

Investigating the floral and reproductive biology of the endangered microendemic cactus *Uebelmannia buiningii* Donald (Minas Gerais, Brazil)

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Abstract The Cactaceae are known to be amongst the most endangered plant families of the world due to reduction of their habitats and activities of collectors. As the species of the family are dependent on animals to perform cross pollination, and hence seed production, their population performance may be further negatively affected by interrupted biotic interactions. For efficient conservation of rare species, knowledge on reproductive biology and pollinators is of prime importance. In our study we focused on *Uebelmannia buiningii* Donald, a microendemic cactus from the Serra Negra State Park, Minas Gerais state,

Brazil. During four field expeditions to three localities of the species between September 2012 and September 2013, we measured flowers, detected nectar-guides and osmophores and performed pollen viability tests. We studied the reproductive system of the species using manual self- and cross-pollination tests and observed pollinators. Our results revealed that the flowering period takes place during the dry season, between April and October, and that the diurnal flowers open between 7:00 a.m. and 5 p.m. The flowers are shortly tubular with yellow perianth-segments. We found neither nectar nor nectar-guides, and osmophores appeared as glands within the flower tube. Whereas pollen viability was 90.25%, manual cross-pollination tests have shown cross-pollination with gametophytic incompatibility. We observed two bee species visiting the flowers and acting as effective pollinators: *Dialictus opacus* and *Plebeia* sp. The combination of low reproductive activity with gametophytic incompatibility, together with the reduced number of individuals in a population and low number of populations, makes the endemic cactus *U. buiningii* a critically endangered species.

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Introduction

Cactaceae, with over 1,450 species in 127 genera (Hunt et al. 2006), distributed almost exclusively in the

Americas, have diversity centres in Mexico and south-western USA, Argentina, Peru and the Bolivian Andean region, and in eastern Brazil (Taylor and Zappi 2004). Amongst Brazilian plant families, the family Cactaceae has both high levels of endemism and is under serious threat, with ca 110 out of 262 species currently considered vulnerable or endangered (Ribeiro-Silva et al. 2011). Genus *Uebelmannia* (subfamily Cactoideae) may serve as a typical example. It has three species, all known only from state of Minas Gerais (BFG 2015) and growing in a single vegetation type, the highlands known as ‘Campos Rupestres’. The Campo Rupestre, listed among OCBIL territories (old, climatically-buffered, infertile seascapes) of the world (Hopper et al. 2016), occurs in the Espinhaço Range and Chapada Diamantina, eastern Brazil and is known by the combination of its small area with very high levels of diversity and endemism, namely in families Eriocaulaceae, Velloziaceae, Apocynaceae and Cactaceae (Giulietti and Pirani 1988, 1997; Rapini et al. 2008).

Species of the genus *Uebelmannia* are found in a few very small populations in rock crevices or on pure quartzitic sands (Zappi and Taylor 2008). Due to the rarity and an interest of specialist plant collectors, all species of this genus are endangered and listed in the official Brazilian Red List of Plants (Martinelli and Moraes 2013; MMA 2014). Endemic species that present small distribution range and local dispersal tend to have low genetic diversity (Gitzendanner and Soltis 2000), but it remains unclear whether the low diversity is a cause or a consequence of their restricted distribution (Rapini et al. 2008). Other factors associated with rare plants, such as low dispersal capacity and lineage age (Gaston 1994) may also influence the size of the area occupied by them. Crucial for such rare plants is their reproductive ecology (Martínez-Peralta and Mandujano 2011). From studies done in the Chapada Diamantina (Conceição 2006), we know that pollination in the flora of the Campos Rupestres is dependent on pollination services provided by pollinators and that gene flow thus depend on their behaviour (Rapini et al. 2008). In this situation, knowledge of pollinators as well as flower and reproductive biology is fundamental for understanding and conserving plant populations (Goettsch et al. 2015).

Studies of reproductive and floral biology exist for only 15 of the 263 species of Brazilian Cactaceae. In the majority of the genera studied, seed production depends on cross-pollination

performed either by specific or generalist pollinators that are rewarded by pollen and/or by nectar: *Cereus* (Silva and Sazima 1995), *Cipocereus* (Rego et al. 2012; Martins et al. 2016), *Gymnocalycium* (Schlindwein and Wittmann 1995), *Micranthocereus* (Aona et al. 2006), *Melocactus* (Colaço et al. 2006; Fonseca et al. 2008; Gomes et al. 2013), *Notocactus* [= *Parodia*] (Schlindwein and Wittmann 1995) and *Pilosocereus* (Rocha et al. 2007; Locatelli et al. 1997). So far we have no specific information about the pollination and reproductive biology of any species from the genus *Uebelmannia*, so the present study intends to shed light on the possibility that reproductive constraints may translate into an extremely reduced distribution and decline of this species’ populations. We aim (i) to verify whether there is a pollinator dependent relationship, (ii) to clarify the role of pollinators in the reproduction of the species, and (iii) to investigate whether this interaction might be responsible for the plant’s rarity.

