

# Short flowers for long tongues: Functional specialization in a nocturnal pollination network of an asclepiad in long-tongued hawkmoths

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## Abstract

Since Darwin, very long and narrow floral tubes have been known to represent the main floral morphological feature for specialized long-tongued hawkmoth pollination. However, specialization may be driven by other contrivances instead of floral tube morphology. Asclepiads are plants with a complex floral morphology where primary hawkmoth pollination had never been described. We detailed here the intricate pollination mechanism of the South American asclepiad *Schubertia grandiflora*, where functional specialization on long-tongued hawkmoth pollinators occurs despite the short floral tube of this species. We studied two plant populations in the Brazilian Cerrado and recorded floral visitors using different approaches, such as light-trapped hawkmoths for pollen analysis, direct field observations, and IR motion-activated cameras. Finally, using a community-level approach we applied an ecological network analysis to identify the realized pollinator niche of *S. grandiflora* among the available niches in the pollinator community. Throughout a period of 17 years, long-tongued hawkmoths were consistently recorded as the main floral visitors and the only effective pollinators of *S. grandiflora*. Flowers rely on highly modified corona and gynostegium, and enlarged nectar chambers, to drive visitors and pollination mechanism. Despite its relative short-tube, network analysis placed *S. grandiflora* in the module including exclusively long-tongued hawkmoth pollinators and the most phenotypically specialized sphingophilous plants in the community. These results represent the first example of functional specialization in long-tongued hawkmoths in an asclepiad species. However, this specialization is uncoupled from the long floral tubes historically associated with the sphingophily syndrome.

Abstract in Portuguese is available with online material.

## KEY WORDS

Apocynaceae, Asclepiadoideae, Cerrado, ecological networks, floral specialization, hawkmoth pollination, nectar, pollinator niche

## 1 | INTRODUCTION

Evolution of extremely long floral tubes represents one of the main morphological adaptations of flowers for long-tongued hawkmoth pollination (Amorim et al., 2014; Johnson et al., 2017; Nilsson, 1988, 1998; Wasserthal, 1997). Darwin (1862) correctly predicted that the sexual reproduction of the Malagasy orchid *Angraecum sesquipedale* was dependent on the extremely long floral spur associated with the occurrence of nectar concealed at the bottom of the floral tube. Consequently, the pollination of this plant would rely on a pollinator with a proboscis long enough to reach the nectar inside the deep and narrow flower tube, later confirmed to be a hawkmoth (see Wasserthal, 1997). In fact, long and narrow flower tubes are the most common morphological traits related to highly phenotypically specialized hawkmoth-pollinated species worldwide, and represent a private niche for long-tongued hawkmoths (Johnson et al., 2017). However, floral contrivances may uncouple flower tubes and pollinator tongues, and the realized pollinator niche of some specialized plant species may be driven by other factors instead of floral tube morphology (Amorim et al., 2013; Drewniak et al., 2020; Soteris et al., 2020).

Asclepiadoideae (the asclepiads or milkweeds) are a group of plants with a very complex floral morphology, which comprises the development and evolutionary appearance of novel characters and structures in the history of the Apocynaceae (Fishbein, 2001; Ollerton et al., 2019). Members of the subfamily possess a range of adaptations involving complex synorganization of floral parts, that is, functional structures formed by two or several floral parts intimately associated and connected to each other (*sensu* Endress, 1990, but see also Endress, 2016). This complexity of the floral morphology led to the development of unique structures and pollination mechanisms (e.g. Cocucci et al., 2014; Heiduk et al., 2016; Pauw, 1998), which play a major role as adaptations to particular guilds of pollinators (Ollerton et al., 2003; Ollerton & Liede, 2003; Wiemer et al., 2012). One of the main distinguishing adaptations in this clade is the presentation of pollen in paired masses (pollinia) together with ancillary structures (collectively the pollinarium) that mechanically clip to the body of a pollinator. This represents a separate, parallel evolution to the orchids (Orchidaceae) and allows researchers to accurately distinguish between floral thieves, who steal nectar from flowers, and true pollinators (Ollerton & Liede, 1997).

