though, the low frequency of pollen collection visits and the little amount of *A. grandifolium* pollen in the corbiculae of *A. mellifera* is consistent with the suspicions that large size, spines and pollenkitt (all characters observed in *A. grandifolium*) would hinder the packing of mallow pollen grains by corbiculate bees (Lunau et al. 2014; Konzmann et al. 2019).

The differences observed in the abundance of the main recorded hymenopteran visitors may have been due to their different foraging and nesting habits as well as degrees of sociability (which influence their food collection). The halictids, at least the ones from genus *Augochlora*, are solitary or semi-social, with a generalist diet and nest on the ground or in decaying wood (Dalmazzo et al. 2014). Many solitary bee species restrict their flights to a few hundred meters around their nest (Gathmann and Tschamtko 2002; Zurbuchen et al. 2010). Therefore, their visits would reflect the presence of nests in a trunk or on the ground in the reserve as well as in the square. In contrast, *A. mellifera* is eusocial (living in colonies of tens of thousands of individuals), can travel several kilometers on its collecting flights and use to temporarily concentrate this activity on one

resource. Besides, at the university campus adjacent to the reserve, there were colonies of these bees, a possible reason for, at least in part, their high frequency of visits. Regarding the less frequent hymenopterans, *Bombus* bumblebees are eusocial, but their colonies comprise a few hundred individuals and they usually nest on the ground under leaf litter (Goulson 2003). *B. pauloensis* has a generalist diet, collecting nectar and pollen from numerous families, including Malvaceae (Abrahamovich et al. 2001). In the square, given the characteristics of the study site and the surrounding blocks, the presence of *B. pauloensis* nests in the vicinity would be rare, which would explain their low abundance in the site, not only their visits to the flowers; anyway, in the reserve, a site with more nesting availability, the visits were not much more than those registered in the square, probably because these bees were visiting assiduously the abundant flowers of many nearby *Solanum sisymbriifolium* individuals. *Xylocopa* are solitary, nest on soft wood and are usually polylectic (Lucía 2011) and polytropic (Amela García 1999). Both of these large bees do not fit comfortably in the flowers of *A. grandifolium* and would prefer more producing nectar or easier pollen collecting resources if available.

Regarding the number of flowers visited per individual, in both sites, *A. mellifera* and *Xylocopa* sp. visited rather more flowers than *B. pauloensis* and halictids, so, these last two would contribute more to xenogamy than the ones that visited successively flowers of the same bush, thus performing geitonogamy to greater degree.

The floral visitor assemblage was similar in the reserve and in the square. This proves that the square is providing conditions that allow the recruitment of floral visitors, at least to *A. grandifolium*, and that the incorporation of this species in this urban green space has been worth it. Even, the species richness was higher in the square. In another comparative study between a reserve and an urban green space with cultivated plants in C.A.B.A. (Mosconi 2020), species richness was also lower in the reserve. This may be because in the recreated urban spaces the floral offer is usually greater or more continuous, probably even superabundant. Anyway, regardless of richness, the square would be functioning as a post, part of a biological corridor between the RECU-CN and the other reserves of C.A.B.A., as well as the nearer ones of the Area Metropolitana de Buenos Aires (AMBA), and the nearby urban green spaces. The finding of pollen from plant species that were not in flower at the study site indicate that floral visitors travel greater distances than within the sampled site and, therefore, would be capable of interconnecting these areas. A home range greater than the area offered in urban green spaces (urban strips planted with sunflowers) was also verified for *A. mellifera* and *Bombus terrestris* through pollen load, suggesting that they forage in other habitats within the urban matrix (Potter et al. 2019). In relation to pollination, biological corridors favor both the presence of floral visitors looking for resources in each one of the nodes and pollen flow between populations, thus contributing to outcrossing.

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Tables

	Spontaneous self-pollination	Induced self-pollination	Free pollination
No. of flowers	49	44	39
No. of fruits	44	40	39
% of mature seeds/ovules	78	79	88
RRS (relative reproductive success)	0.69	0.72	0.87

Table 1. Fruit and seed set from different pollination treatments in *A. grandifolium*. Relative reproductive success (RRS index) calculated from the number of fruits, flowers, seeds and ovules.