Material and methods

Study species

Uebelmannia buiningii occurs in the ‘Campos Rupestres’ of the Serra Negra region in the Municipality of Itamarandiba. It has a very narrow distribution range and is occasionally targeted by plant collectors (IUCN 2015). Despite being found within a protected area, the Parque Estadual da Serra Negra, habitat destruction to extract sand and gravel for building work and habitat degradation through cattle farming and hunting are amongst the negative pressures that affect the populations (Moreira et al. 2012; Souza 2014). Given this situation, the species is considered critically endangered (IUCN 2015; Martinelli and Moraes 2013). Individuals of *U. buiningii* are globose to elongated cacti with epidermis varying between greenish, reddish and dark-purplish brown, with ca 3–5 flowers per cephalium, ribs broken into tubercles crowned by areoles, each with 4 lateral spines (Schulz and Machado 2000; Taylor and Zappi 2004). The species rarely sprouts from the base of from the sides and is not capable of clonal growth, being dependent on seed production for its regeneration.

Study area

The study was carried out in the region of the Parque Estadual da Serra Negra and surroundings, an area of 13,654.31 ha in the Municipality of Itamarandiba, Jequitinhonha Basin (IEF/MG 2014), which encompasses the central part of the Espinhaço Mountain Range in Minas Gerais. The study species grows in the highlands known as Campos Rupestres (Silveira et al. 2015) found at and above 1,000 m and forming a large enclave (IEF/MG, 2014) inserted in the limit between Cerrado and Caatinga, two of the six major Brazilian Biomes (Forzza et al. 2012). Collection permits were issued by the 'Sistema de Autorização e Informação a Biodiversidade' (SISBIO: n° 35570-1) and the 'Instituto Estadual de Florestas' (IEF-MG: n° COL 116/12) granted authorizations for accessing the Parque Estadual da Serra Negra.

The local climate fits within the definition of the altitude subtropical climate with dry winters (lower temperatures below 18°C) and hot summers (temperatures higher than 22°C), Cwa type according to Köppen (1948), with mean precipitation of 1,036 mm, using data collected over the last 30 years by the Brazilian National Institute of Meteorology (INMET 2014).

During the study period (2012: September, 2013: March, April and July), the lowest precipitation was recorded between April and the beginning of August, with a long drought (65 dry days) between June and August. During July no precipitation was recorded, and this was also the coldest month, with the lowest temperature recorded (12°C). During the rainy season, between September and March, the total precipitation was 657 mm, peaking during November (302 mm) and January (190 mm). The total year's precipitation reached 741 mm, and maximum mean temperatures during September to December were around 34°C (INMET 2014).

The only three known extant localities (L) for *U. buiningii* were visited (Figs. 1 and 2) during five field expeditions: September and October 2012; March, April and July 2013. The map of the occurrence locations was prepared using ArcGIS 10 software. The first locality (L1), at a mean altitude of 1,050 m a.s.l., with steep terrain hosted 45 young to mature individuals (29 mature/45 total), the second locality (L2) at around 1,160 m a.s.l., hosted ca 268 individuals (186 mature/268 total), and finally, the third locality (L3), was on very steep terrain altitude around 1,080 m a.s.l., hosted

500 individuals (211 mature/500 total; Fig. 1). All three localities face north and receive sunlight in the morning until mid-afternoon, when they become shaded by the mountain.

Floral biology

Floral biology studies were carried out by bagging and marking four flowers in each of the three populations ($n = 12$) and observing them from anthesis until senescence. For the morphologic study, flowers ($n = 12$), fruits ($n = 7$) and seeds were collected from different individuals. Morphology used for the floral parts follows Radford et al. (1974) and Taylor and Zappi (2004).

Osmophore presence was tested by applying the technique of Dafni (1992), where a longitudinally sectioned randomly selected flower was coloured with Neutral Red 0.1%. Nectar guides were checked by exposing a longitudinally sectioned flower to ammonium hydroxide (NH₄OH)-soaked cotton (Scogin et al., 1977). Nectar production was verified by introducing a microcapillar (5 µL) in the nectariferous chamber ($n = 12$) every two hours from 7 a.m. at anthesis to flower closure.

The Pollen/Ovule ration (P:O) was calculated following Cruden (1977) by randomly selecting a flower and counting the total number of pollen-grains from one anther ($n = 5$) and the total number of ovules per ovary ($n = 5$) in pre-anthesis, where the number of pollen grains per flower was estimated by calculating the mean number of grains per anther multiplied by the mean number of anthers per flower.

Pollen viability test was carried out by randomly selecting a flower and counting up to 300 pollen grains collected randomly from 12 flowers and coloured with acetic carmine 1%, according to Dafni (1992) and Kearns and Inouye (1993), considering viable only the coloured pollen-grains. From the 12 resulting slides, the mean and the standard deviation were calculated.