Despite being pervasive in many angiosperm groups, pollination by hawkmoths is extremely rare among asclepiads (Ollerton et al., 2019). Most asclepiad species from both New and Old Worlds that have been studied so far are pollinated by flies (Diptera), or bees and wasps (Hymenoptera), while pollination by butterflies and moths (Lepidoptera) is much less common in this subfamily (Ollerton et al., 2019). Although butterflies, and less frequently settling moths, have been recorded as pollinators of some asclepiad species (Mochizuki et al., 2017), in most cases they are part of a diverse array of floral visitors and do not act as primary pollinators (Ollerton et al., 2019). Primary pollination by long-tongued hawkmoths has never been described for the Asclepiadoideae (see Johnson et al., 2017; Ollerton et al., 2019). In contrast, hawkmoth pollination, including long-tongued hawkmoths, is common

in the less derived Rauvolfioideae and Apocynoid grades within Apocynaceae (Ollerton et al., 2019).

In this study we describe a novel and intricate morphological adaptation for long-tongued hawkmoth pollination in the South American asclepiad *Schubertia grandiflora* Mart. (Asclepiadoideae, Gonolobinae), which operates despite the relatively short floral tube. Furthermore, since ecological interaction networks are a useful tool to help identify the realized pollination niche of a given species in relation to all available pollination niches in the community (Amorim, 2020; Phillips et al., 2020), we used information about the community-level interactions between hawkmoths and plants to test whether this plant species exhibits niche specialization for long-tongued hawkmoths.

