



# Beyond taxonomy: anther skirt is a diagnostic character that provides specialized noctuid pollination in *Marsdenia megalantha* (Asclepiadoideae–Apocynaceae)

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## Abstract

Taxonomic diagnostic floral structures can be very informative about details of the floral mechanism, promoting baselines for inferences about pollination system. In a number of species in the milkweed tribe Marsdenieae, the already complex basic asclepiad flower structure is further elaborated by the evolution of one morphological novelty: the anther skirt. Since the functional significance of this trait is poorly understood, we investigate its role in the pollination mechanism on *Marsdenia megalantha*. We tested the phenotypic integration of anther skirt; we described its functional anatomy; we identified and recorded the function of its nectaries; we described and tested in laboratory its contribution to the pollination mechanism; and we studied the pollination effectiveness of this system. The characteristics of an anther skirt are essential in the noctuid pollination of *M. megalantha*. This is due to its particular morphological and anatomical features related to its ability both to secrete at night and to contain nectar independently from the stigmatic chamber. The skirt also functions coordinately with the corona as a funnel that directs the noctuid legs to gynostegium. Its effectiveness in producing pollination was confirmed by field observations and manipulative experiments. We suggest that nectar offers at night and mechanical adjustment may be applicable to other representatives of the tribe as a feature that determines noctuid pollination, assigning a functional value for the anther skirt beyond the taxonomy.

**Keywords** Anther skirt · Asclepiadoideae · Gynostegium · Lepidoptera pollination · Marsdenieae · Substitutive nectaries

## Introduction

Flowers are the most informative organs of angiosperm diversity. They are instrumental to assess taxonomic identity due to their high variability, evolutionary relatedness and phylogenetic signal (Kohn et al. 1996; Singh and

Subramaniam 2008; Prenner et al. 2010). At various taxonomic levels, particular floral features are often diagnostic of certain groups. Additionally, if such features represent an acquisition with adaptive value, pollination biology should be able to explain the maintenance of structures in a lineage (see Gould and Lewontin 1979). Some studies go beyond the simple characterization of morphological features and seek to understand their functions. These include many examples such as the elaiophores on the sepals of most neotropical Malpighiaceae (Vogel 1990), which produce oil reward (Possobom et al. 2015); the labellum, an exclusivity of Orchidaceae and other lilioid monocots (Rudall and Bateman 2002), which modulates interaction animal pollinators (Teixeira et al. 2004); the particularly trichome-covered staminode in the subtribe Jacarandinae (de Vattimo 1984), which generates mechanical adjustment and floral display (Dieringer and Cabrera 2001); or the configuration of reproductive organs of *Allamanda* within Apocynaceae (Fallen 1985), promoting secondary presentation of pollen and pollen transport on probosces

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(Araújo et al. 2014). We highlight the anther skirt, an autapomorphy of Marsdenieae tribe absent in other related Asclepiadoideae (Apocynaceae). This structure adds complexity to the already elaborated milkweed flowers, since the basal parts of the anther margins are convolute and fused, forming a skirt with nectariferous function (e.g., *Hoya*, Kunze and Wanntorp 2008a, b). We present here evidence of the functional role of this structure associated with the unusual pollinarium transfer mediated by the legs of noctuids.

Asclepiadoideae (Apocynaceae) are noteworthy for having outstanding structures already well enlightened about their functionalities. This group has the most complex flowers of the Eudicots, with three exclusive structures: corona, gynostegium and the pollinarium, which collectively represent a strong taxonomic specificity and have key biomechanical roles in the pollination. The corona and gynostegium morphology contributes to visitor attraction, mechanically precise and species-specific pollinarium transfer (Ollerton and Liede 2003; Meve and Liede 1994; Wolff et al. 2008; Wiemer et al. 2011; La Rosa and Conner 2017). The guide rails and pollinarium system are considered part of a complex mechanical contrivance (Vieira and Grabalos 2003; Wiemer et al. 2011). They allow remarkable interactions such as ant pollination (Domingos-Melo et al. 2017), male-to-male confrontation through pollinaria concatenation (Cocucci et al. 2014), pollinia deposition on the tongue of birds (Pauw 1998) and pollination by mutilation of pollinator appendages (Shuttleworth and Johnson 2009). In addition to these peculiarities, one tribe of Asclepiadoideae has conspicuous structures whose function remains unknown which is the anther skirt of Marsdenieae.

The anther skirt in Marsdenieae develops laterally from each lobe of the fleshy staminal corona, and lobes are always independent from each other (Fig. 1d–g). The skirt merges with the anther and, in some species, anthers have revolute margins below it. These margins stand out from the filament and surround the gynostegium in various configurations associated with the shape of a skirt (Morillo 1978; Fishbein 2001; Wanntorp et al. 2006; Kunze and Wanntorp 2008a, b). In addition, secondary nectaries are frequently present under this structure (Omlor 1998). Although this anther skirt is a morphologically well-characterized structure and has a recognized taxonomic importance in the well-supported Marsdenieae tribe, nothing is known about its function in the pollination mechanism (Morillo 1978; Wanntorp et al. 2006; Rapini et al. 2007, 2008a, b; Wanntorp and Kunze 2009). In addition to this knowledge gap, there is a strong lack of information about pollination biology in the whole tribe. Since nectar is not concealed, Ollerton and Liede (1997) have suggested that fly pollination should predominate, with a lower contribution of Hymenoptera, Lepidoptera and Coleoptera. This suggestion was recently been partly

confirmed with a list of up to ten different pollination systems for this clade (Ollerton et al. 2018).

