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Floral morpho-anatomy and reproductive biology of *Mammillaria lasiacantha* Engelm. (Cactaceae) at Sierra of Juárez, Chihuahua

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Abstract. *Mammillaria lasiacantha* is a small cactus that inhabits at Sierra of Juárez, Chihuahua, Mexico. Their populations have been affected by habitat destruction, illegal extraction, and uncontrolled tourism in the region that steps on the seedlings; therefore, knowing its reproductive biology is necessary for its conservation. Our main questions were: What is the floral morphology and anatomy like? What type of sexual and mating system do *M. lasiacantha* present? Do the seeds produced by the crosses germinate, how and in what percentage? Sixty individuals from a population of 120 plants were studied at Sierra of Juárez, Juárez Chihuahua from January to August 2021. The study of the floral morpho-anatomy was carried out. Four treatments were applied: natural selfing, forced selfing, geitonogamy and forced outcrossing plus a control. Pollinated flowers, fruits, number of seeds and germination percentage were recorded. Flowers have naked pericarp, funnel-type nectary, nectarostomata, closed style and collateral vascular bundles. The anatomical characteristics of *M. lasiacantha* agree with those reported for other species of *Mammillaria*. The flowers of *M. lasiacantha* present herkogamy, with an obligate outcrossing system. The seeds produced from the outcrossing and control group germinated in 32 and 76%, respectively. The herkogamy and strict outcrossing system of *M. lasiacantha* should be confirmed in other populations. The seeds are positive photoblastic, without dormancy, but the study of the seed bank and seedling survival in the field would be pertinent. The *ex-situ* conservation of *M. lasiacantha* in the site will be feasible, since species high percentage of germination may contribute to produce plants for restoration programs.

Keywords: *Floral anatomy, outcrossing, germination, herkogamy*

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Introduction

Mammillaria is the largest genus of tribe Cactaceae with more than 120 species (Korotkova *et al*., 2021) which are mainly distributed in the arid and semiarid regions of Mexico and the United States of North America (Hernández and Gómez-Hinostrosa, 2015). A distinctive character of *Mammillaria* is the occurrence of having an areole split into two clearly separated parts, one at the base and the other occurring at the apex of the tubercle. Some *Mammillaria* species are mainly distributed across the Chihuahuan Desert as for *Mammillaria lasiacantha* Engelm. with three subspecies (Guzmán *et al*., 2003). This species is distributed from Texas, New Mexico (United States), Chihuahua, Coahuila, Durango, Zacatecas, San Luis Potosí and Sonora (Mexico). This research focuses on a population at the Sierra de Juárez, Chihuahua. The vegetation is mainly xerophyllous and rosetophilous scrub, the most abundant species are *Fouquieria splendens* Engelm., *Agave lechuguilla* Torr., *Larrea tridentata* (D.C.) Coville, *Vachellia constricta* (Benth.) Seigler & Ebinger and *Neltuma glandulosa* (Torr.) Britton & Rose. Cactaceae is one of the best represented families, the most numerous species are *Echinocactus horizonthalonius* Lem., *Echinocereus dasyacanthus* Engelm., *Echinocereus coccineus* Engelm., *Opuntia phaeacantha* Engelm., *Cylindropuntia leptocaulis* (DC.) F.M.Knuth and *Ferocactus uncinatus* (Galeotti) Britton & Rose. *Mammillaria lasiacantha* inhabits rocky limestone massifs of marine sedimentary origin, composed of shales and clays, from 1,400 to 1,700 m asl (Dena-Ornelas *et al*., 2011). The species has low population sizes and compared to other cacti species it is scarce. The number of individuals is greater on the solid limestone peaks, exposed to strong winds and direct sun, at more than 1,500 m asl. The species is not listed in the NOM-059-SEMARNAT-2010, but it is included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) along with the rest of the cacti, and in the International Union for Conservation of Nature (IUCN) in the category of least concern. *M. lasiacantha* is a plant with high demand in the ornamental market, which encourages its extraction from the environment. Moreover, the species habitat is being destroyed by anthropogenic activities such as the advance of the urban extension and the activity of the cement industry.

The floral morphology and anatomy are completely related to function, external floral characteristics are associated to the pollination syndrome, but also the spatial separation of the stigma and anthers indicates herkogamy (Mandujano *et al*., 2010). On the other hand, anatomy allows us to understand the processes of organ development, the presence of nectaries, even the receptivity of the stigma, and the presence of pollen in the anthers (Crang *et al*., 2018).

The reproductive biology is crucial in the life cycle of species since new individuals depends on it. The sexual systems (morphologically) refer to how the gynoecium and androecium are positioned functionally in the individuals of a population, expressing themselves as hermaphrodites, dioecious, gynodioecious, androdioecious (Barrett, 2002). The mating system in hermaphrodite plants is usually defined as the relative frequency of cross-fertilization (outcrossing) and self-fertilization (selfing) in a population (Barrett, 2014). The reproductive biology of *Mammillaria* is still little known. Some previous studies have reported some aspects related to reproduction in the genus *Mammillaria*. For example, *M. blossfeldiana* Boed. and *M. dioica* K. Brandegee present a gynodioic sexual system (Rebman *et al*., 2002; Sánchez and Vázquez-Santana, 2018) and *M. neopalmeri* R.T. Craig. have a trioic sexual system (Lindsay and Dawson, 1952; Camacho-Velázquez *et al*., 2016). In *M. huitzilopochtli* D.R. Hunt and *M. grahamii* Engelm. (Bowers, 2002) an outcrossing system has been reported, while for *M. prolifera* (Mill.) Haw. (Ross, 1981) and *M. humboldtii* Ehrenb. (Martínez-Ramos *et al*., 2016) mixed mating system had been registered. In addition, various species of the genus *Mammillaria* show melithophilous pollination (Camacho-Velázquez *et al*., 2016). Mating and breeding systems affect the genetic structure of populations and the success of the subsequent processes of seed germination and seedling establishment. Therefore, the study of reproductive biology in cacti is of special importance to generate information on reproductive requirements and factors that could limit the persistence of natural populations (Mandujano *et al*., 2010; Matias-Palafox *et al*., 2017). The floral anatomy and which sexual and mating system has *M. lasiacantha* is unknown, thus the aims of this study were to analyze floral morphology and anatomy, the sexual and mating systems, and germination for all treatments.