	Diameter	Length	Major diameter	Intermediate diameter	Minor diameter
Grain (n=6)	51.58 ± 3.12	-	-	-	-
Warts (n=4)	0.20 ± 0.02	-	-	-	-
Perforations (n=3)	0.28 ± 0.05	-	-	-	-
Spines (n=10)	-	2.96 ± 0.41	2.37 ± 0.45	1.10 ± 0.07	0.31 ± 0.02

Table 2. Characterization of *A. grandifolium* pollen grain. Dimensions in microns (μm).

	Floral visitors (order, family, species)	Visitors number	Visits number	Visits per hour	Flowers visited by individual	Relative abundance (%)	Visit duration (s) (mean ± SE)	Type of visit (%)		Reward collected
								L	I	
Ecological Reserve	Hymenoptera									
	Apidae									
	<i>Apis mellifera</i>	91	553	10.40	6.08	73.39	5.9 ± 0.2	52	48	P, N
	<i>Xylocopa sp.</i>	5	30	0.57	6.00	4.03	3.4 ± 0.3	100	0	N
	<i>Bombus pauloensis</i>	3	4	0.34	1.33	2.42	2.0 ± 0.4	100	0	N
	Halictidae									
	Halictidae sp. 1	20	52	2.29	2.60	16.13	11.4 ± 1.2	98	2	P, N
	Diptera									
	Syrphidae									
	Syrphidae sp. 1	4	42	0.46	10.50	3.23	3.0 ± 0.1	100	0	P
Coleoptera										
Curculionidae sp. 1	1	1	0.11	1.00	0.81	nd	1	0	N	
TOTAL	124	682	14.17							

Square	Hymenoptera									
	Apidae									
	<i>Apis mellifera</i>	306	1348	26.24	4.40	88.6	13.3 ± 0.7	77	123	P, N
	<i>Xylocopa sp.</i>	1	18	0.06	18.00	0.3	7.3	94	6	P, N
	<i>Bombus pauloensis</i>	2	3	0.16	1.50	0.5	51.8 ± 40.8	67	33	N
	Hymenoptera sp. 1	4	4	0.46	1.00	1.1	73.3 ± 49.3	100	0	P, N
	Halictidae									
	<i>Augochlora sp.</i>	17	19	1.36	1.12	4.6	85.6 ± 18.2	nd	nd	nd
	Halictidae sp. 2	14	14	1.12	1.00	3.8	100.6 ± 31.1	91	9	P, N
	Diptera									
	Syrphidae									
	Syrphidae sp. 2	2	2	0.16	1.00	0.5	22.5 ± 2.5	100	0	P, N
	Syrphidae sp. 3	1	2	0.08	2.00	0.3	397.5	100	0	P
Coleoptera										
Coccinellidae sp. 1	1	1	0.08	1.00	0.3	10	nd	nd	P	
TOTAL	370	1505	29.60							

Table 3. Total number of floral visitors and total number of flowers visited, number of visitors relativized to the total time of each census, flowers visited per individual, relative abundance, average duration with its standard deviation of the visit, type of visits and rewards collected by floral visitors of *A. grandifolium* surveyed in 35 (8 hours 45 minutes) and 50 (12 hours 30 minutes) censuses carried out in an ecological reserve and a square, respectively. I = illegitimate visit, L= legitimate visit, N = nectar, P = pollen; nd= no data.

Figure legends

Figure 1. Study sites of *A. grandifolium* in green spaces of the Ciudad Autónoma de Buenos Aires. (A) Ciudad Universitaria-Costanera Norte Ecological Reserve. (B) Clemente Square.

Figure 2. Phases of development of flowers and fruits of *A. grandifolium*. A) bud about to open. B) flower in phase 1. C) flower in stage 2, corolla bell-shaped; the anthers (yellow) and the stigmas (purple) are observed. D) flower in stage 3, bowl-shaped corolla. E) flower in phase 4, corolla closed and dry. F) flower with recently fallen corolla. G) fruits with different degrees of development: one green immature (left) and two mature, dry and dehiscent (right).

Figure 3. A) Neutral red staining test positive for anthers. B) Photograph of the nectaries, formed by glandular trichomes; C-D) Pollen stained with C) sudan IV and D) iodine solution; note pollenkitt among grains (arrows).

Figure 4. UV. Photographs of the different floral phases taken with an analogical camera of *A. grandifolium* without (A) and with (B) an UV filter. From left to right: stages 1 to 4.

Figure 5. Morphology of *A. grandifolium* pollen A) general appearance of the grain; B) ornamentation of the exine, with (widespread) simple and (rare) double (arrow) spines; C) detail of perforations (black arrow) and papillae (white arrow); D) detail of the papillae (arrowhead) and conical spines, with apex slightly curved (black arrow) or straight (white arrow); E) detail of spine cushion.