Reproductive biology

Self-pollination tests were made with pollen and ovules of the same flower ($n = 12$) and cross-pollination using pollen of randomly selected flowers from different and distant individuals ($n = 12$) between individuals to the same locality were performed in May 2013. The low number of flowers used in each treatment was due to plants rarity and low number of flowering individuals. After the treatments the flowers collected were placed in

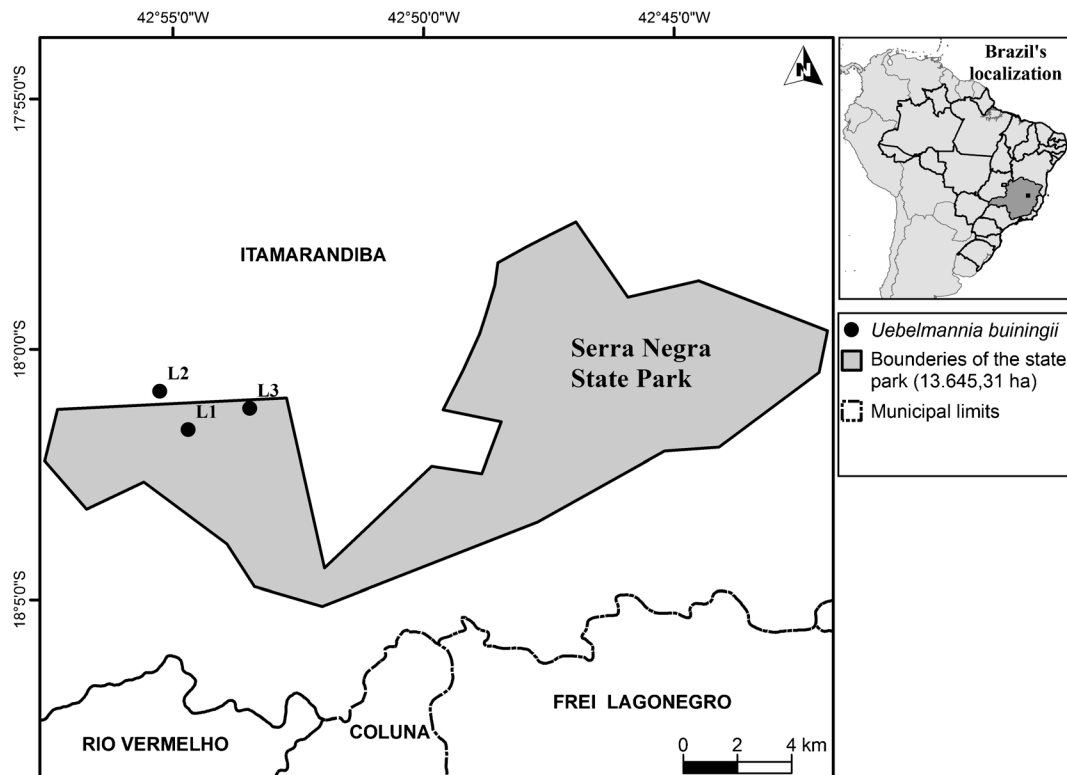


Fig. 1 Map comprising three localities (L) of *Uebelmannia buiningii*, at and nearby the Parque Estadual de Serra Negra, Minas Gerais

3% agar. Every six hours, two flowers of each batch were fixed in 50% FAA until they completed the 72-h cycle. After fixation, the material was stored in alcohol 50%.

The pollen tube growth was verified by isolating the style and stigma from a randomly selected flower and washing them four times with 20 min gaps with sodium hypochlorite (NaClO) in a hot water bath at 60°C until the plant material looked transparent. Afterwards the material was washed five times with distilled water at room temperature, in 20 min intervals. Following removal of NaClO, style and stigmata were placed on slides with 2–3 drops of aniline blue 0.2% and then pressed. The slides were stored at 4°C during 24 h, with two additions of aniline blue drops to reinforce colouring (Kearns and Inouye 1993). The resulting material was analysed under fluorescent microscope at the Cytotaxonomy and Plant Evolution Laboratory of the Biology Department at the Universidade Federal do Ceará (UFC).

Floral visitors

Observations of floral visitors were carried out from 7 a.m. to 5 p.m. during three days in May 2013 in L3.

Visitors were collected using net bags and pinned using insect pins and deposited in the collection of the Laboratório de Bionomia, Biogeografia e Sistemática de Insetos (BIOSIS), an associate unit of the Museu de História Natural / Zoologia – MHNBA/MZUFBA of the Biology Institute at the Federal University of Bahia.

Results

Floral biology

During the April–August flowering season, less than 15% of plants from all population flowered (59 out from 426 mature individuals), usually with one (rarely two) flowers per individual (Table 1). *Uebelmannia buiningii* has day-opening flowers that last three days. Anthesis begins at 7 a.m. and flowers stay open until closing time at 5 p.m., and this process is repeated over three days, with the flowers closing completely and wilting on the last day. The stigma is central to the flower and, as the flower opens in its first morning, the stigma lobes unfold, remaining extended for the duration of the anthesis.