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Material and Methods

Study area

The research was carried out at Sierra de Juárez, located in the municipality of Juárez, Chihuahua, at coordinates 31° 40.887' N and 106° 33.537' W, at an altitude of 1,400 to 1,700 m asl. The vegetation is microphyllous desert scrub, rosetophyllous scrub and halophyllous vegetation (Rzedowski, 2006). The population studied here is in microphyllous desert scrub.

Studied species

Mammillaria lasiacantha, small pincushion cactus, is an inconspicuous cactus with apically rounded globose stem, from 1 to 3.5 cm wide and 1.5 to 7 cm tall, with whitish appearance given by the presence of 40 to 80 radial spines, pubescent or not, and with no central spines; their flowers are white or cream, 1 to 2.5 cm wide and the tepals with a median line arranged longitudinally (Bravo-Hollis and Sánchez-Mejorada, 1991).

Collection

The flowers in different stages of development to pollinated ones were collected during the months of February and March 2021 in 10 plants. A botanical specimen was also collected and deposited in the Herbario UACJ Universidad Autónoma of Ciudad Juárez, Mexico with the number 002543 (Collector: Coyolxauhqui Figueroa 511). The floral samples were fixed in standard FAA (formaldehyde, acetic acid and ethanol; Ruzin, 1999) for 48 hours and stored the samples in 50% ethanol in the refrigerator at 4˚C until processing.

Scanning electron microscopy (SEM)

The longitudinal dissections of the pre-anthesis phase and pollinated flowers were carried out to analyze morphological characters and post-pollination changes. The samples were dehydrated in ethanol (50 to 100%), one hour for each concentration. The samples were then critically dried with CO_2 in a tumble dryer Emitech K850 (Quorum Technologies Ltd, Lewes, UK); fixed to aluminum specimen holders with double-sided tape and coated with gold in a metallizer/evaporator Quorum Q150R ES (Quorum Technologies Ltd, Lewes, UK). The coated samples were observed and photographed with the scanning electron microscope Hitachi S-2460 N (Hitachi, Tokyo, Japan) operated at 15 kv at Instituto of Biología, Universidad Nacional Autónoma de México (UNAM).

Floral anatomy

In order to analyze floral anatomy, such as stigma papillae, anthers containing pollen, nectary and ovules/seeds, the fixed flowers (longitudinal sections) were dehydrated in a tissue processor TP1020, (Leica, Wetzlar, Germany) for 12 hours at each ethanol concentration (50 to 100%) and embedded in paraplast. The sections 12 to 14 µm thick were made with a rotary microtome Leica RM2125 (Leica, Wetzlar, Germany) and stained with safranin/fast green (Ruzin, 1999). The floral tissues were evaluated through bright microscopy and to determine the morphology and the type of crystals; the sections were observed under polarized light using an Olympus BX 51 microscope (Olympus, Tokyo, Japan), when crystals bright with polarized light they are classified as calcium oxalate. The photographs were obtained with the Image-Pro Plus 7.1 software (Media Cybernetics, MD, USA). Crystal types follow the terminology proposed by De la Rosa-Tilapa *et al*. (2022).

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Sexual system

The flowers in anthesis were morphologically characterized; the arrangement and functionality of the androecium and the gynoecium of 30 flowers were observed. The separation between androecium and gynoecium was measured with a digital vernier. In addition, free-hand dissections of the anthers were carried out to observe pollen and the stigma was considered receptive when it showed extended lobes with sticky adaxial surface.

Mating system

Following the proposal of Flores-Martínez *et al*. (2013), four treatments and a control group were applied to evaluate the mating system. The experiment had six flowers for the five groups, giving a total of 30 flowers. The number of plants in the population is low and the individuals selected were of difficult access to guarantee no human intervention. The flowering was not simultaneous therefore experiments were performed during weekly visits with available flowers. Additionally, some flower buds were eaten by rodents.

Before applying the treatments, an organdy protection bag was placed on each of the young buds, to prevent them from being pollinated during anthesis. The treatments were as follows: (1) natural selfing, where the covered flowers were not manipulated; (2) manual selfing, pollen was placed on the stigma of the same flower; (3) geitonogamy, pollen was placed on the stigma of another flower of the same plant; (4) manual outcrossing, pollen was deposited on the stigma of a flower from a different plant. The distance between the flowers used for outcrossing was 30-50 m. The plants in the control group remained unchanged during flowering and pollination and the fruits were only collected when they were ripe.