Our aim is to clarifying the role of the anther skirt and its secondary nectaries during pollination process. To this end, we use as model *Marsdenia megalantha* Goyder & Morillo, whose anther skirt morphology is already well studied (Goyder and Morillo 1994; 2008a, b), to (1) perform morphometric study and test the relationship between the anther skirt and other floral parts; (2) describe the floral anatomy and locate the different nectaries; (3) portray how the anther skirt interacts with pollinators through field observations and describe the process of pollinaria transfer between flower and pollinator; (4) confirm the functionality of these structures through manipulative experiments; and finally (5) document the pollination efficiency by quantifying flowers pollinated, fruit and seed set.

## Materials and methods

### Model species and populations studied

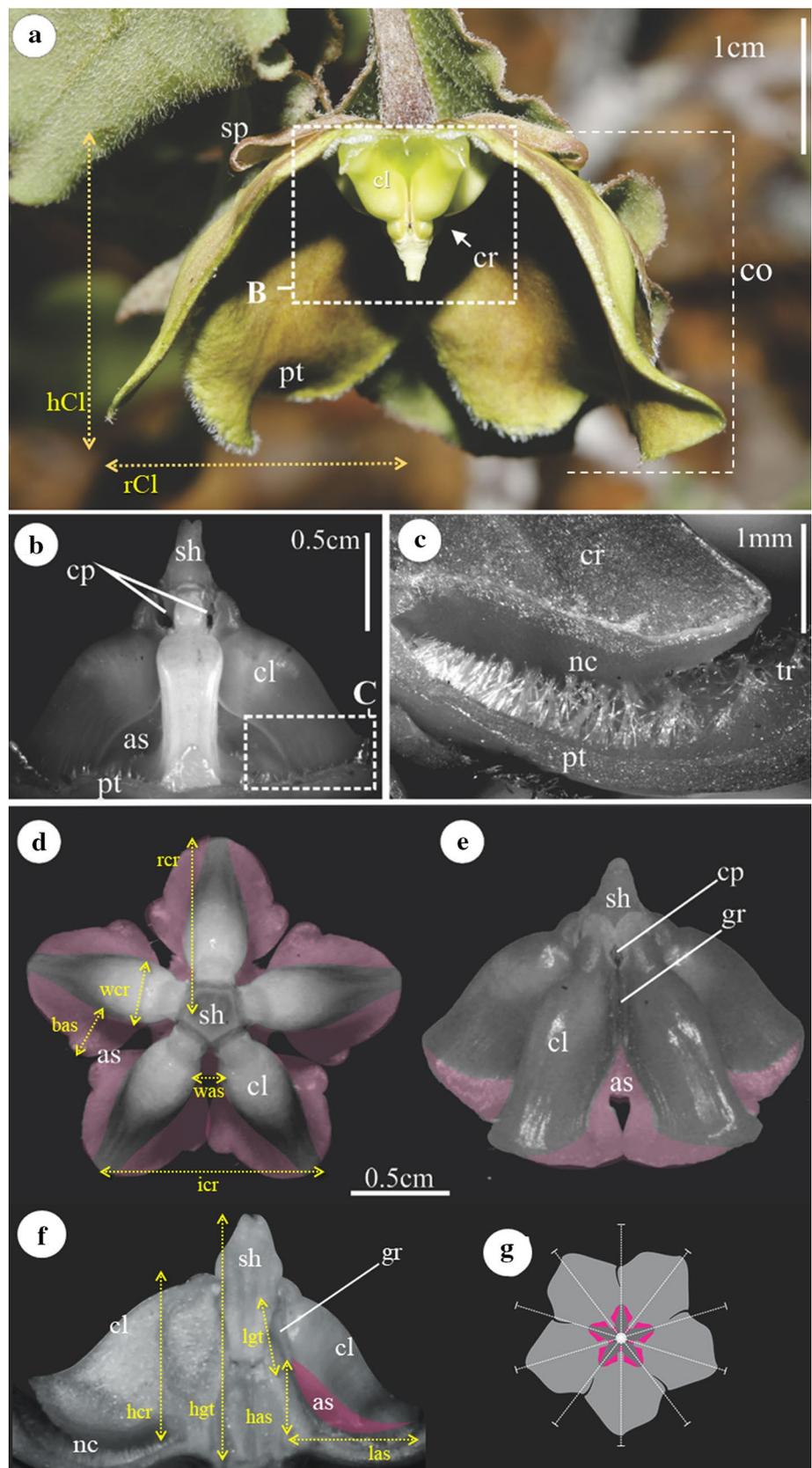
*Marsdenia megalantha* occurs on some inselbergs in the Caatinga from Brazilian Northeast. Its ranges from east Ceará to the central Bahia (speciesLink 2016). *Marsdenia megalantha* presents one of the largest flowers in the genus (~3.5 cm). These are greenish-brown, subcampanulate, pendulous (Fig. 1a) and are scented exclusively at night, when they smell like rotten bananas. Results of a scent analysis support this human subjective olfactory perception (A. Domingos-Melo unpublished data). These traits suggest a phenotypic specialization in the pollination system (Faegri and van der Pijl 1979; Ollerton et al. 2007), although the necessary pollinators remain unknown.

We conducted our studies in two populations, one located on a sandstone outcrop (8°30'57"S, 37°13'58"W) in the Parque Nacional (PARNA) do Catimbau (Pernambuco—Brazil), an area of shrubby Caatinga with a semiarid climate whose average annual temperature and precipitation are 25 °C and 1095.9 mm, respectively (Andrade et al. 2004). The second population was located on a set of rocky outcrops (7°28'51"S 36°54'40"W) in the Reserva Particular do Patrimônio Natural (RPPN) Fazenda Almas (Paraíba—Brazil), an area of arboreal or shrubby Caatinga, where average annual temperature and precipitation range from 26 to 30 °C and between 500 and 800 mm, respectively (Velloso 2002).