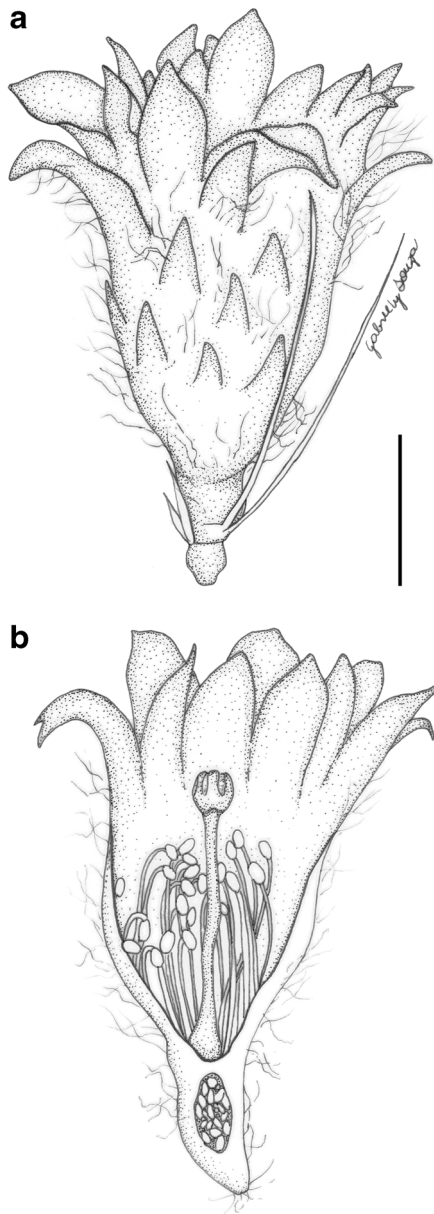


Fig. 2 Flower of *Uebelmannia buiningii*. **a** – Outside view showing floral-tube scales. Note in A flower still attached to a detached areole with spines, and that the hairs that appear outside the flower became detached from the floriferous areoles; **b** – Longitudinal section. Scale: 2.2 cm

Each location (L1, L2 and L3) was observed for 30 h. During the observed time, L1 had 44.82% of adult individuals with flowers; the percentage found during the same period was 6.99% for L2 and 15.64% for L3.

Flowers of *U. buiningii* are hermaphroditic, have radial symmetry and are tubular, measuring $18.25 \pm$

$1.76 \times 13.17 \pm 1.85$ mm. The outside of the tube has scales (Fig. 3) and the outer and inner perianth-segments are yellowish, turning pale-orange or pinkish when old. The number of stamens varies between 60 and 70. Stamens are included, 10.33 ± 2.01 mm long. Style measure 14.50 ± 1.09 mm and the stigma is divided into six lobes, while 38 is the mean number of ovules found in the unilocular ovary.

During the field visits to the populations, fruit formation in *U. buiningii* appeared to be very low. Only seven fruits were observed in May 2013 and five in July 2013 (these were not necessarily related to the flowers observed earlier in the experiment). Fruits are clavate and succulent, measuring $6.57 \pm 1.27 \times 5.00 \pm 1.29$ mm, the epicarp is reddish when ripe and contain on average 30 seeds per fruit (Table 1). Seeds are oblong to ellipsoid, ca 1 mm long. The colour varies from black to reddish-brown, and the testa is smooth, shiny, with depressions.

The NH_4OH test confirmed the absence of nectar guides. The osmophore test showed the presence of glands in the anthers and stigma. No nectar was detected in the nectar-chamber of *U. buiningii* flowers.

Longitudinally-slit anthers of *U. buiningii* (Fig. 3a) release tricolpate pollen-grains with a continuous exine (Fig. 3c). The P:O ratio was of 626.13:1, with high pollen-grain offering (24,105) per flower (Table 1). Pollen viability was high, at 90.25% with a mean of 270 viable pollen-grains (Table 1).

Reproductive biology

Pollen tube growth after manual pollination was observed for 12 self-pollinated flowers and for 12 cross-pollinated flowers. After 6 h after manual pollination pollen-tube reached around half of the style (Fig. 4a and b) in both treatments (Fig. 4d). Considering that style length was 14.5 mm, the estimated growth rate was of 335.65 nm/s. Twelve hours after manual pollination, the pollen-tube reached the ovary for both treatments; however, only in the cross-pollinated flowers the pollen-tube reached the ovules. After 18 h the pollen-tube of cross pollinated treatment penetrated the micropyle (Fig. 4f). Incompatibility in self-pollinated flowers of *U. buiningii* was confirmed as the pollen-tube growth was interrupted when it penetrated the ovary, or when the pollen-tube extremity became deformed while entering in contact with the ovule (Fig. 4e), unable to access the micropyle.

Table 1 Biometric features of flowers, fruits and seeds of *Uebelmannia buiningii* collected in three studied localities. *SD* – Standard Deviation.

Character	Number of flowers/fruits	Mean	<i>SD</i>
Flower length [mm]	12	18.25	1.76
Flower diameter [mm]	12	13.17	1.85
Number of external perianth segments	12	25.83	3.49
Stamen length [mm]	12	10.33	2.01
Stamen number	12	67.92	6.21
Pollen-grains / anther*	12*	354.92	9.70
Pollen-grains / flower	12	24,105.94	658.80
Style length [mm]	12	14.50	1.09
Stigma-lobe number / stigma	12	6.58	0.51
Ovule number	12	38.50	7.25
Pollen-grain viability	12	270.75	20.51
Fruit length [mm]	7	6.57	1.27
Fruit diameter [mm]	7	5.00	1.29
Seed number	7	30.14	4.74

Pollen-grain viability: 90.25%

Pollen/Ovule ratio: 626.13:1

*Each anther comprises two thecae

Floral visitors

Two visiting bees were observed in all three localities: *Dialictus opacus* Moure, 1940 – Halictidae (Fig. 5a, c and e) and *Plebeia* sp. Schwarz, 1938 – Apidae (Fig. 5b, d and f). The foraging activity was not continuous, with bees staying on the flowers for approximately three minutes without accessing the inner part of the flower-tube and making contact only with anthers and stigmas when the bees were collecting pollen. A total of 10 visits were recorded per day between 11 a.m. and 1 p.m. However, these visits were not subdivided by specific visitors as

these were not identified until later in the process. During the remaining anthesis period, no other floral visitors were observed.