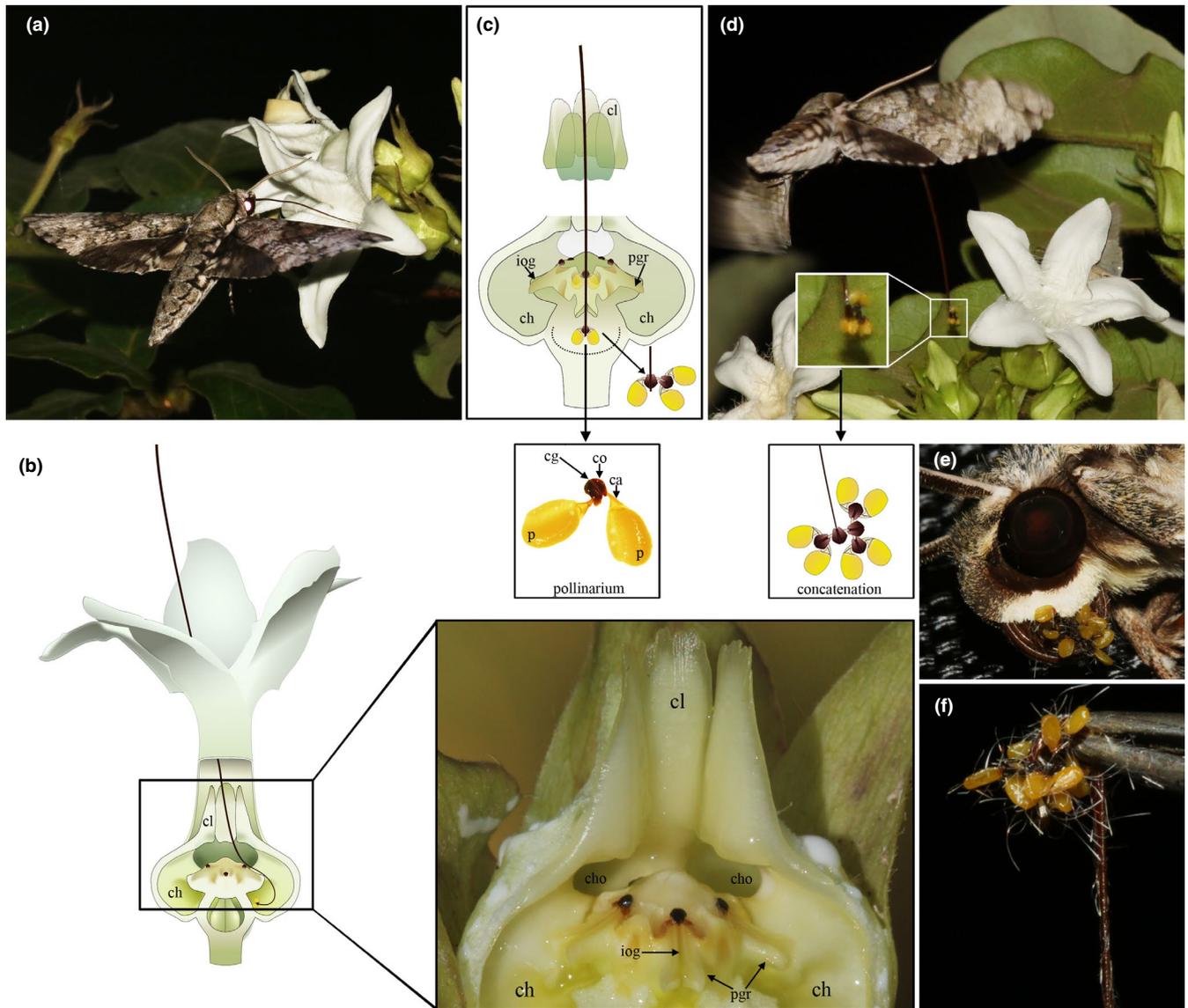
## 2 | METHODS

### 2.1 | Study sites and species characterization

This study was carried out in two Cerrado areas (Neotropical savanna of Central Brazil) in the municipality of Uberlândia, Minas Gerais state, southeastern Brazil: Panga Ecological Station (19°09'20" S, 48°23'20" W) and Glória Experimental Farm (18°51'25" S, 48°13'47" W). Observations were carried out irregularly between 2003 and 2020. *Schubertia grandiflora* is a heliophyte vine that grows in open areas and blooms from December to February. Flowers are whitish, pentamerous, and possess a corolla fused at the base forming a short tube (Figure 1a–c). The base of the corolla tube is widened, forming five large independent nectar chambers (Figure 1b; see also the 3-D microtomography reconstruction in Video S1).

### 2.2 | Floral biology and functional mechanism of pollination

Floral anthesis was observed in 40 marked flowers in five plants (10 flowers per plant), following the flowers from opening until senescence, when the corolla started becoming dark and scent production (from the perspective of human olfaction) ceased. Flower tube length was measured as the distance between the corolla tube opening and the base of the nectar chamber using a digital caliper (error 0.1 mm) in four plants (totaling 31 flowers). We measured the accumulated nectar volume of 15 newly open unvisited flowers bagged for about 16 hours using a graduated (50 µl) syringe (Hamilton, NV, USA), and sugar concentration was measured with a pocket refractometer (0–90%; Instrutherm - RT-280). These flowers were bagged as soon as they started opening in the evening, during which we also measured the standing crop of nectar (volume and sugar concentration) in 12 additional flowers naturally exposed to flower visitors during the previous nights. To understand the functional pollination mechanism, we simulated the interaction between the pollinator tongue and flower using a human hair (following Cocucci et al., 2014). With aid of a forceps, we used an eyelash to extract the pollinarium and then inserted a single pollinium into the stigmatic chamber, which allowed us to observe in detail the pollinarium concatenating one to another (see Video S2).



**FIGURE 1** Morphology and functional pollination mechanism of *Schubertia grandiflora*. (a) The long-tongued hawkmoth, *Manduca florestan*, with the proboscis inserted into *S. grandiflora* flower tube. This moth species is the main pollinator of *S. grandiflora*. (b) A schematic representation of the tip of the moth's proboscis inside the nectar chamber (ch) contacting the gynostegium and with pollinarium attached to the thin tip of the proboscis. Note in the detail a flower with the corolla and part of the corona removed exposing the three of the five corona lobes (cl), two nectar chamber openings (cho), the long prolongation of the guide rail (pgr), and the inner opening of the guide rail (iog). Also, note the intersection between the adjacent corona lobes forming a groove that vertically coincides with the nectar chamber opening. (c) A schematic representation of the pollinator proboscis guided by the intersection of the corona lobes to the coincident prolongation of the guide rail, which eventually conducts the proboscis to the corpusculum, where it gets attached to the pollinarium. Note below the detail of an intact pollinarium showing the corpusculum (co), the corpusculum groove (cg), the two caudicle (ca), and the two pollinia (p). (d) A *M. florestan* after withdrawing the proboscis from the flower tube with three intact concatenated pollinaria (inset) attached to the tip of the proboscis. The detail below shows the schematic representation of the concatenated pollinaria observed in the image, where five different pollinaria are attached to the pollinator's proboscis. (e) A *M. florestan* netted while visiting *S. grandiflora* with a chain-like concatenated pollinaria attached to the proboscis. (f) The detail shows a large number of concatenated pollinaria (at least 10)

### 2.3 | Floral visitors and pollinators

Indirect determination of *S. grandiflora* floral visitors was made through pollen analyses of light-trapped hawkmoths (see Sazatornil et al., 2016). Hawkmoths were monthly sampled at Panga Ecological Station from August 2003 to July 2004 (Amorim et al., 2009). We analyzed the pollen load from each sampled hawkmoth ( $n = 267$ ), and used a digital

caliper (error 0.1 mm) to measure the length of their proboscises. We built a reference pollen collection from the studied area, and used pollen morphology analyses to identify the plant species visited by hawkmoths in the community.