Figure 6. Visitation rate of the most frequent floral visitors of *A. grandifolium*. Number of visits per flower per hour estimated from 50 censuses. (A) Types of visits performed by honey bees either, exclusively collecting nectar (HB.nectar) or nectar and/or pollen (HB.pollen) in the successive visits, and halictids (HAL). (B) Visits by the mentioned groups of visitors in relation to time of day (morning, 10 a.m. to 1 p.m.; afternoon, 2 to 5 p.m.).

Figure 7. Number of visits received by *A. grandifolium*, according to the floral phase and time of day (morning, 10 a.m. to 1 p.m., n = 26 censuses; afternoon, 2 to 5 p.m., n = 22 censuses).

Figure 8. Pollen load carried by floral visitors of *A. grandifolium* analyzed with MO. The numbers next to each floral visitor name identify the specimen.

Figure 9. Floral visitors of *A. grandifolium*: A) *Apis mellifera* licking nectar illegitimately; B) *Apis mellifera* licking nectar legitimately and contacting pollen with thorax dorsum; C) *Apis mellifera* collecting pollen; D) halictid licking nectar; E) halictid walking around staminal column; F) *Xylocopa* sp. arriving at a flower; G) non identified hymenopteran; H) syrphid hovering in front of a flower; I) curculionid about to lick nectar.

Figure 10. Pollen carried by floral visitors analysed with SEM. A-D, *A. mellifera* (one specimen). A) *A. grandifolium* pollen between the antennae; B) *A. grandifolium* and *S. bonariensis* pollen on the corbicula. C) Detail of the former. D) detail of indeterminate pollen grain on the corbicula. E-H, Halictidae (sp. 1, two specimens). E), ventral area of the head with grains of *A. grandifolium*. F) surroundings of the proboscis base with *A. grandifolium* and *S. bonariensis* pollen grains; G) ventral view of the abdomen with *A. grandifolium* and *S. bonariensis* pollen grains; H) hind leg with *S. bonariensis* pollen grains. I-L, Sirphidae (two specimens, each one from different species). I) detail of the edge of the eyes with grains of *A. grandifolium*; J) ventral view of the end of the abdomen with pollen of *A. grandifolium*, *S. bonariensis* and *Conium* sp.; K) detail of hind leg with *Conium* sp. pollen; L) detail of pollen grains of *Conium* sp. in polar and equatorial view between hairs of the hind leg.