### Morphometry

We collected fresh flowers of *M. megalantha* in the field and fixed them in 70% ethanol. We defined 12 external morphometric characters of corolla, corona, anther skirt, gynostegium and pollinarium insertion mechanism (Fig. 1a,

**Fig. 1** Flower of *Marsdenia megalantha* **a** Lateral view, part of the corolla was removed. **b** Detail of corona and gynostegium. **c** Longitudinal section showing the interior of the nectar chamber. Anther skirt marked in pink in **d** upper view, **e** lateral view and **f** lateral section. **g** Schematic drawing of the five planes of actinomorphic symmetry. (Abbreviations: as—anther skirt; corolla—co; cr—corona; cl—corona lobe; cp—corpusculum; gr—guide rail; nc—nectariferous chamber; pt—petal; sh—stigma head; sp—sepal; tr—trichomes/ dotted yellow lines indicate the 12 external morphometric characters measured: rcl—corolla radius; hcl—corolla height; las—anther skirt length; has—anther skirt height; was—anther skirt width; bas—anther skirt brim width; rcr—corona radius; hcr—corona height; wcr—corona lobe width; icr—corona inter-lobes length; hgt—gynostegium height; lgt—gynostegium guide rail length)



d, f; Table 1). We photographed 60 fresh flowers (one per individual) in the field in the RPPN Almas population. The photographs consisted of transversal images of the adaxial view of the whole flower (Fig. 1d, g) and longitudinal views of the flowers sectioned across one of the five planes of actinomorphic symmetry (Fig. 1f, g). We obtained measures from the scaled images using ImageJ software which we used to build a matrix of correlations using Biostat5.3.

### Floral anatomy—nectar localization

We performed permanent longitudinal and cross histological sections of the collected flowers using the standard paraffin embedding procedures and staining with Astra Blue and Basic Fuchsin (Ruzin 1999) to locate flower secretory structures in fresh flowers; we used a neutral red and tap water solution (1: 10,000) as vital stain (Kerns and Inouye 1993). To do so, we bagged pre-anthesis flower buds and then collected open flowers at 22 h and submerged them 4 h in neutral red solution. Then, we washed the flowers with tap water and dissected them to identify the colored areas.

### Pollination mechanism

To record floral visitors of *M. megalantha*, we carried out focal observations and active searches in shifts of four to 10 h, between 04:00 p.m. and 05:00 a.m. (we used red lights for nocturnal observations). For both populations, we accumulated 400 observations hours during two consecutive years (03/2013 to 04/2015). We considered as pollinators animals that removed pollinaria and deposited pollinia. The pollination effectiveness is evident by the presence of pollinaria attached to the body of animals that lack one or both pollinia. Pollinators were dry-mounted for identification and deposited in the collection of flower visiting insects of the

Floral Biology Laboratory, Recife—Pernambuco, Brazil (Universidade Federal de Pernambuco).

We made detailed observations and photographed flowers and pollinators during their visits to describe the pollination mechanism. We took into account how they accessed the floral rewards and how they positioned and moved in the flower and paid special attention to the moment they removed pollinaria or deposited pollinia.

### Records and experiments of the secretory and mechanical function of anther skirt

We conducted simulation tests in the laboratory to confirm how the anther skirt participates in the pollinating mechanism. We used flowers fixed in FAA 70% because floral structure and stiffness are preserved in good conditions without loss of its mechanical functioning and allowing its manipulation. The use of fresh flowers became impracticable, because a large amount of latex exuded when the anthers skirt was removed. We removed the corolla from the flowers, held them on metal rods and slipped the legs of the pollinators into the guide rail system with the help of tweezers. In this way, we simulated the pollination mechanism and checked in detail the path of the legs along the guide rail. This assay was performed with intact flowers and other flowers that had their anthers skirt removed with a scalpel.

We performed focal observations recording the time and location of nectar accumulation throughout the anthesis to determine how nectar is presented. We quantified volume and concentration of nectar of the secondary nectaries when the amount allowed collection. For this, we bagged pre-anthesis flowers ( $n = 18$  flowers/6 individuals) and removed the nectar 3 h after the beginning of the secretory activities with the help of graduated microsyringes and measured sugar concentration with a pocket refractometer. We performed nectar-staining experiments to test whether there

**Table 1** External morphometry and relationships between floral structure measurements of *Marsdenia megalantha* Gray cells represent significant correlation values

	Mean $\pm$ standard deviation (coefficient of variation)	Correlation												
		(rcl)	(hcl)	(las)	(has)	(was)	(bas)	(rcr)	(hcr)	(wcr)	(icr)	(hgt)		
Corolla Radius (rcl)	16.42 $\pm$ 2.66mm (16.23)													
Corolla height (hcl)	11.10 $\pm$ 1.78mm (15.91)	-0.35												
Anther skirt length (las)	2.63 $\pm$ 0.36mm (13.82)	-0.14	0.09											
Anther skirt height (has)	3.12 $\pm$ 0.40mm (12.91)	-0.32	0.28	0.42										
Anther skirt width (was)	0.88 $\pm$ 0.19mm (21.02)	0.21	-0.17	-0.06	-0.12									
Anther skirt brim width (bas)	0.85 $\pm$ 0.17mm (20.19)	-0.12	-0.05	0.43	0.27	0.45								
Corona radius (rcr)	4.90 $\pm$ 0.52mm (10.73)	0.06	0.03	0.49	0.29	0.30	0.42							
Corona height (hcr)	4.67 $\pm$ 0.38mm (8.23)	0.11	0.26	0.29	0.52	-0.16	0.22	0.24						
Corona lobe width (wcr)	1.86 $\pm$ 0.18mm (9.99)	0.17	0.13	0.09	-0.16	0.28	-0.10	0.20	-0.06					
Corona inter-lobes length (icr)	6.35 $\pm$ 0.67mm (10.59)	0.16	0.07	0.66	0.26	0.36	0.37	0.77	0.15	0.40				
Gynostegium height (hgt)	7.10 $\pm$ 0.71mm (10.13)	0.25	0.21	0.14	0.25	0.28	0.32	0.38	0.62	0.13	0.41			
Gynostegium guide rail length (lgt)	1.89 $\pm$ 0.20mm (10.85)	-0.20	0.36	0.31	0.44	-0.02	0.37	0.09	0.42	0.18	0.23	0.47		