For direct determination of *S. grandiflora* floral visitors, we also carried out field observations of floral visitors during the blooming seasons of 2013, 2016, 2018, 2019 and 2020, totaling about

40 hours of observations. Additionally, in January 2020 we used an IR motion-activated camera (Bushnell Nature View camera, model 119740, Bushnell Corporation, Overland Park, Kansas; see Johnson et al., 2020) to record hawkmoth visitation in *S. grandiflora* during four consecutive nights, totaling 48 hours.

## 2.4 | Pollinator niche

To test whether *Schubertia grandiflora* explore a similar pollinator niche as the phenotypically specialized (i.e., species with a functionally specialized pollination system, *sensu* Ollerton et al., 2007) sphingophilous species in the community, we used the pollen loads found on the hawkmoth community to build a weighted hawkmoth-plant interaction network. Hawkmoth-plant interactions were compiled into a quantitative matrix, where rows (*i*) represent plant species and columns (*j*) represent hawkmoth species. The quantitative matrix was weighted by the interaction frequency for each pair of species ( $a_{ij}$ ), as the number of individual hawkmoths *j* carrying pollen from a given plant species *i* (Sazatornil et al., 2016). Then, we used modularity analysis to identify the realized pollinator niche of *S. grandiflora* among the available pollination niches in the community (as in Amorim, 2020; Phillips et al., 2020). Despite *S. grandiflora* blooming only from December to February, we considered the whole year for modularity analysis because hawkmoth species interacting with *S. grandiflora* also occur in other months interacting with several plant species throughout the year.

We used the weighted modularity index ( $Q_w'$ ), which calculates whether species from the same subset interact more with each other than with species composing other subsets in the hawkmoth-plant community. The modularity index was estimated using the DIRTLPAwb+algorithm (Beckett, 2016). Because the value of  $Q_w'$  might vary among sequential algorithm runs, we ran the algorithm 20 times to find the module conformation with the highest value of  $Q_w'$ , and set the number of Markov chain Monte Carlo moves to  $10^{10}$  (Dormann & Strauss, 2014; Maruyama et al., 2018). Additionally, to test whether the lengths of the hawkmoth proboscises differ among modules resulting from the modularity analyses, we performed a one-way analysis of variance (ANOVA), and used the Tukey *post hoc* test for multiple comparisons of means among pairs of modules.

## 3 | RESULTS

### 3.1 | Floral biology and functional mechanism of pollination

*Schubertia grandiflora* flowers have a corolla fused at the base forming a short hypocateriform floral tube averaging  $2.26 \pm 0.18$  cm ( $\bar{x} \pm \sigma$ ) in depth, with lobes covered sparsely by trichomes on the distal and internal part (Figure 1a,b,d). Flower opening occurred in the evening between 18:00h and 20:00h, followed by a strong sweet scent production detected by human olfaction, and flowers remain open for four to five days. Scent production occurred exclusively



**FIGURE 2** Two recently open *Schubertia grandiflora* flowers showing the trichomes, as well as the shining and wet appearance of the corolla lobes where flower scent is released

during the night at the corolla lobes at the region of the trichomes, which became markedly wet and shining (Figure 2). Accumulated nectar production in the five nectar chambers varied between 46.7 to 160.0  $\mu\text{l}$  ( $\bar{x} = 83.1 \pm 30.53 \mu\text{l}$ ) with a mean sugar concentration of  $13.7 \pm 1.84\%$ . Standing crop of nectar varied from 1.8 to 24.8  $\mu\text{l}$  ( $\bar{x} = 9.75 \pm 6.98 \mu\text{l}$ ) with mean sugar concentration of  $13.5 \pm 3.43\%$ .

The functional mechanism of pollination depended on the nectar chambers that form five independent pollination sectors (Figure 1b,c, Video S2). These chambers are partially isolated by the development of spongy tissue in the corona that encloses the corolla against the base of the gynostegium (Figure 1b). The corona is fused with the corolla tube at the base of the gynostegium and they differentiate only toward the staminal part where corona lobes are prolonged and rise above the gynostegium (Figure 1b). The tube formed by the corolla is internally surrounded by the five corona lobes, and the intersection between two adjacent lobes forms a groove that vertically coincides with a single nectar chamber, leading a pollinator's proboscis to one of the chamber openings (Figure 1b). A pollinator's proboscis is unable to pass laterally from one chamber to another, thus the proboscis must be entirely withdrawn from the nectar chamber before it can be inserted again into another.