Figure 1

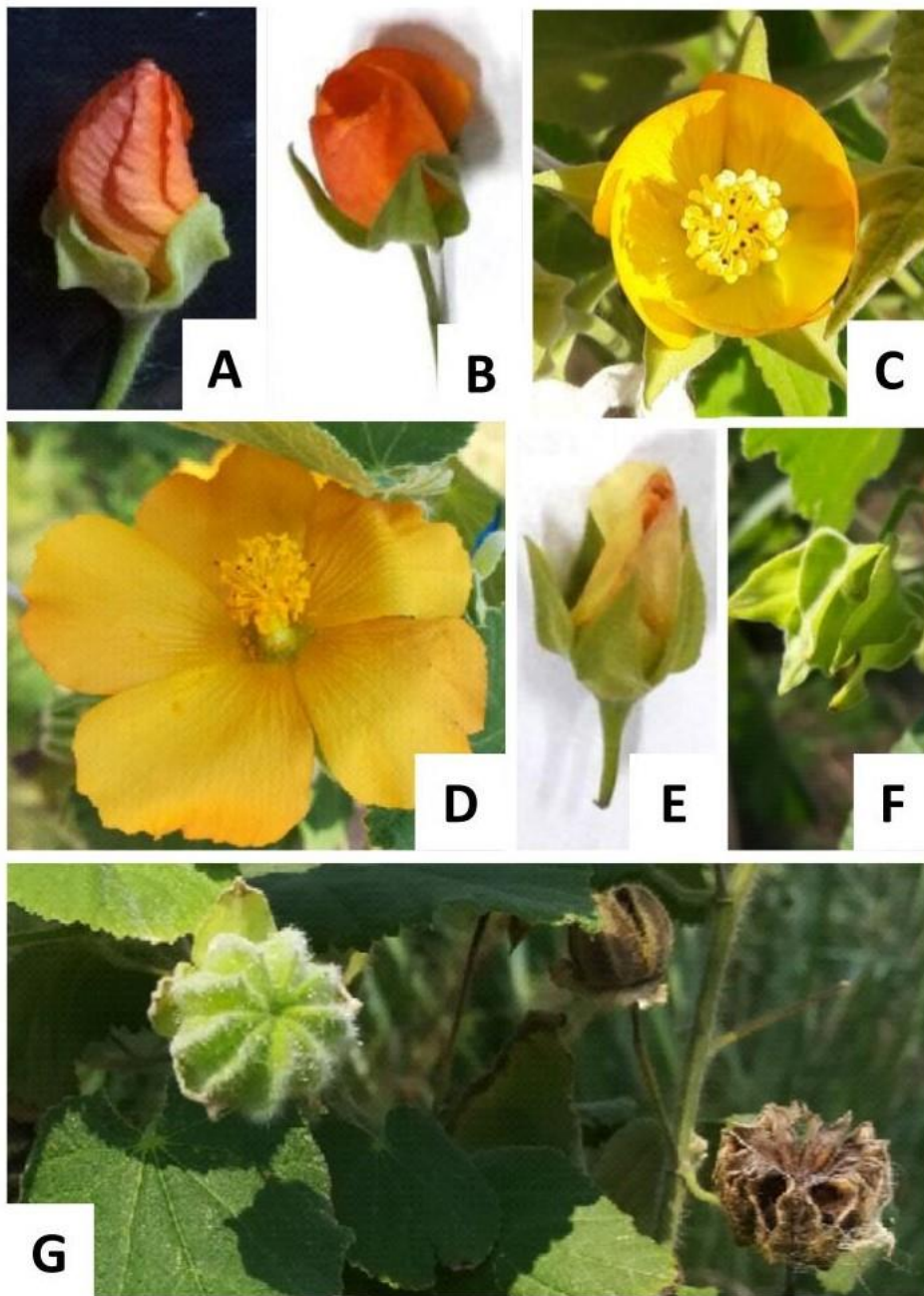


Figure 2

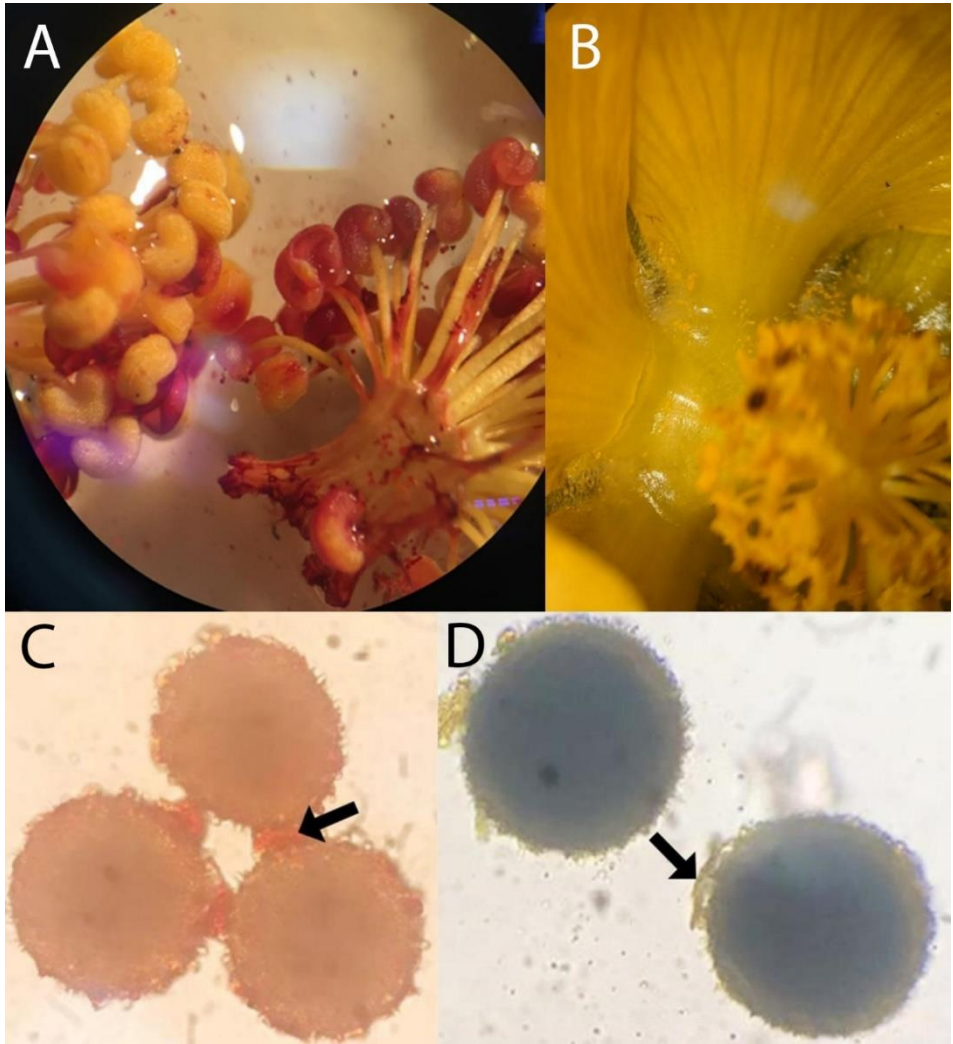