Subsequently, the treated flowers and the control group were monitored every eight days for six months (February to July 2021) to record the morphological changes that occurred in the flowers through fruit development. During the field work, a couple of insects were captured and observed in the laboratory using a stereomicroscope and identified with the Michener keys (1994, 2007).

During June and July, the fruits, if formed, were collected from each treatment, and the seeds of each fruit counted. The mean was obtained, and the Mann Whitney U test was performed with a confidence level of 95% using the SPSS version 28.0 program (SPSS®, Chicago, USA).

Germination

In order to know if the seeds were viable and to understand the germination process, the seeds of each treatment were submerged in fungicide 2 g L^{-1} (Captan®) for two hours. Subsequently, seeds were sown in separate containers with a substrate composed of coconut fiber and tezontle (1:1) and maintained in a rustic greenhouse with temperatures between 25°C in the morning and a maximum of 40°C in the afternoon. The containers were manually watered every day to maintain constant humidity and kept under natural light conditions. They were monitored three times a week and the number of germinated seeds for each treatment was counted.

Results

Floral morphology in anthesis

The flowers of *M. lasiacantha* originate subapically forming a crown. They are actinomorphic, hermaphrodite, homochlamydeous, epigynous, infundibuliform, 12 to 15 mm long and 16 to 20 mm wide (Figure 1A; Harris and Harris, 1994); pericarpel is naked, greenish (4 mm long), tepals lanceolate-oblong, apex acute, margin entire, white to cream in color with a pinkish-brown midline extending to the throat (Figure 1B); stamens are numerous (70-80), pale yellow to whitish filaments, basifixed yellow anthers (Figure 1B, C); stigma has 5 to 6 lobes, yellowish-green, with finger-shaped papillae in the adaxial portion (Figure 1B-D) and without papillae abaxially (Figure 1B).

Fruit and seed morphology

The fruits are berry, clavate, red color, 1.4 to 2.5 cm long, with remnants of the tepals in the apical portion (Figure 1E). The seeds are black-brown, matt, pyriform, 0.9 to 1.1 mm long by 0.8 to 0.9 mm wide, cells uniform, anticlinal boundaries inconspicuous, relief par-concave, appearance pitted, with more than 50 pits per face (Figure 1F-G). The hilum-micropylar region is elliptical with brown trichomes and expanded edges around hilum, disjunct micropyle, distally located in the narrowest region of the seed (Figure 1F-I). In addition to the testa, there is a brownish membranous layer of tegmen, with parallel thickenings and a darker area in the distal portion that agrees with the hilum-micropylar region (Figure 1F, I-J).

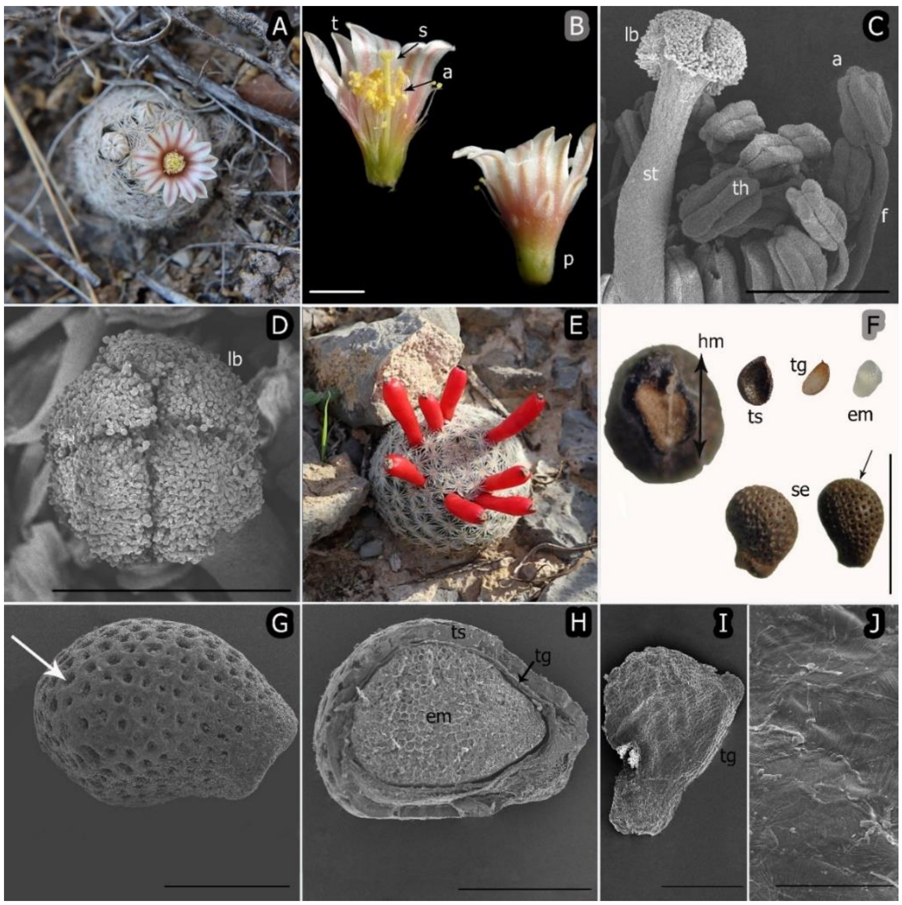

Figure 1. *Mammillaria lasiacantha* floral and seed morphology. (A) Wild plant with flower in anthesis. (B) Longitudinal section of the flower. (C) Close-up of the anthers (SEM). (D) Close-up of the stigma (SEM). (E) Plant with ripe fruits. (F) Seed and its parts. (G) Lateral view of seed and with pits (arrow) (SEM). (H) Longitudinal section of seed (SEM). (I) Tegmen (SEM). (J) Tegmen microrelief. a= anther, em= embryo, f= filament, hm = hilum-micropyle region, lb= stigma lobes, p= pericarpel, s= stigma, se= seed, st= style, t= tepal, tg= tegmen, th= theca, ts= testa. Scales: B= 5 mm, C-D= 1 mm, F= 2 mm G-H= 500 µm, I= 300 µm, J= 50 µm.