Cells with highlighted blue border indicate anther skirt correlations

was communication between places of accumulation in the same flower. We conducted this experiment in the field with flowers we cut through the pedicel and immediately inserted into a colloidal array of Agar–Agar and 2% water in acrylic boxes. In this way, we avoided any external interference, such as shaking of the branches or contact with visitors, that could spread the dye between the nectar compartments by means other than the nectar flow itself. In the acrylic box, the nectar secretory activity is maintained, which allows to observe nectar transfer within flowers. We placed Methylene Blue dye on one of the nectar accumulation sites and observed its diffusion. After 4 h, we checked if the dye had extended from one compartment to the other.

### Pollination effectiveness

To determine pollination effectiveness, we recorded (1) pollinia deposition and pollinaria removal in the flowers and (2) fruit and seed set due to open pollination. To this end, we randomly collected a sample of flowers from the PARNA Catimbau population (120 flowers/30 individuals) during the flowering peak period (April and June 2014) and preserved them in 70% alcohol. In the laboratory, we counted under stereomicroscope the number of removed pollinaria and deposited pollinia.

To determine the natural fruit set, we marked flowers that were left exposed to pollinators (120 flowers/30 individuals) and monitored the development of the fruits. To ensure that fruit formation was due to the pollinator action alone, we verified whether there was fruit set by spontaneous self-pollination monitoring flowers bagged at pre-anthesis (100 flowers/30 individuals). We also compared the number of ovules per flower in relation to the number of seeds per fruit ( $n = 15$  individuals).

## Results

### Morphometry

Flowers are pendulous and bear a subcampanulate corolla concealing the corona and gynostegium in the center (Fig. 1a). The corona lobes are robust and rounded; between them the system of rails belonging to the gynostegium is evident. Beneath each guide rail, there is a stigmatic chamber and above it the pollinarium (Fig. 2b). The anther skirt surrounds the base of the gynostegium, and it is a continuous structure, extending from the lower part of the guide rails through the rough edges on lower margins of the corona lobes, under which it forms a small protrusion (Fig. 1d, e). Under the anther skirt, chambers are located, that are delimited internally by the base of gynostegium and corolla tube,

externally by trichomes of the corolla and at the upper part by the corona lobes (Fig. 1c).

Among the 12 measured characters, those related to anther skirt and corolla presented the highest variation coefficients and there was greater conservatism in the measurements of corona and gynostegium (Table 1). The correlations of the anther skirt measurements are similar to other correlations among floral parts ( $t = 0.38$ ;  $p = 0.35$ ), which may indicate that the flower is integrated in general. Even so, the anther skirt has strong correlations with some particular traits, especially the corona (Table 1).

### Floral anatomy—nectar localization

The anther skirt and corona lobes are formed by a thick spongy parenchyma apparently responsible for its robust structure. Their surface consists of a shiny, smooth and thick hydrophobic epidermis, unlike the petal epidermis which is mat, thin and rough (Fig. 2a, b).

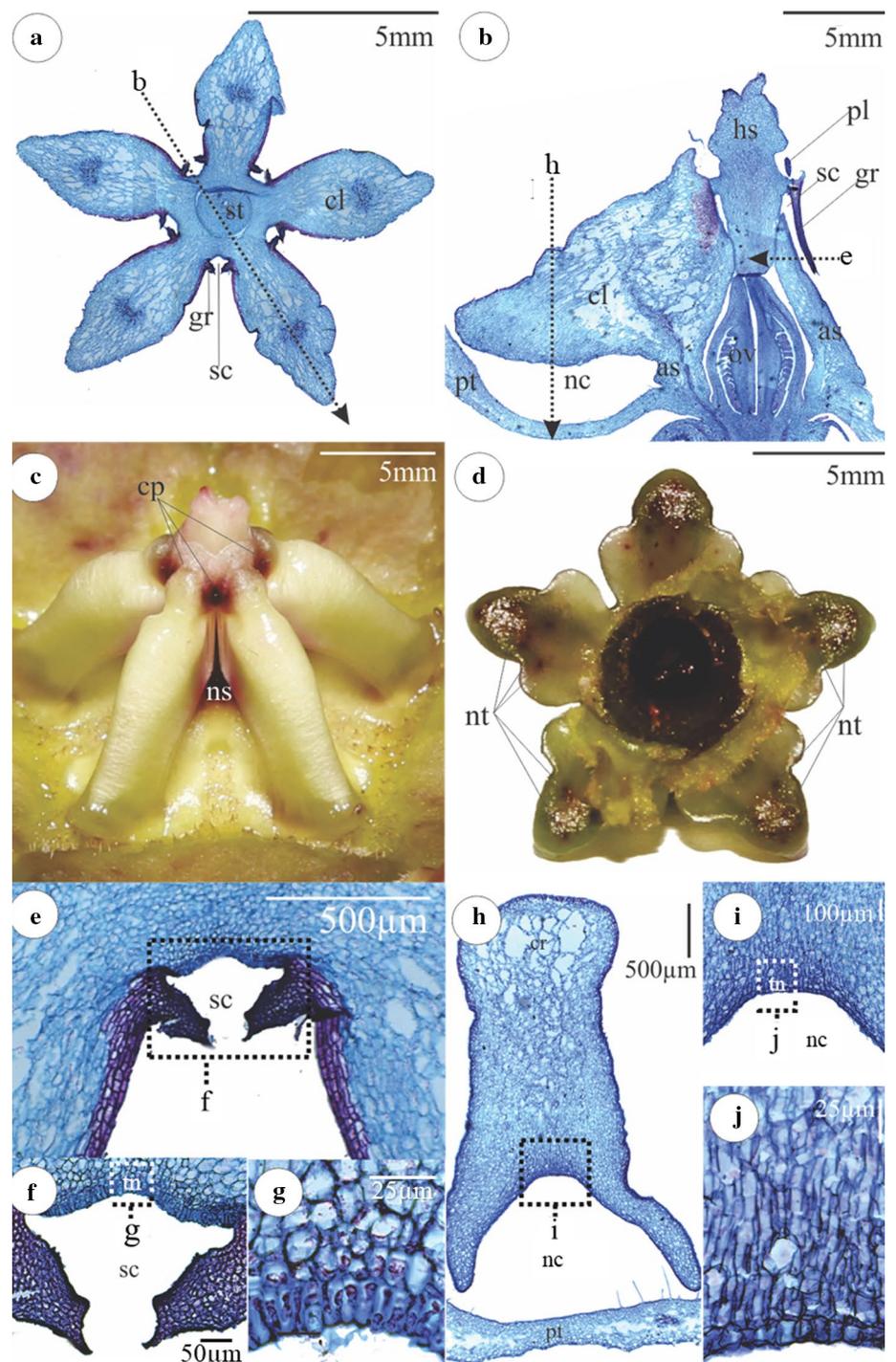
Neutral red test revealed presence of secretory tissue in two flower compartments: inside each of the five separated stigmatic chambers, where coloration was uniform and intense (Fig. 2c), and on the underside of skirt, in the nectar chamber, with discontinuous spotty and less intense staining (Fig. 2d). In both regions, a secretory tissue is evident in the histological sections (Fig. 2e–i). The cells in stigmatic chamber are rounder than those in the nectar chamber; in anther skirt secretory tissue cells are rather long and narrow (Fig. 2g, j).