Figure 3



Figure 4

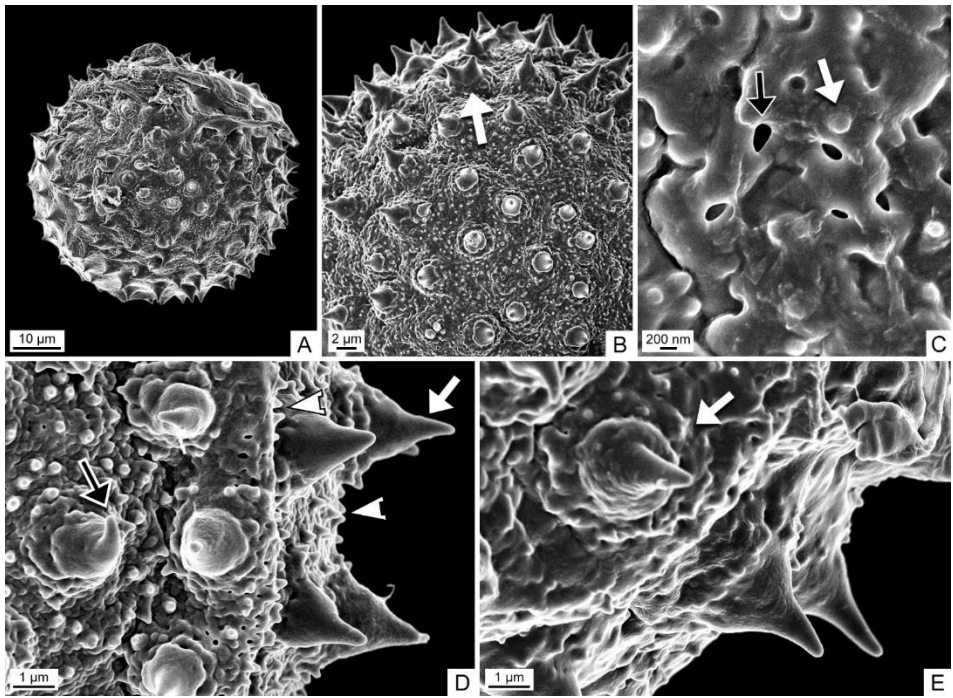


Figure 5