Random visits by grasshoppers from the genus *Eurotettix* sp. Bruner, 1906 (Fig. 5g) to the flowers were also observed. These visitors remained continuously on the flowers for up to five minutes during 1–2 day of anthesis. The grasshoppers were seen predated perianth segments, anthers and the stigma. Another type of visitor were unidentified ants (Fig. 5h). All flower visiting insects recorded are active only during the day. No night-time observation was carried out during the project, as the flowers were closed.

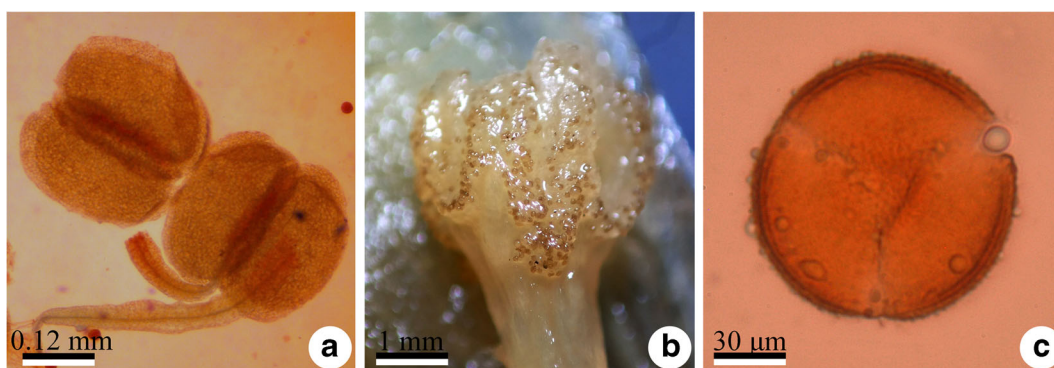


Fig. 3 Pollen aspects of *Uebelmannia buiningii* at and nearby the Parque Estadual da Serra Negra – MG. **a** – Emptied out anthers; **b** – Stigma with pollen-grains attached (without colouring); **c** – Polar