### Pollinators and pollination mechanism

Moths of the family Noctuidae (Lepidoptera) that appeared to be of different unidentified species (Fig. 3a, b) were the only pollinators recorded in both populations (PARNA Catimbau e RPPN Almas). These insects performed the pollination, i.e., they were the only ones seen removing pollinaria or depositing pollinia. Collected specimens carried pollinaria with and without pollinia. Corpuscula were always attached to the tarsal claws (Fig. 3c). Occasionally we found severed legs of these animals trapped in the flowers by the guide rails.

The noctuids land inside the corolla where they easily find footing onto the rough petal surface (Fig. 3d). To access the nectar, they grasp the corona and insert their proboscis into the nectariferous chamber beneath the skirt (Fig. 3e). In this position, they place their legs between the lobes of the corona and over the anther skirt. Thus, the anther skirt leads the noctuid to get positioned in the flower to contact the gynostegium between corona lobes, the correct spot to perform pollination. Because the surfaces of these structures are slippery, the noctuid legs are caused to slide directly into the entrance of the guide rail. After entering into the

**Fig. 2** Identification of secretory structures and anatomical sections of *Marsdenia megalantha*. **a** Cross and **b** longitudinal sections of the structure formed by corona and gynostegium. **c, d** Identification of secretory areas with neutral red, lateral and inferior view, respectively. Cross section showing **e** position and **f** detail of the stigmatic chamber with **g** stigmatic nectary. Longitudinal section showing **h** position and **i** detail of the nectar chamber with **j** nectarioles. Dotted arrows indicate section planes, and dotted frames indicate structure in detail. (Abbreviations: *as* anther skirt; *cc* corpuscular chamber; *cl* corona lobe; *cp* corpusculum; *gr* guide rail; *nc* nectar chamber; *nt* nectarioles; *ns* stigmatic chamber nectary; *ov* ovary; *pl* pollinium; *pt* petal; *sc* stigmatic chamber; *hs* stigma head; *sn* stigmatic nectary; *st* style; *tn* nectar tissue)