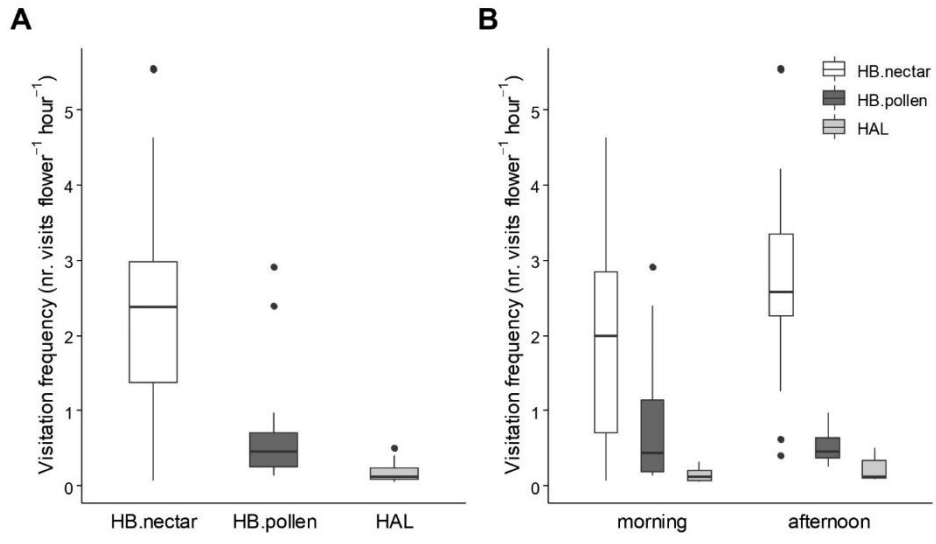


Figure 6

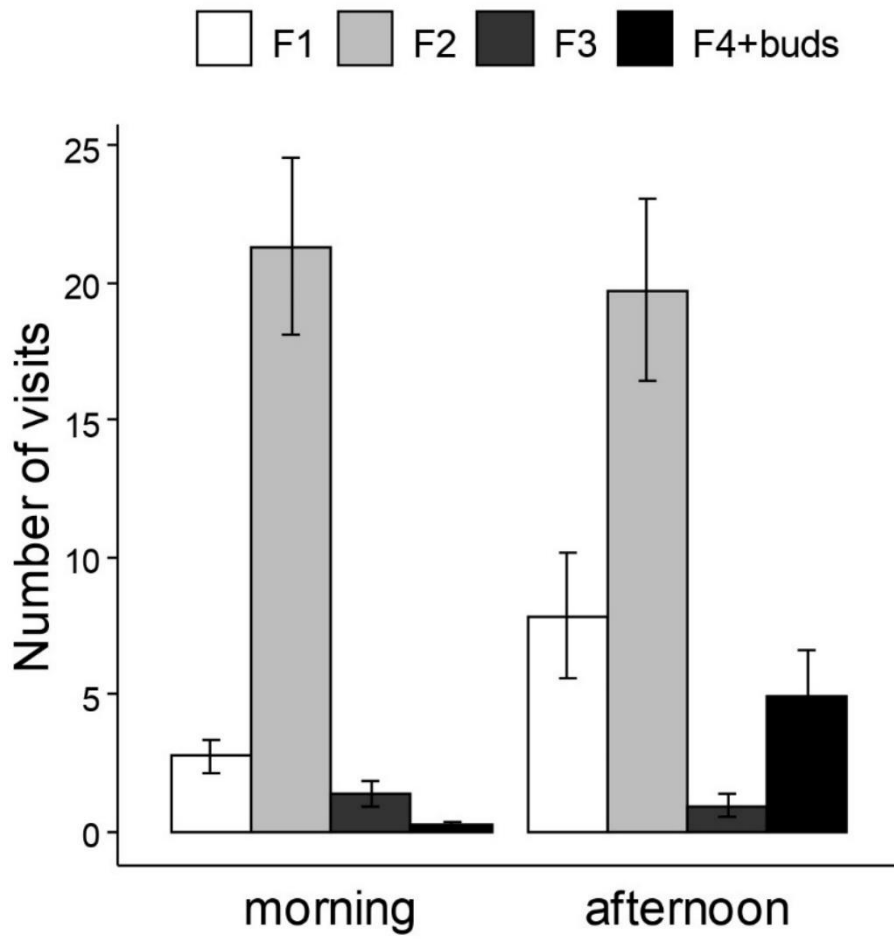