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Floral anatomy

Pericarpel. It presents smooth cuticle; epidermis is simple with rectangular cells and parallelocytic stomata (Eggli, 1982; Figure 2A-B); cortex is composed of reserve parenchyma with large, elongated, or isodiametric cells (10 strata; Figure 2A), prismatic aggregates crystals and mucilage cells.

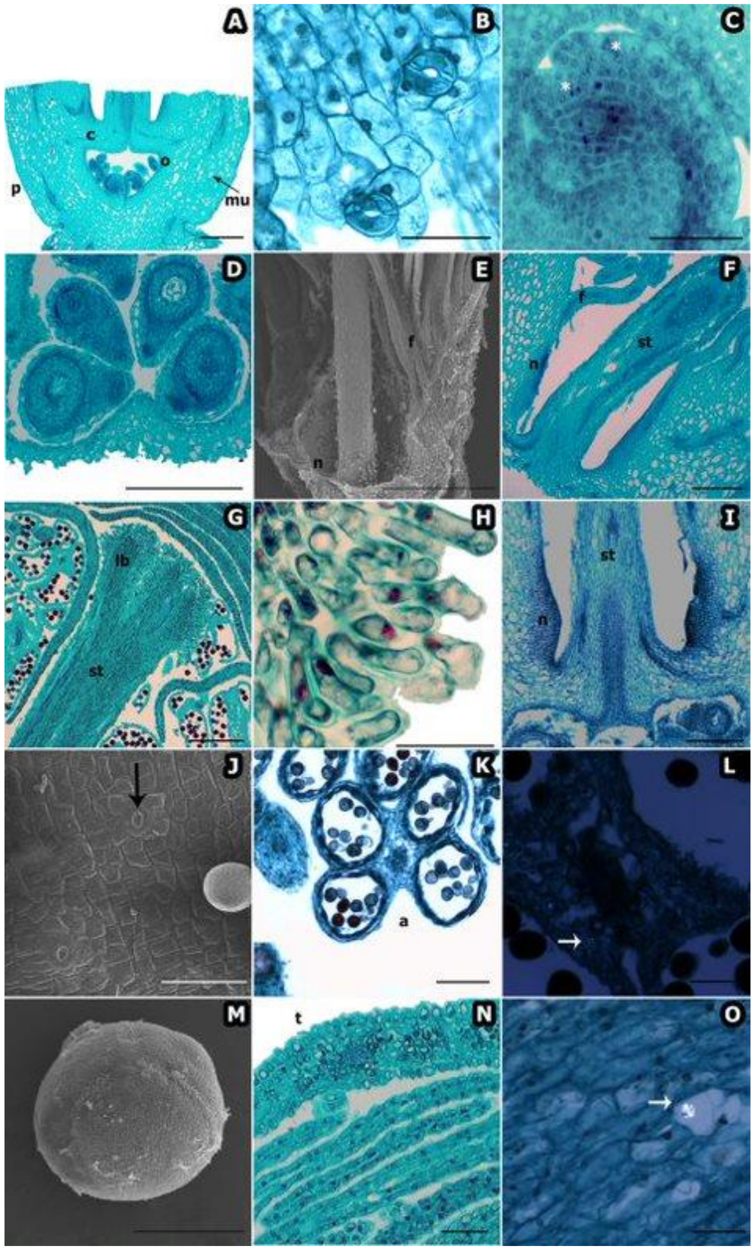

Figure 2. *Mammillaria lasiacantha* floral anatomy. A) Long-section of the basal region of the flower. (B) Parallelocytic stomata in the pericarpel. (C) Young ovule with two integuments (*). (D) Mature ovules. (E) Flower in anthesis showing style, nectary, and filaments (SEM). (F) The style is closed. (G) Apical portion of the style and stigma showing papillose lobes. (H) Stigmatic papillae. (I) Furrow type nectary. (J) Nectarostomata (arrow). (K) Tetrasporangiate anther with pollen grains. (L) Aggregated crystals (polarized light) of connective tissue. (M) Tricolpate pollen grain (SEM). (N) Transverse-section of tepals. (O) Close-up of the crystal in tepal with polarized light. a= anther, c= column, f= filament, lb= stigma lobes, mu= mucilage, n= nectary, o= ovary p= pericarpel, s= style, t= tepal. Scales: A, D, F, G, I= 300 µm, B, O= 50 µm, C, H, K= 100 µm, J-N= 50 µm, E= 1 mm, L= 10 µm, M= 20 µm.