Each chamber opening coincides exactly with one of the five guide rails that lead to a pollinarium, with the stigmatic surface hidden below. The pollinarium is positioned at the proximal end of the guide rail, which guides the pollinator proboscis to the corpusculum groove that mechanically attaches the pollinarium to the proboscis (Figure 1a–c). The simulation of pollinarium extraction and pollinium deposition using a human hair revealed that when the proboscis enters the nectar chamber, the inner opening of the prolongation of

the guide rail captures the tip of the proboscis, leading it toward the pollinarium (video S2). The thin tip of the proboscis then gets attached to the corpusculum groove, removing the pollinarium as the insect retracts its proboscis. Pollination occurred when a proboscis with a pollinarium attached to its tip is inserted again into another nectar chamber, leading a single pollinium to the stigmatic sector, where it breaks off the caudicle. The remaining caudicle may then attach to the corpusculum groove of a new pollinarium, concatenating one to another (Video S2, Figure 1c–e). The concatenation process can proceed sequentially until the concatenated pollinaria form a large chain (Figure 1c–f). Our experimental pollinarium removal and concatenation (Video S2) revealed the need of the pollinator to exert a force in order to release the proboscis after it gets attached to the corpuscle (see also Video S3).

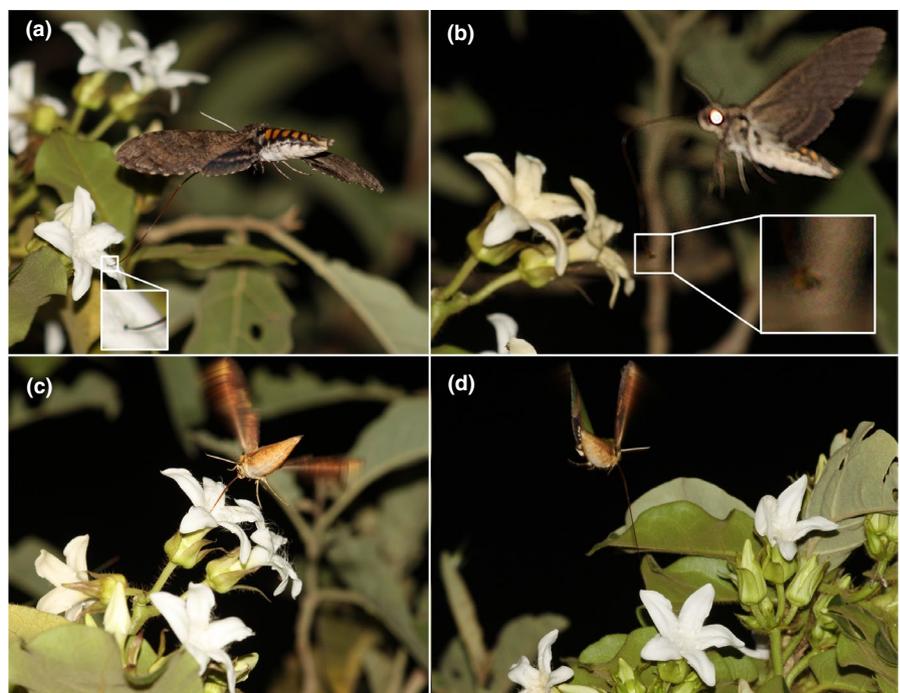
### 3.2 | Floral visitors and pollinator niche

Throughout a period of 17 years of observations, we recorded five hawkmoth species visiting *Schubertia grandiflora*: *Cocytius lucifer* (proboscis length  $9.2 \pm 0.7$  cm), *Manduca diffissa* ( $7.5 \pm 1.1$  cm), *M. florestan* ( $6.1 \pm 0.5$  cm), *Protambulyx strigilis* ( $2.9 \pm 0.3$  cm) and *Xylophanes chiron* ( $4.7 \pm 0.2$  cm). Only three light-trapped individuals belonging to three of the 24 species, totaling 103 hawkmoth individuals collected between December 2003 and February 2004 were carrying *Schubertia grandiflora* pollinaria. However, only the two long-tongued species, *C. lucifer* ( $n = 6$  light-trapped individuals) and *M. florestan* ( $n = 2$ ) were carrying concatenated pollinaria attached to the proboscis, revealing effective pollinium deposition into the stigmatic sector (Figure 1c–e). Of 13 light-trapped individuals of *P. strigilis*, only one was carrying a single intact pollinarium suggesting that it did not act as an actual pollinator, despite being the most abundant hawkmoth species