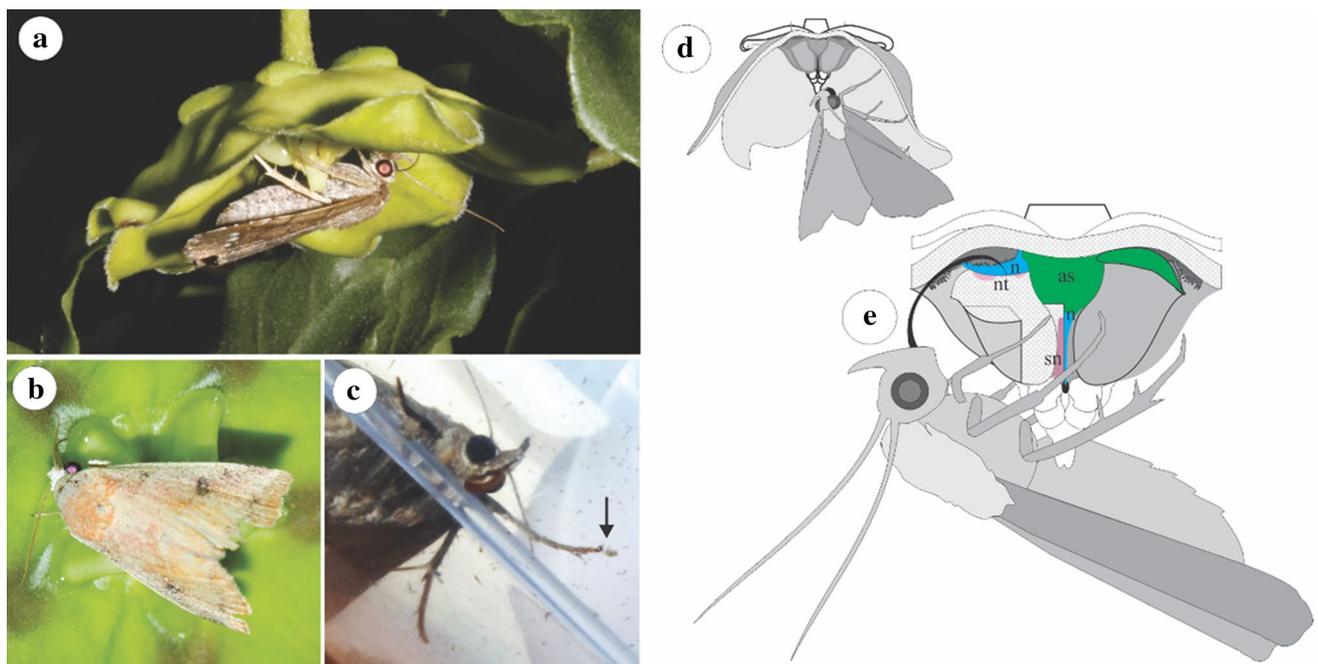


rail, the leg needs to continue sliding along the guide rail to the upper end to be released. In an attempt to release the leg from the guide rail, the noctuids direct the tarsal claw into the corpuscular chamber which is continuous with the corpusculum. Then, the corpusculum clings onto the tarsal claw and finally is set free by pulling out the whole pollinarium. For pollinium deposition, the mechanism works in the same way, but instead of the leg being insert, one pollinium is

completely inserted in the guide rail and the pollinium is set free once it is cut off from the caudicle.

### Records and experiments of the mechanical and secretory function of anther skirt

When sliding the noctuid legs experimentally over the anthers skirt, we see that their slope between the corolla



**Fig. 3** Noctuid pollinators of *Marsdenia megalantha* and its behavior of visit. **a** Sp. 1. **b** Sp. 2. **c** Detail of the attachment site of the pollinarium (arrow) in the body of the noctuid. **d** Arrival of the noctuid to the flower leaning in the rough corolla. **e** Position assumed to access the nectar, here one corona lobe is longitudinally sectioned and blue

areas indicate the nectar, green areas indicate the anther skirt, and pink areas indicate nectariferous tissue: nectariferous (light pink) and stigmatic nectary (dark pink) (Abbreviations: *as* anther skirt; *n* nectar; *nt* nectariferous; *sn* stigmatic nectary)

lobes and their smooth surface favor the legs to slip easily toward the entrance of the guide rail. When the skirt is removed with a scalpel and the noctuid legs are experimentally slid over the gynostemium as above, the legs get stuck in the  $\sim 70^\circ$  corner between the corona lobes, thus making it difficult to reach the guide rail.

We observed throughout anthesis a small and immeasurable amount of nectar secretion in the inner part of the stigmatic chamber. In the skirt chamber, nectar was present only at night, when the droplets started to form and started to emerge from below the skirt at about 07:00 p.m. (Figure 4c). Total nectar amount accumulated until 10:00 p.m. was  $3.4 \pm 1.5 \mu\text{l}$  and concentration  $24.1 \pm 3.4\%$ , and this production is maintained throughout the night. Nectar coloring showed that there is no communication between the anther skirt chambers and stigmatic chambers, since there was no spreading of the dye between them (Fig. 4a, b).

### Pollination effectiveness

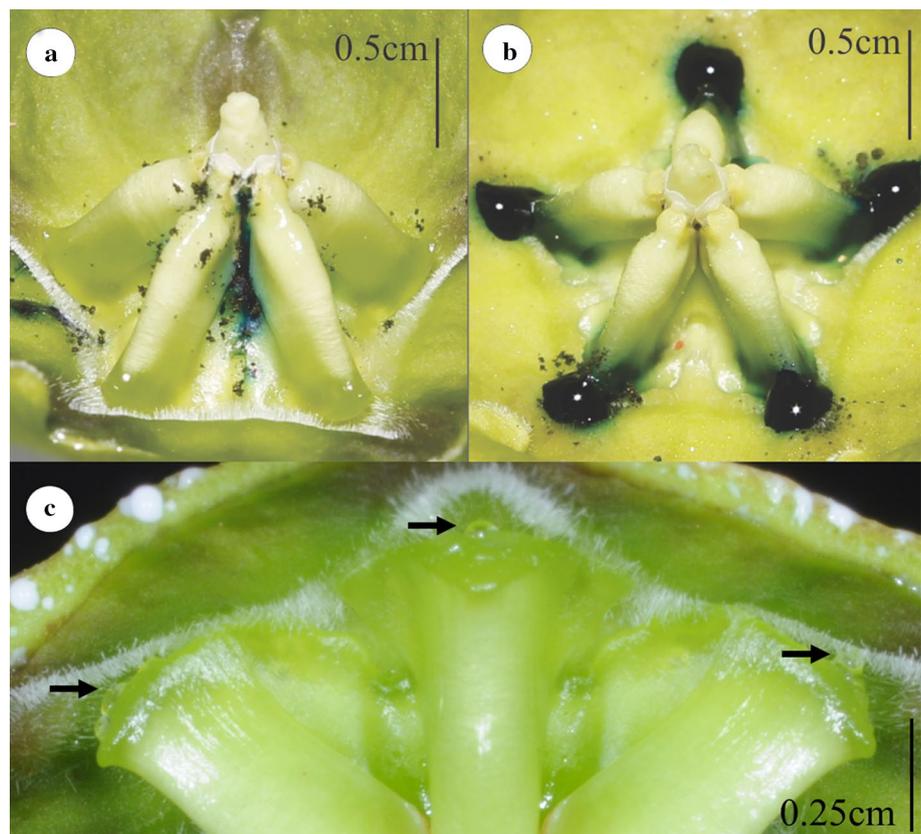
We found 29% of the 120 flowers with one to three pollinaria removed and 5% of the flowers had one pollinium deposited. In total, 58 pollinaria had been removed and six pollinia deposited, resulting in a deposition/removal ratio of about 1/10.