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Gynoecium. The ovary is surrounded by the pericarpel, which is naked, having a simple epidermis with parallelocytic stomata, 10 strata of parenchyma cells, vascular tissue and abundant mucilage cells; the ovarian cavity has semicircular-depressed shape (Figure 2A); ovules are campylotropous, bitegmic, crassinucellate and with a long funicle (Figure 2A, C, D); the column is parenchymatous (Figure 2A) with vascular tissue, mucilage cells, and prismatic aggregate crystals. The style is straight with the apical part broadened (Figure 2E, F); stylar epidermis slightly papillose with thin cuticle (Figure 2G); the stylar cavity is closed, parenchymatous, with mucilage cells; the transmission tissue has elongated rectangular thin-walled cells with dense protoplasts (Figure 2F, G) and some small calcium oxalate prismatic crystals. The stigma has 5 triangular lobes seen in transverse section, with papillae in the adaxial portion, a central vascular bundle per lobe (Figure 2G, K).

Nectary. In longitudinal section, the nectary is located around the base of the style, immediately below of filaments and is furrow type; nectariferous cells are slightly above the base, distinctive because their smallest size with dense protoplast and conspicuous nucleus; nectary epidermal cells are more or less elongated squared with nectarostomata (Figure 2J).

Androecium. The free filaments are inserted ascendingly above the nectary tissue (Figure 2E, F), cuticle is slightly rough, epidermis simple with rectangular cells and the underlying tissue of the filament is composed of parenchyma cells and a central vascular bundle. The tetrasporangiate anthers have papillose epidermis (Figure 2K), the endothecium shows one layer, and the connective tissue has parenchyma cells with rare calcium oxalate prismatic aggregate crystals (Figure 2L) and also a vascular bundle. The pollen is spherical, granular-stippled, tricolpate, with micro-warty colpi (Figure 2M).

Tepals. They present a simple epidermis with a slightly papillose cuticle in both surfaces, having the abaxial smaller cells and parallelocytic stomata (external tepals); the mesophyll shows exclusively isodiametric parenchyma cells (Figure 2N) with abundant plastids, mucilage cells, and rare calcium oxalate prismatic aggregate crystals (Figure 2O). The collateral vascular bundles are abundant.

Sexual system

Morphologically the sexual system is hermaphroditism. This system was observed in the thirty flowers analyzed and both sexual organs are mature simultaneously (Figure 3A). In addition, a spatial separation of 4-5 mm was observed between the androecium and the gynoecium of all the flowers, with stigmatic lobes above the anthers “herkogamy” (Figure 3B).

Mating system

In the pollinated flowers an invagination produced by the adaxial recurvation of the tepals was observed (Figure 3C-D). In contrast, non-pollinated flowers had dehydrated tepals (Figure E). In addition, after pollination the open stigmatic lobes showed a change in position, causing the receptive portions of the stigma to remain attached and unavailable to other insects (Figure 3F, G). During the field work, bees of the genus *Apis* (Figure 3H) were observed visiting the flowers. These bees were observed interacting with the sexual organs of the flower and with pollen on their body, therefore it is considered a possible pollinator. In addition, a wasp of the genus *Sphecodes* (Figure 3I) was observed submerged to the base of the flower only taking nectar.

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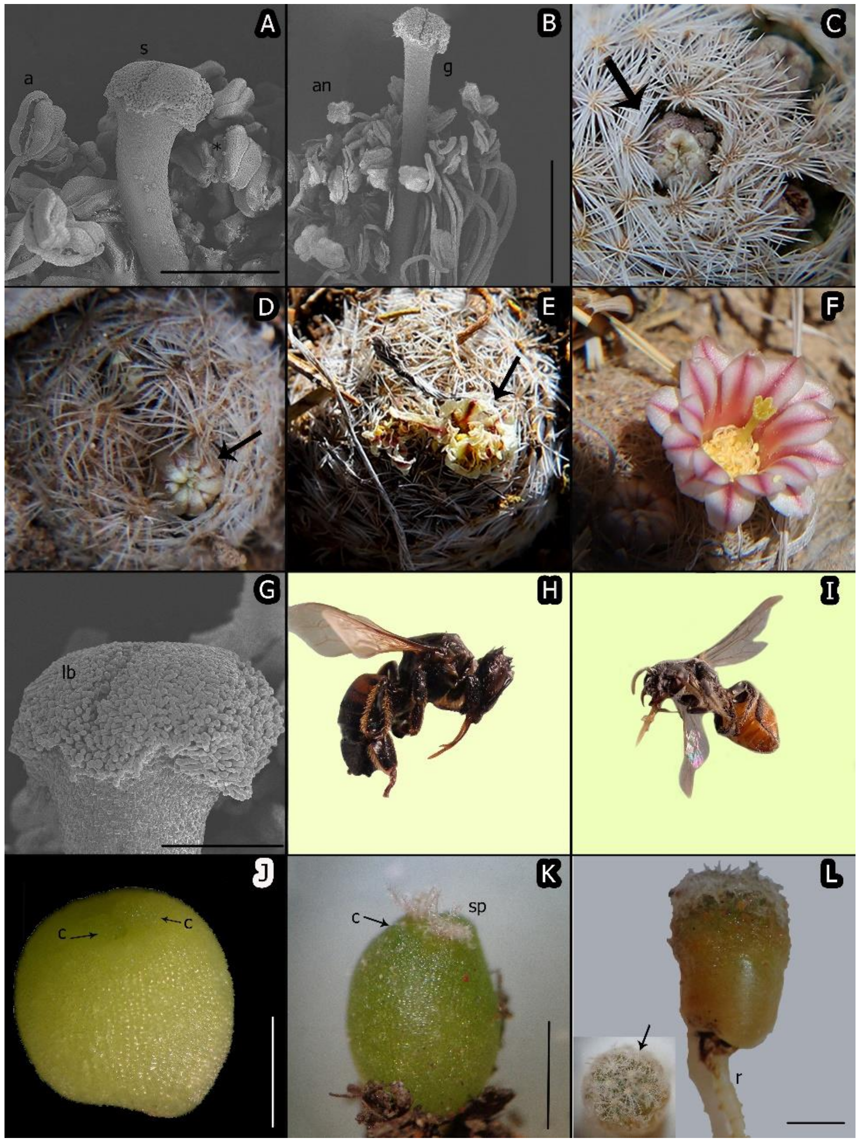