view of pollen-grain. Anthers and pollen grains coloured with Neutral Red 0.1%

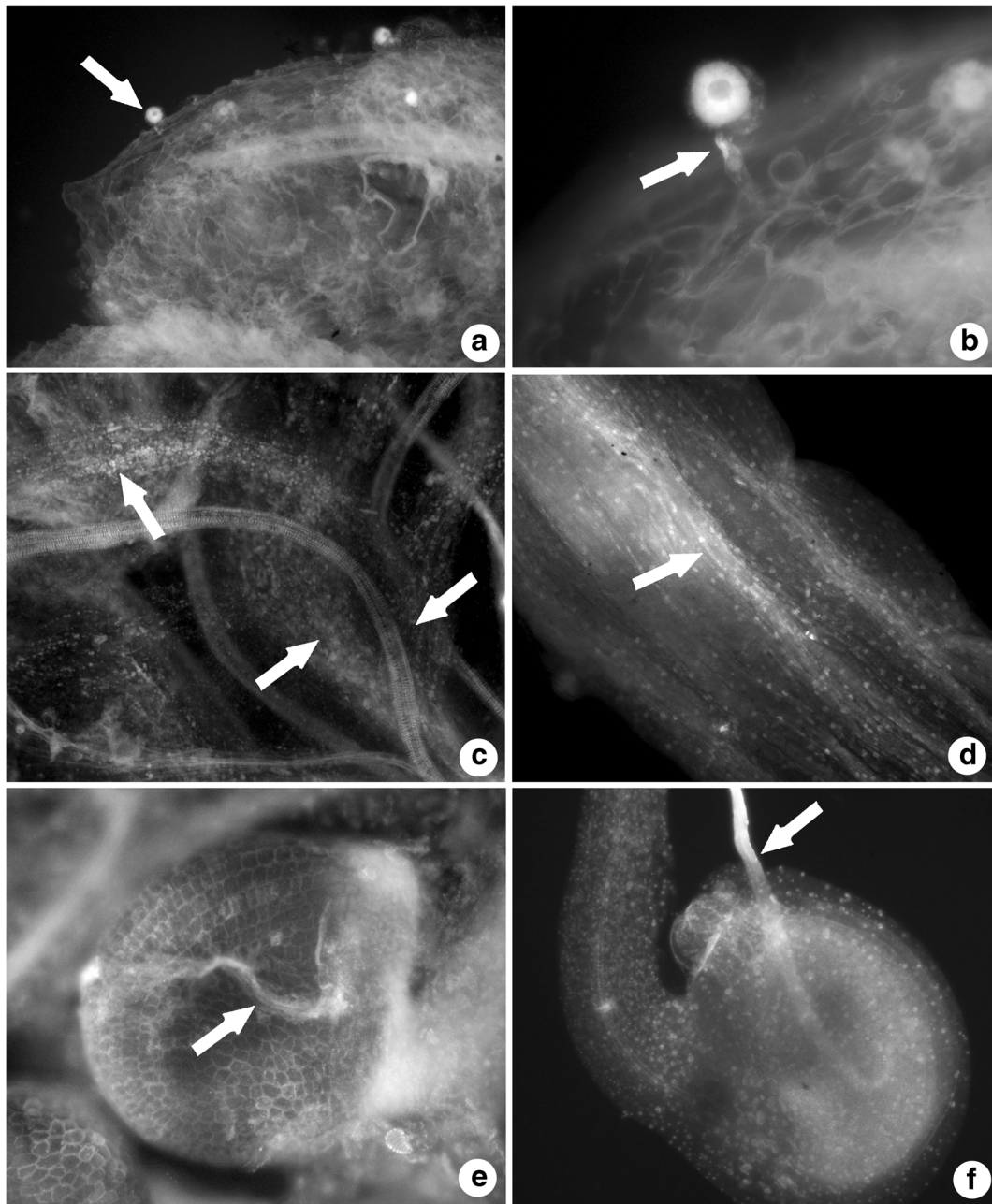


Fig. 4 Pollen-tube growth evidenced by aniline blue 0.2% in fluorescent microscope, in self- and cross-pollination treatments in *Uebelmannia buiningii* at and nearby the Parque Estadual da Serra Negra – MG. **a** – Pollen grain adhered to the stigma (5×); **b** – close-up of pollen-grain adhered to the stigma (20×); **c** – Pollen

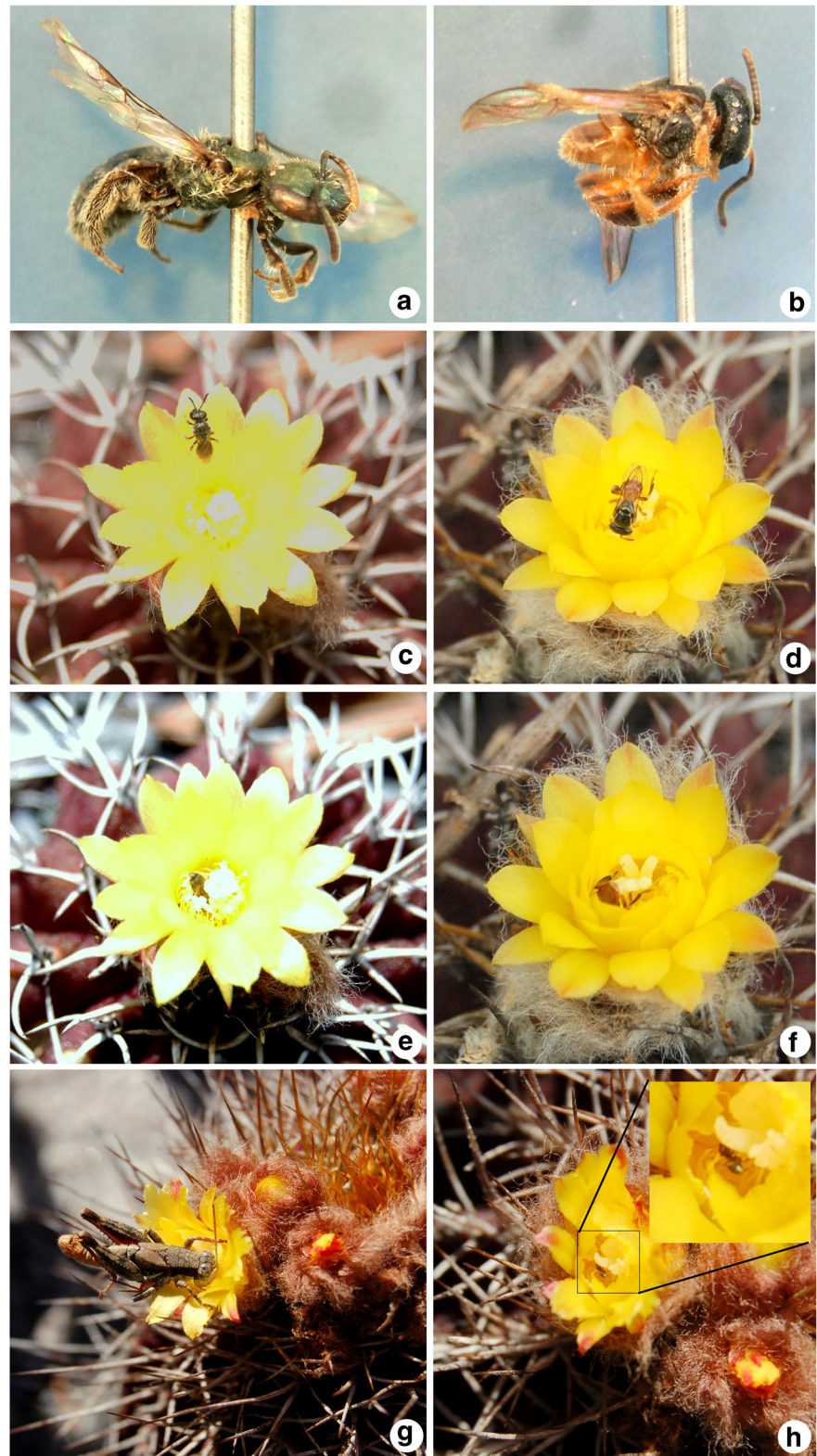
tube growing towards the style (5×); **d** – Pollen tubes interrupted by the FAA 50% treatment six hours following self-pollination (5×); **e** – Deformed pollen-tube near an ovule 18 h after self-pollination (10×); **f** – Pollen-tube fertilizing an ovule 24 h after cross-pollination (10×)