Fruit set under natural pollination is 6% of the flower number, and this is consistent with the proportion of pollinated flowers in the population ( $\chi^2 = 296$ ,  $gl = 1$ ,  $p = 0.5866$ ). We verified in the field that fruits formed by two carpels were atypical. The fruits formed by a single carpel had  $121 \pm 20$  seeds, and this equals to the number of ovules ( $135 \pm 26$ ) per carpel ( $t = 2.11$ ;  $gl = 11$ ;  $p = 0.058$ ). There was no fruit set due to spontaneous self-pollination.

### Discussion

The present results show the role of the anther skirt in *M. megalantha* and its nectaries (nectariferous tissue inside the nectariferous chambers) in relation to functional specialization to Noctuidae pollination. The skirt acts aligning the pollinator, guiding its legs into the guide rail to the slippery surface and producing additional nectar at night. This is due to its particular morphology and anatomy related to its ability to both secrete and contain nectar independently (and secondarily) from the stigmatic chamber, the primary nectary. In addition, the anther skirt can be understood in the light of the pollination mechanism as coordinated with the corona and gynostegium to direct the noctuid legs toward the guide rail. While the nocturnal secretion of nectar is an adaptation to nocturnal pollinators, the anther skirt might

**Fig. 4** Coloration experiments with methylene blue after 4 h demonstrating that there is no connection between the two secretory sites of *Marsdenia megalantha*: **a** secretion of the stigmatic chambers and **b** secretion of the nectariferous chambers. **c** Nectar drops starting to form and emerging from below the anther skirt, around 07:00 p.m



guide whatever thin structure of any pollinator into the guide rail. The interaction of this structure with the noctuid moths, confirmed both by field observations and by manipulative experiments, is effective in bringing about pollination.

### Funnel mechanical function of anther skirt

The anther skirt of *M. megalantha* is functionally closely associated with other flower structures essential for pollination: the corona and gynostegium. The corona is a whorl derived from the androecium showing extensive variation in configurations across groups of Asclepiadoideae; the gynostegium represents the organ of pollinarium removal and pollinium deposition (Liede and Kunze 1993; Fishbein 2001). The conservatism in the structure of these flower parts in *M. megalantha* can be explained by their engagement in mechanical aspects of pollination, because they interact equally well with the extremities of insects independent of the kind of insect (Fishbein 2001; Wanntorp et al. 2006; Baranzelli et al. 2014; La Rosa and Conner 2017). The anther skirt leads the noctuid to a favorable position in the flower, because the mechanical match between the bodies of the pollinator and flower is fundamental for a successful pollination (as in Campbell et al. 1996, for hummingbird beaks; Nilsson 1988).

Anther skirts differ in anatomy from the corolla and resembles corona. This may provide clues about its function and its origin. While the corolla of *M. megalantha* has a rough surface, which allows pollinators to move around on the flower without slipping (see Whitney et al. 2009), the anther skirt and corona are massive and rounded due to their spongy parenchyma and their surface smooth due to their thick and flat epidermis. These features make them slippery and help to funnel the insect's extremities toward the rail system. With both field observations and manipulation experiments, we recorded that the anther skirt deflects the legs of noctuids toward the gynostegium. We hold the view that one important effect of the anther skirt in *M. megalantha* is to mechanically guide the pollinator. We believe that the same is possible in the other genera of the Marsdenieae tribe that share this structure with *M. megalantha* (e.g., *Gongronema*, *Gymnema*, *Hoya*, *Sarcolobus*—Kunze and Wanntorp 2008a, b). In this direction, it would be worthwhile to test both how specifically this skirt structure associates to Noctuid pollination and whether or not it works in the same way with other pollinator groups.

### Substitutive nectaries in anther skirt

A disk nectary surrounding the ovary (with nectar at the base of floral tube) is the basal condition in the Apocynaceae

(Thomas 1992; Galetto 1997). In the course of the evolution of the Asclepiadoideae, there was loss of the disk and relocation of the nectar production function to nectariferous chambers inside the gynostegium from where it can be led by capillarity to different floral parts: inside the corona, below or surrounding the gynostegium, etc. (Galil and Zeroni 1965; Christ and Schnepf 1985; Endress and Stevens 2001; Galetto 2006). These stigmatic nectaries are regarded as the primary nectaries in Asclepiadoideae. In *M. megalantha*, we verified a new situation. The secondary nectaries are additional because the primary nectaries are maintained, but the function of production and accumulation of nectar as reward to pollinators occurs only for the secondary nectaries in the anther skirt.

Remarkable substitutive nectaries appear in flowers whose ancestral group lost the functionality of their original nectaries (Vogel 1997). Generally, they arise in non-homologous structures, but have a similar role to the lost past nectaries (Vogel 1997, 1998a, b; Lopes et al. 2002). The secondary nectaries of *M. megalantha* can be treated as nectarioles (i.e., small indefinite areas that produce nectar—sensu Vogel 1998a, b). Thus, we recorded a very particular case of reward production by an another additional substitutive nectary.