Figure 3. Pollination and germination in *Mammillaria lasiacantha*. (A) Simultaneous development of sexual organs (*pollen grains) (SEM). (B) Herkogamy (SEM). (C-D) Recurved tepals after pollination. (E) Non-pollinated flower, see dehydrated tepals. (F) Stigma with separated lobes (G) Stigma with attached lobes (SEM). (H) *Apis* sp. (I) *Sphecodes* sp. (J) Seedling 3 days after germination. (K) Seedling 2 weeks after germination. (L) Plant 1 year old, spines (arrow). a= anther, an= androecium, c= cotyledon, g= gynoecium, lb= stigma lobes, r= root, s= stigma, sp= spines. Scales: A, J= 1 mm, B= 2 mm, G= 400 um, K-L= 1.5 mm.

Fruit set and seed set

The fruit maturation coincided with the presence of rainfall in the region (66.4 mm; CECATEV-UACJ 2022), from June 7 to July 18, 2021. The flowers of the control (without treatment) and the forced outcrossing treatment developed fruits (Table 1). The four fruits of the forced outcrossing varied in their length (fruit 1= 1.9 cm, 2= 1.5 cm, 3= 2.3 cm, 4= 2.5 cm). In the natural selfing treatment, only 2 out of 6 flowers presented recurved tepals, but none produced the fruit. Therefore, it is inferred that *M. lasiacantha* presents a strict outcrossing system since fruits were only produced in natural and forced outcrossing.

In the control, the six flowers had fruits and seeds (26-55), as did the four flowers that were successfully pollinated in the forced outcrossing treatment (37-61; Table 1). A mean of 56 seeds per fruit was

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obtained. The Mann Whitney U test did not show significant difference (*P*= 0.873) for seed set between the control and forced outcrossing treatment.

Table 1. Fruit set and seed set in *Mammillaria lasiacantha.*

	Treatment				
	Control group (0)	Natural selfing	Forced selfing	Forced outcrossing	Geitonogamy
Fruits					
1	46	0	0	0	0
2	45	0	0	0	0
3	55	0	0	37	0
4	26	0	0	65	0
5	50	0	0	62	0
6	51	0	0	61	0

Germination

In the control treatment, 75.82% of the seeds geminated (207 out of 273 seeds); whereas only 31.55% germinated in the forced outcrossing treatment (71 out of 225 seeds). The maximum number of germinated seeds occurred at 23 days in the control and at 30 days in the forced outcrossing.

The morphologically germination begins with imbibition, then the testa breaks, the radicle emerges, followed by the growth of the embryonic axis, and two reduced cotyledons, with the first pubescent spines emerging between the cotyledons (Figure 3 J, K). After a year of germination, the plant is slightly ovate, with pubescent white spines that develop sequentially from the apex to the base shoot, but at this age the hypocotyl remains distinctive from the root (Figure 3L).

Discussion

Floral morphology and anatomy. The present research provides a more accurate characterization of flower morphology. Based on the results, the flowers of *M. lasiacantha* emerge subapically and form a crown around the apex of the stem. The flowers are infundibuliform, the outer and inner tepals have entire margins, and the stigma show 5-6 lobes. In contrast, Bravo-Hollis and Sánchez-Mejorada (1991) mentioned that the flowers of *M. lasiacanth*a measure 12 × 12 mm, with serrate margin in the outer tepals and has 4-5 lobes in the stigma. The flower length is consistent with the range reported by Roberts (2011), which goes from 12 mm to 20 mm long.

The naked pericarpel had already been mentioned for some *Mammillaria* species by Buxbaum (1953) and later this trait recognized for the genus by Bravo-Hollis and Sánchez-Mejorada (1991). The type of ovules described here as campylotropous, bitegmic and crassinucellate, coincides with those reported in *M. rhodantha* Link & Otto and *M. compressa* DC. by Hernández-García and García-Villanueva (1991) and in general for other cacti such as *Pachycereus militaris* (Audot) D.R. Hunt by Núñez-Mariel *et al*. (2001) and some species of the genus *Opuntia* (Fuentes-Pérez *et al*., 2009).
This research corroborates the closed style for *M. lasiacantha*, previously observed for the genus *Mammillaria* by T. Terrazas (unpublished data). Likewise, *M. lasiacantha* possessed furrow type nectary, also documented in *M. ortizrubioana* (Bravo) Werderm. and *M. camptotricha* Dams by Buxbaum (1953). The collateral bundles presented by *M. lasiacantha* agree with that reported by Tiagi (1955) for *M. tenuis* DC. Prismatic aggregate crystals occurring in the different floral whorls of *M. lasiacantha* have also been reported by De la Rosa-Tilapa *et al*. (2022), in the pith of *M. candida*

Scheidw. and cortex of *M. polythele* Mart. However, in this same study, other types of crystals were reported for other *Mammillaria* species. For example, sandstones (SS) in epidermis-hypodermis and cortex of *M. geminispina* Haw, *M. tegelbergiana* H.E.Gates ex G.E.Linds. and *M. candida*. In addition, spherulites in the pith of *M. candida* and *M. formosa* Galeotti ex Scheidw. subsp. *formosa*. The study of crystals in the flower of other species of *Mammillaria* will contribute to evaluate if this is a diagnostic character or not as it has been suggested for other organs of different angiosperm groups (Lersten and Horner, 2011; Horner *et al*., 2012).