Discussion

Some attributes of the floral biology of *U. buiningii* are in line with the strategy of other eastern Brazilian cacti, such as the winter-flowering and fruiting,

enabled by the water-storing capacity. Cactus plants therefore provide resources to their pollinators during the most difficult time of the year (Taylor and Zappi 2004). However, some of the attributes differs from other eastern Brazilian cacti, for example flowering

Fig. 5 Floral visitors of *Uebelmannia buiningii* at and nearby the Parque Estadual da Serra Negra – MG. **a, c** and **e** – *Dialictus opacus*; **b, d** and **f** – *Plebeia* sp. **g** – *Eurotettix* sp. grasshopper; **h** – unidentified ant (detail)



prolonged over three days combined with flower closure during the night. The majority of representatives of the Browningiaae-Cereeae-Trichocereae clade in eastern Brazil have flowers lasting for a single night or day. So far only *Micranthocereus* has been reported to have flowers lasting two or three days, but not closing during the night (Aona et al. 2006). The bee pollination syndrome (yellow, short tubular day flowers offering only pollen as a reward) contrasts with a majority of bat, hummingbird and hawkmoth syndromes seen in other genera from that region (bat: *Pilosocereus*, *Cipocereus*, *Brasilicereus*, *Stephanocereus*, hummingbird/bat: *Melocactus*, *Arrojadoa*, *Micranthocereus* and hawkmoth: *Cereus*, *Discocactus*, *Arthrocereus*; Taylor and Zappi 2004). The floral biology of *U. buiningii* shows similarities in its pollination syndrome with species of *Parodia* and *Rebutia* from southern Brazil, which, however, are not related to *Uebelmannia* but are currently placed in tribe Notocacteae (Hernández-Hernández et al. 2011).

Floral biology

Flowering of *Uebelmannia* in populations was low and this was in contrast with rich flowering in cultivation (Olsthoorn. pers. comm.), where plants were remaining in bloom for a continued period of several months. The perceived shortage of flowers in nature may result from stressful conditions in the wild populations, and certainly reflects negatively in the reproductive ability of the species.

The reflective yellow colour of the inner perianth segments of *U. buiningii* is probably visible at a certain distance and attracts bees to the open flowers, despite the lack of nectar guides (and of nectar). Once landed, the bees are stimulated by the osmophores found in the areas they immediately reach, such as anthers and stigma, encouraging contact between these two structures and pollen exchange.

Few bee-pollinated cacti, such as *Opuntia lindheimeri* Englem. (Grant et al. 1979) and *Opuntia monacantha* (Willd.) Haw. (Lenzi and Orth 2011) do not produce nectar. Scogin (1985) records this feature as rare within the family as a whole, with only eight nectarless species out of 43 species studied, amongst them a single eastern Brazilian representative, *Pereskia grandifolia* Haw., from the basal subfamily Pereskioideae. This is the first record of totally

nectarless flowers in subfamily Cactoideae in South America, as the Mexican senita cactus, *Pachycereus schottii* (Fleming and Holland 1998) produces little or, sometimes, no nectar.

While it is possible that the reduced availability of resources (low flower number, small populations) from *U. buiningii* within the study area might lead to a low visitation rate and consequent limited pollination, it represents a saving for the plant that may be offset by the provision of abundant pollen. While nectar is sought after by a large diversity of floral visitors, pollen is a more specific reward that attracts bees. Bees are attracted from the distance by flower colour and guided into the flower by osmophores, resulting in an intense contact between visitors and floral parts, which increases the effectiveness of pollen transfer. Considering the globose to shortly cylindrical habit of the plants and their open, easily accessible flowers, it is possible that the presence of nectar would attract ineffective or predatorial floral visitors such as ants, detrimental to the species reproduction. An interesting question would be to investigate phylogenetically whether the absence of nectar represents a loss or a secondary character of *Uebelmannia* rather than a plesiomorphic character state shared by other basal cacti.

Reproductive biology

Uebelmannia buiningii is an obligate cross-pollinated species and does not form fruits and seeds without the help of pollinators. Most species of cacti are obligate cross-fertilized and reproductively self-incompatible (Ross 1981; Mandujano et al. 2010), making them totally dependent on pollination services for reproduction. Self-incompatibility observed in *U. buiningii* took place with the interruption of the pollen-tube growth when entering the ovary, after 12 h of self-pollination, characterizing a case of gametophytic self incompatibility.

Considering that only 5% of all cactus species have been studied reproductively, it is reported that the vast majority have mechanisms that promote cross-fertilization of different types, including GSI (Mandujano et al. 2010). Strong and Williamson (2007) suggest that self incompatibility system is frequent in cactus species, being found in 31 genera of cacti. Boyle (2003) detected gametophytic self incompatibility system reproductive system in several epiphytes of the endemic Brazilian genera *Hattiora* and *Schlumbergera*. It is possible that the gametophytic self

incompatibility system found in *U. buiningii* and *U. pectinifera* Buining (Sousa 2013) is linked to the restricted distribution of this genus. When compared to self-compatible, inbreeding taxa, self incompatibility system is more likely to lead to new genetic recombinations that could in due course improve their adaptation to a changing environment (Strong and Williamson 2007). On the other hand, self-compatible lineages of *Rhipsalis* diversified and spread throughout the Brazilian Atlantic Rainforest to other Neotropical countries and to the Old World, eventually reaching Madagascar and Sri Lanka (Grosse-Veldmann et al. 2016).