Figure 7

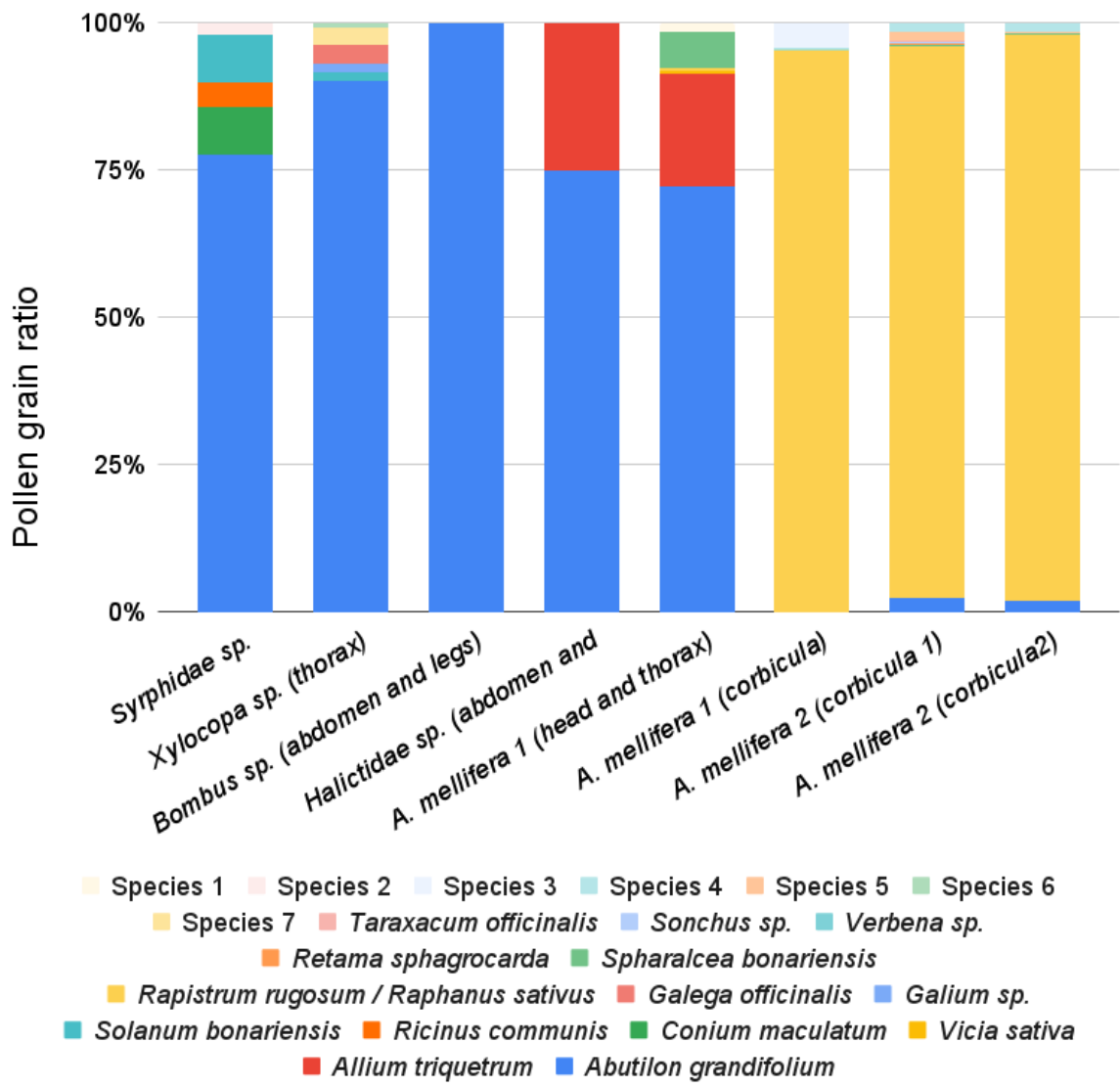


Figure 8