Seed morphology. In this study was observed that the seeds are pyriform and pitted; notably, the shape does not match the previous description made by Barthlott and Hunt (2000) where seeds were described as circular. In addition, the size of the seeds in this research is 1.1 × 0.8 mm, less than the 1.4 × 1.2 mm reported by the same authors. The seeds have testa and tegmen, although there are few data on the internal structure of the seed, the tegmen was recently reported in *M. parkinsonii* Ehrenb. (Uribe-Salazar, 2022).

Sexual system. In the present study, was determined that *M. lasiacantha* like most cacti, shows hermaphroditic flowers as previously recorded for *M. grahamii* and *M. huitzilopochtli* (Bowers, 2002; Flores-Martínez *et al*., 2013). However, this genus is diverse in terms of its sexual system, for example, two sexual systems (gynodioeous and trioecious) have been reported in *M. neopalmeri* (Lindsay and Dawson, 1952); whereas *M. blossfeldiana*, *M. magnimamma* Haw. and *M. dioica* are gynodioecious, with hermaphrodite and female individuals (Callejas-Chavero *et al*., 2021; Rebman *et al*., 2002; Sánchez and Vázquez-Santana, 2018). Would be pertinent to study different populations of *M. lasiacantha*, to analyze whether there is variation in the sexual system as in other species of the genus.

Herkogamy. *Mammillaria lasiacantha* showed herkogamy in all the flowers analyzed with a separation between the stigma and the stamens that ranged from 4-5 mm both organs were functional at the same time. Some of the herkogamous species cited in previous studies are *Mammillaria deherdtiana* subsp. *dodsonii* (Bravo) D.R.Hunt (Rendón-Aguilar *et al*., 2022) and *M. pectinifera* F.A.C. Weber (Valverde *et al*., 2015). This feature appears to be not common; however more species need to be studied to support this assertion.

Mating system. Based on the results of the present study, *M. lasiacantha* exhibits a strict outcrossing system, as in *M. grahamii* (Bowers, 2002), *M. huitzilopochtli* (Flores-Martínez *et al*., 2013), *M. magnimamma* (Callejas-Chavero *et al.,* 2021), *M. pectinifera* (Valverde *et al*., 2015), *M. bocasana* Poselg., *M. candida* Scheidw., *M. compressa* DC., *M. melaleuca* Karw. ex Salm-Dyck., *M. parkinsonii* C.Ehrenb., *M. pennispinosa* Krainz., *M. spinosissima* Lem., *M. uncinata* Zucc. ex Pfeiff., *M. wildii* A.Dietr. and *M. zeilmanniana* Boed. (Ross, 1981). However, has been observed that in some species self-fertilization and geitonogamy can exist, but the fruits are not formed and if they do, they have few seeds (Flores-Martínez *et al*., 2013). Some species have an outcrossing system and selfing, such as*:* *M. humboldtii* (Camacho-Velázquez *et al*., 2016), *M. prolifera*, *M. tenuis* and *M. zeilmanniana* Boed. (Ross, 1981). The self-pollination has been hypothesized to be related to polyploidy (*M. multiceps* Salm-Dyck: Remski, 1954; Ross, 1981). Although other polyploid species such as *M. blossfeldiana*, *M. dioica*, *M. fittkaui* Glass & R.A. Foster and *M. saboae* Glass have been reported, no mating system experiments have been performed (Johnson, 1980).

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Fruit set. The plants of *M. lasiacantha* that produced fruits were those in which the manual outcrossing treatment was carried out and the control. These results are consistent with *M. grahamii*, where 100% of the outcrossed flowers develop fruits (Bowers, 2002). Although, in the present study, two of the flowers subjected to manual outcrossing aborted the fruits. This abortion could be due to the crosses of these two flowers were carried out during a dry period and in hours with high temperatures during February and March (0 mm; 35°C, CECATEV-UACJ 2022) compared with the other four (León De la Luz and Domínguez, 1991; González-Machorro and Navarro-Carbajal, 2011). Another possible explanation that could cause the abortion of fruits is the mechanical damage caused during handling, since the stamens were dissected with forceps and there could be damage to surrounding tissues. Other authors reported that damages in flower buds cause their abortion and fruits do not develop (Cárdenas-Ramos and Mandujano, 2021). In *M. lasiacantha* with natural selfing treatment, in this study were observed two flowers with recurvated petals, an apparent sign of pollination, but no fruits developed. There are other species of *Mammillaria* that self-fertilize. Flores-Martínez *et al*. (2013) pointed out that *M. huitzilopochtli* have fruits in the natural and forced selfing treatments; however, only 1.13% of these showed seeds. In contrast, in *M. pectinifera* the possibility of fruit and seed formation by self-fertilization in the absence of a pollinator was reported (Valverde *et al*., 2015), but Ross (1981) mentioned that this species is self-incompatible.