Differences in the functioning of these nectaries are the main factor to characterize this substitution, which seems to be advantageous for flower pollination. In most species of Asclepiadoideae, there is a single nectary type in the stigmatic chambers which is responsible both for the secretions that stimulate the pollen germination and for those that serve as a floral reward. To perform these distinct functions, nectar usually flows through capillary ducts to nectar containers in locations of the flower other than where they are produced (Kunze 1997; Galetto 2006; Wiemer et al. 2011; Vieira et al. 2012). In *M. megalantha*, the primary nectar is not used as reward any more, which became the exclusive function of the secondary nectaries under the anther skirt. One possible advantage of separating such functions in different secretory structures is to minimize the danger that germinating pollen tubes are disturbed by foraging insects, since pollen tubes take up to 48 h to reach the ovules (Vieira et al. 2012). Another advantage would be the eventual production of specific compounds in each type of nectar, considering that pollen germination has specific demands that are different or eventually conflicting with pollinator attraction demands (Christ and Schnepf 1985; Wunnachit et al. 1992).

### Noctuid-specialized pollination

Despite their high floral complexity, Asclepiadoideae include species with different levels of generalization in the pollination system. This occurs in phenotypic, functional and ecological terms (Ollerton and Liede 1997; Ollerton et al.

2007; Wolff et al. 2008). *Marsdenia megalantha* attracts only nocturnal visitors due to evident phenotypic specializations. On the other hand, its mechanical adjustment to noctuids evidences functional specialization. Beyond the odor emission at night, the anther skirt and its substitutive nectaries are apparently essential for these levels of specialization, since they control the time of reward availability to the pollinators and the mechanical adjustment to pollinators. Ecological specialization requires the study of greater number of populations to be confirmed.

Caution must be taken extrapolating the specialized pollinating system results of *M. megalantha* to other species of Marsdenieae tribe. However, the features that ensure the pollination by moths in this species are common in the group. This is the case of nocturnal emission of sweet odor, presence of open or campanulate pendulous corollas, fleshy corona and anther skirt with secondary nectaries that intensify nectar production at night (Altenburger and Matile 1988; Pott et al. 2002; Kunst and Wanntorp 2008a).

There are other reliable records of pollination by noctuids in Marsdenieae for *Hoya* (Forster 1992; Mochizuki et al. 2017). In fact, Mochizuki et al. (2017) suggest that the transport of pollinaria on the legs of noctuids is common in the genus due to the morphological similarity between its flowers. Attachment to the proboscis of these lepidopterans may not be the most effective way for pollinaria deposition, considering the fact that the proboscis needs to be adjusted to a floral tube (Barrios et al. 2016). Thus, it is possible that pollinaria attachment on legs in this Marsdenieae is a strategy to ensure pollination effectiveness. We suggest that much of this pattern owes the functionality of the anther skirt, since a floral structure is often maintained in a taxonomic group when it is associated with a group of specialized pollinators (Anderson 1979; Fenster et al. 2004; Fenster and Martén-Rodríguez 2007). In this context, the genus *Hoya* stands out for having the most evident anther skirts of the whole tribe (Kunze and Wanntorp 2008a).

### Pollination efficiency

*Marsdenia megalantha* is an outcrossing species. In addition, since pollen is packaged in only five units per flower (pollinaria), each of which can deliver only two pollen loads (this means only 10 opportunities to donate pollen loads per flower). Pollen transfer between flowers seems to be particularly sensible to efficiency of the pollination mechanism (Cruden 1977; Endress and Stevens 2001). Spontaneous self-pollination seldom occurs in the Asclepiadoideae due to a strong herkogamy which requires pollinators for reproduction (Endress 1994; Webb and Lloyd 1986; Yamashiro and Maki 2005). The post-pollination process is very efficient since the proportion of pollinated flowers is similar to fruit set. However, only one of the two ovaries in the

flower develops as at most only one pollinium is deposited per flower (Vieira et al. 2012).

The pollination efficiency of *M. megalantha* is comparable with other American Asclepiadoideae. Generally, these species present low proportions of pollinated flowers or fruit production (Willson and Rathcke 1974; Liede 1994; Krings 1999; Wolff et al. 2008; Wiemer et al. 2011). In addition, the limitation of resources can affect the reproductive success, a process already recorded for the Caatinga (Aronne and Wilcock 1994; Leite and Machado 2010). In Asclepiadoideae, there is evidence that fruit and seed production are affected by resource limitation (Willson and Price 1980). Therefore, because *M. megalantha* is an Asclepiadoideae of Caatinga, it is expected that it has a low fruit formation.

Due to the high complexity of the Asclepiadoideae flowers, their morphological variation and integration modulate the pollination efficiency (Baranzelli et al. 2014). It is undeniable that the anther skirts and their secondary nectaries are fundamental to the functioning of the system, but so far we do not know how they affect efficiency directly. Future studies comparing fitness among species of the group with different configurations of this structure or the effect of intra-specific variation can be used to answer these questions (La Rosa and Conner 2017).

## Conclusion

If we resort to the metaphor of considering the revolute margins of Marsdenieae anthers as “skirt,” we may further speak of this structure as a “fashion trend” in the functional specialization for pollination in the Marsdenieae tribe. Only eight *Marsdenia* species occur in NE Brazil (Rapini and Pereira, 2011). We present the first pollination system studied in one to them. Here, we draw attention to the function of the anther skirt that promotes a suitable flower–pollinator fit. Moreover, we show for the first time how its substitutive nectarioles allow for a split in nectar functions, i.e., aid for pollen germination and pollinator reward. These characteristics are essential in the specialized noctuid pollination of *M. megalantha*. As anther skirt is recurring in the Marsdenieae tribe (Kunze and Wanntorp 2008a, b), we suggest that similar features may be applicable to them. On the other hand, while the nocturnal secretion of nectar is an adaptation to nocturnal pollinators, the anther skirt might guide whatever thin structure of any pollinator into the guide rail. Therefore, future studies are needed to test how specific this skirt structure really is for Noctuid pollination and whether it would work in the same way for other groups of pollinators. Finally, we emphasize the importance of the study of taxonomic diagnostic floral structures under a functional perspective of pollination, because this type of association

can support the understanding of evolutionary modifications of functional traits in some groups.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest regarding the publication of this article.

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