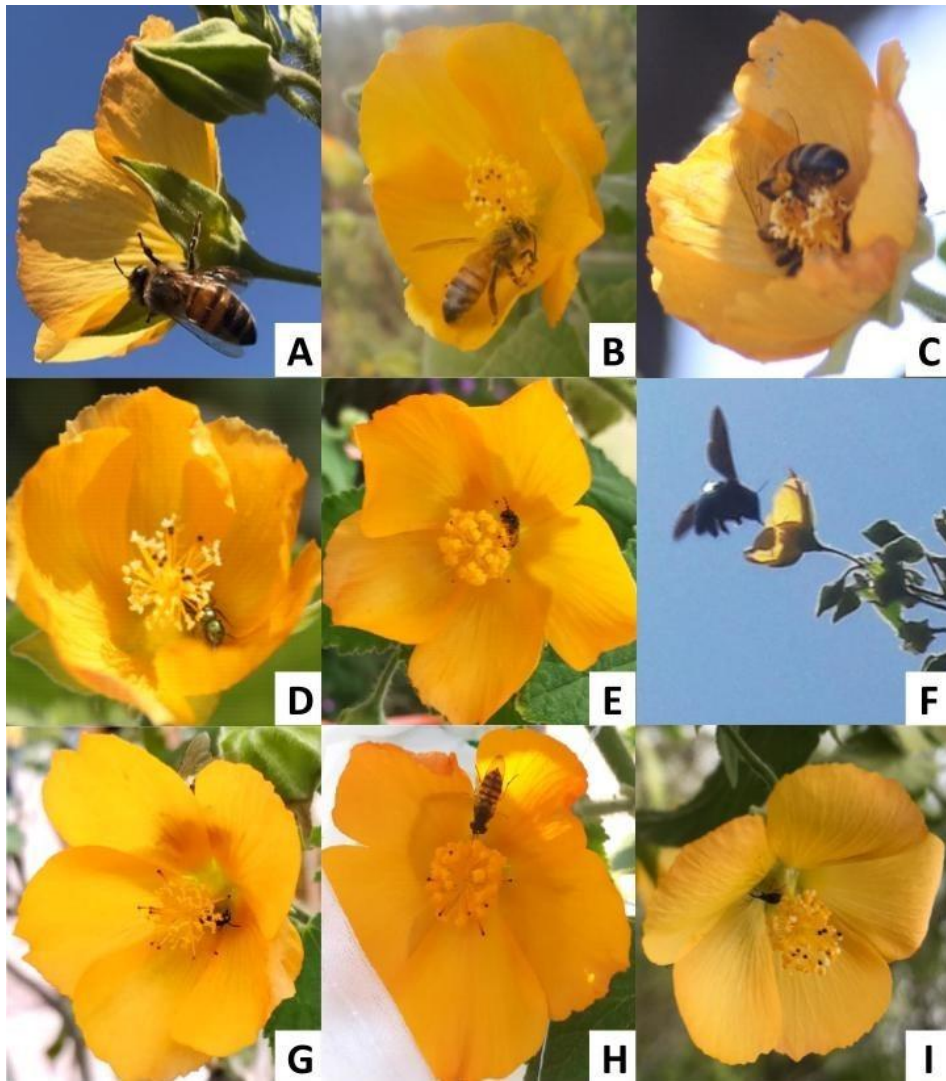


Figure 9

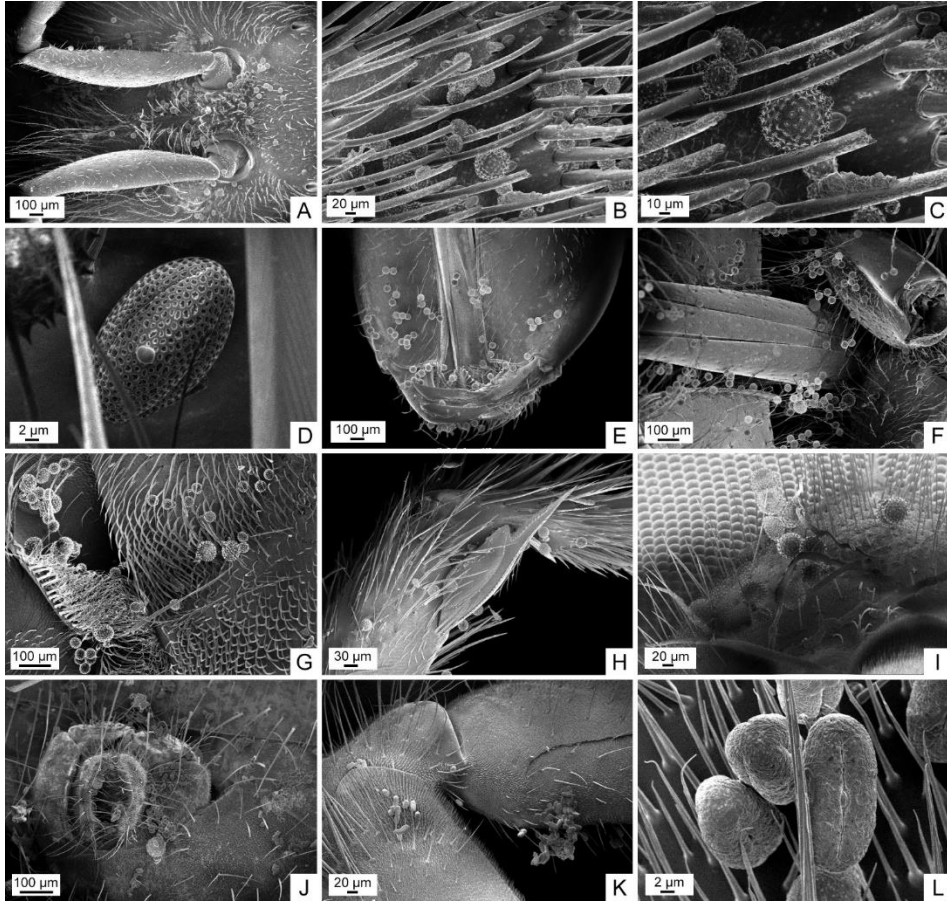


Figure 10