Seed set. In *M. lasiacantha* a mean of 56 seeds per fruit was obtained, similar to *M. polythele* Mart. with 51. However, in the genus a high variation in the number of seeds per fruit has been reported having 21 in *M. pectinifera* (Valverde *et al*., 2015) and 22 in *M. crinita* DC. (Larios-Ulloa *et al*., 2015) to 38 in *M. huitzilopochtli* (Flores-Martínez *et al*., 2013) and 96 seeds in *M. uncinata* (Larios-Ulloa *et al*., 2015). Apparently, *M. lasiacantha* produces an intermediate number of seeds, at least in the population analyzed.

Seed germination. In *M. lasiacantha*, a germination percentage of 75.82 and 31.55% was achieved in the flowers with natural and forced outcrossing, respectively. The high percentage of germination in controls coincides with previous reports for *M. aureilanata* Backeb., *M. bocasana*, *M. crinita*, *M. longimamma* DC., *M. mazatlanensis* K. Schum., *M. orcutti* Boed., *M. plumosa* F.A.C. Weber and *M. uncinata* (Flores *et al*., 2006; Sánchez-Soto *et al*., 2010; Loza-Cornejo *et al*., 2012). However, the low percentage of germinated seeds in the forced outcrossing could be related to the immaturity of the four fruits collected. The smallest fruits had fewer seeds as well as a brown-reddish color, indicating their immaturity. Further studies in which fruits should be maintained longer time during the experiments are necessary, probably seed maturation is not related either with color or fruit size as results presented here suggest.

The newly formed seeds of *M. lasiacantha* germinated with high percentages in transparent plastic containers at 40°C and constant humidity, without any additional treatment. Based on this, it is determined that they are positive photoblastic and that they do not present dormancy. It would be pertinent to carry out survival studies of seedlings in the field to understand the persistence of the population.

Pollination. A bee of the genus *Apis* was found feeding on the nectar of *M. lasiacantha* flowers and interacting with the sexual organs of the flowers. Similarly, this pollinator was reported in the species *M. grahamii* (Bowers, 2002) and *M. magnimamma* (Callejas-Chavero *et al*., 2021). Likewise, different species of the genus *Mammillaria* have melitophilous pollination syndrome as in *M. columbiana* Salm-

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Dyck (Cardeño-Londoño and Rodríguez-Herrera, 2020), *M. hernandezii*, *M. kraehenbuehlii*, *M. napina* (Téllez-Valdés *et al*., 2015), *M. humboldtii* (Martínez-Ramos *et al*., 2016), *M. huitzilopochtli* (Flores-Martínez *et al*., 2013) and *M. pectinifera* (Valverde *et al*., 2015). In addition, a wasp of the genus *Sphecodes* was observed in the flowers of *M. lasiacantha*, submerged in the nectary located at the base of the style, this wasp did not touch the sexual organs. Bogusch and Straka (2012) mentioned that the species of *Sphecodes* are nectar robbers and that they are not pollen collectors. A precise study of floral visitors and determination of effective pollinators in *M. lasiacantha* is needed to support the observations here reported.

The Sierra of Juárez shows habitat fragmentation caused mainly by urbanization and cement industry activity. Furthermore, recently there has been the extraction of flora and fauna, even the population used in this research was diminished by illegal extraction (personal observation). The populations of *M. lasiacantha* are very small, just a few dozens, and separated by kilometers or from one hill to another. Isolated small populations, coupled with its narrow niche breadth, removal of individuals from wild populations, and projected climate change, leave this population at a critical level of risk (Carrillo-Angeles *et al*., 2016, Martorell *et al*., 2015).

The *M. lasiacantha* flowers the first months of the year, when plants from other families are at rest, therefore, it does not have competition for pollinators. Although it was not the main purpose of this study, bees touching the stigma and entering through of the filaments of the stamens to take nectar were observed, a very important food resource in times of flower shortage.

In consideration that *M. lasiacantha* needs cross-pollination, a collection of seedlings because of this research was established, to have a good number of adult individuals to conserve and propagate the species *ex situ*. In the future, manual crosses will be carried out to produce seeds, seedlings, and juvenile individuals in greenhouse. The juveniles obtained could be reintroduced to Sierra of Juárez or could be used to cover the commercial demand for this plant and reduce pillage from wild populations.

Conclusions

Mammillaria lasiacantha has hermaphroditic flowers with herkogamy and these exclusively form fruits when there is outcrossing consistent with previous reports for other *Mammillaria* species. The seeds of *M. lasiacantha* are photoblastic, positive, and non-dormant. The seed is pyriform and presents testa and tegmen. The floral morphology is associated with melitophilous pollination syndrome coherent with the observation of floral visitors. The flower anatomy shows characteristics shared with other cacti. The *ex situ* conservation of *M. lasiacantha* is possible, but a high number of individuals must be present to carry out outcrossing, seed production, propagation, and reintroduction in Sierra of Juárez.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Coyolxauhqui Figueroa participated in the field work, integrated the first version of the manuscript, and directed the project. Laura J. Prieto carried out the mating experiments in the field and participated in the structuring of the first version of the manuscript. Sheila de la Torre participated in the field work and the processing of the samples for light and scanning electron microscopy, preparation of slides and structuring of the article. Teresa Terrazas the advice to process the samples in the Structural Botany laboratory of the Institute of Biology of the UNAM, interpretation, structuring, and revision of the manuscript. The authors reviewed and approved the manuscript.

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