The pollen/ovule ratio (P:O) found in *U. buiningii* (626:1) is even higher than what was observed for *U. pectinifera* subsp. *pectinifera* (485:1), where 9.216 pollen grains were found for 19 ovules (Sousa 2013). Following Cruden (1977) classification, the allogamous reproduction observed in *U. buiningii* is corroborated by its P:O ratio.

Genetic diversity between individuals and subpopulations is ensured by SI in *U. buiningii*, ensuring effective genetic exchange. However, the number of individuals is crucial to ensure the success of this type of reproduction and, in xeric environments, there is a trend of low recruitment of young individuals (Mandujano et al. 2007) as well as low seed production rates. Despite the fact that the fruit and seed production was not considered by this study, preliminary studies from a demographic study of *U. buiningii* show that its populations have aggregated distribution and low recruitment, as the proportion of young plants in the studied populations is low (Teixeira in prep.).

Floral visitors

Both *Dialictus opacus* and *Plebeia* sp. were considered effective pollinators of *U. buiningii* as they make contact with the flower's reproductive structures, promoting successful pollination. The flight activity of bees coincided with increased flower visitation as temperatures rose between 11 a.m. and 1 p.m., as also reported by Silveira et al. (2002). Bright yellow flowers exposed to sunlight and the intense volatilization of the substances found in the osmophores was effective in the attraction of *Dialictus opacus* and *Plebeia* sp. bees. Some bee visits were rapid, probably due to the absence of nectar in *U. buiningii* flowers. Similar results with small size bees were reported by Sousa (2013) for *U. pectinifera* subsp. *pectinifera*.

Belonging to the subfamily Halictinae, comprising solitary and social basal bees that nest in holes on the ground and rotting wood, *D. opacus* (Halictidae) is well represented in southern, southeastern and northeastern Brazil. Pacheco-Filho et al. (2015), studying Neotropical bee-plant associations, identified *D. opacus* as a generalist bee that interacts with a large number of plant species in the caatinga. Prior to this research, there were no records of this species visiting Cactaceae, though it was observed in other localities of Campo Rupestre of the Chapada Diamantina, Bahia (Juncá et al. 2005).

Plebeia sp. (Apidae) belongs to subfamily Meliponinae, alongside very small bees that build their nests in tree-trunks and abandoned nests from other animals (Silveira et al. 2002) and are truly social, forming perennial colonies with large numbers of workers (Michener et al. 1994), needing vast quantities of resources throughout the year, favouring the diversified use of floral resources (Pacheco-Filho et al. 2015). Even though they make no specific mention of *Plebeia*, Pacheco-Filho et al. (2015) assume that species from this tribe are generalist bees. It is worth pointing out that solitary bees, on the other hand, are more active during certain times of the year and tend to present more restricted diet, while the duration of their colonies is linked to the flowering period of the plants it pollinates (Biesmeijer and Slaa 2006).

Florivory in *U. buiningii* was carried out by *Eurotettix* sp. This grasshopper was observed ca 2–3 times predated on flower parts, probably diminishing the number of flowers available for cross pollination. Studies carried out with *Ariocarpus fissuratus* flowers predated by beetles show that herbivory negatively affects species reproduction as it affects flower attraction and destructs reproductive structures (Martínez-Peralta and Mandujano 2011). Reproductive success was limited in *Opuntia microdasys* due to flower-bud predation by moth larvae of *Olycella* aff. *junctolineella* (Piña et al. 2010). It is important to monitor populations of *U. buiningii* regarding grasshopper predation to quantify the possible decrease in reproductive success and to investigate whether the attacks are frequent and/or periodic.

The low number of pollinators seen in *U. buiningii* during this study, together with the low flower production per individual allied to the allogamous reproductive system, may interfere in the reproductive dynamic of the species, leading to a decrease in the abundance of

individuals in the locations under study. It is therefore necessary to have more information regarding the conservation status of the pollinators and of the interaction between plant and pollinator in order to adopt efficient strategies to aid the recovery of *U. buiningii* in its natural habitat.

Conclusion

Uebelmannia buiningii was assessed as critically endangered based on the small overall size of its population and their low number, added to the fact that, due to its rarity, it continues to be a target for illegal collection of plants and seeds, and suffers from habitat disturbance and possible decline. During the present study the flower availability and fruit set were found to be low, and the reproduction was allogamic with gametophytic self-incompatibility, meaning that the species is dependent on its pollinators in order to set seeds. Two of the floral visitors observed were effective pollinators (*D. opacus* and *Plebeia* sp.) with generalist requirements, and a floral predator (*Eurotettix* sp.) with detrimental effect to pollination and fruit set was also recorded. For the future we need to protect all three populations and ensure their seed production by considering also their pollinators in protection to ensure genetic diversity of the existing populations. Population structure studies are under development and a genetic analysis of the three known populations needs further research in order to assist in situ recovery of the species.

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Compliance with Ethical Standards

Competing interests The authors declare there are no competing interests.

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