in this community (Amorim et al., 2009). During focal observations between 2013 and 2020 we recorded four hawkmoth species visiting *S. grandiflora* flowers: *C. lucifer* ( $n = 2$ ), *M. diffissa* ( $n = 4$ ), *M. florestan* ( $n = 20$ ) and *X. chiron* ( $n = 5$ , Figure 3). However, *X. chiron* was never recorded carrying any pollinaria, thus, it possibly acts only as a nectar thief such as the short-tongued *P. strigilis*. Settling moths were also commonly observed visiting *S. grandiflora* flowers, but their proboscises were too short (about 1.0 cm long) to reach the nectar chamber. Thus, these moths only accessed the nectar accumulated in the mouth of the floral tube. *Manduca florestan* was the most common flower visitor, accounting for about 65% of total recorded hawkmoth visits, and 76% of the long-tongued hawkmoths. It was also the only species recorded (by pollen analysis, cameras and direct observations) visiting *S. grandiflora* flowers throughout the entire study period, and carrying concatenated pollinaria attached to the proboscis (Figure 1, Video S3). Our observations also revealed that only some of the visits made by long-tongued hawkmoths resulted in pollinarium removal (Video S3).

Community-level analysis of the hawkmoth-plant interactions revealed a modular structure of the network ( $Q = 0.32$ ,  $p < .001$ ) with five distinct modules (Figure 4a). *Schubertia grandiflora* belonged to the module that included exclusively long-tongued hawkmoth species (Figure 4a), and the most phenotypically specialized sphingophilous plants in the community. ANOVA showed significant differences in proboscis lengths among modules ( $F_{4,34} = 16.02$ ,  $p < .0001$ ). In addition, the Tukey *post hoc* test showed that the module of which *S. grandiflora* belongs also harbors hawkmoth species with the longest proboscises in the community (Figure 4b). Thus, the short-tubed *S. grandiflora* occupied the same realized pollinator niche as *Tocoyena formosa* and *Hedychium coronarium*, two phenotypically specialized plant species with long hypocrateriform corolla tubes ( $>7.0$  cm) that rely on long-tongued hawkmoth species for pollination (Figure 4a).

**FIGURE 3** Floral visitors (pollinators and nectar thieves) of *Schubertia grandiflora*. (a) The long-tongued *Manduca diffissa* approaching the flower with a corpusculum without pollinia attached to the tip of the tongue, suggesting pollinia deposition and pollination. (b) Another individual of *M. diffissa* approaching the flower with concatenated pollinaria attached to the proboscis. (c) and (d) *Xylophanes chiron*, a hawkmoth species with a medium-size proboscis ( $4.7 \pm 0.2$  cm) visiting the flowers without removing any pollinaria. Our focal observations along different years showed that this moth is the second most common hawkmoth visiting *S. grandiflora* flowers, but it acts only as nectar thief, since this moth species was never recorded with pollinaria attached to the proboscis



## 4 | DISCUSSION

The small length of the corolla tube means that *Schubertia grandiflora* can interact with most hawkmoth species in the studied community. However, it is surprising that the realized pollinator niche of *S. grandiflora* relies exclusively on long-tongued hawkmoths, similarly to the phenotypically specialized sphingophilous plants. Morphological match and phenology are some of the main determinants of hawkmoth-plant interaction networks (Lautenschleger et al., 2020; Sazatornil et al., 2016). Nevertheless, plants belonging to the module reflecting the long-tongued hawkmoth pollinator niche are not blooming simultaneously in the study area. Similarly, long-tongued hawkmoth species recorded visiting *S. grandiflora* occur over a longer period than the blooming season of this plant species. In this sense, other factors such as the amount of nectar may also influence modularity in this hawkmoth-plant interaction network. Hawkmoths, including long-tongued species, form mixed modules with bats because they are not restricted to the typical sphingophilous flowers (see Queiroz et al., 2021) and frequently visit bat-pollinated species, such as *Caryocar brasiliense*, which belongs to the same module of *S. grandiflora* and flowers produce copious amounts of nectar. Hence, the highly modified corona and gynostegium, and the presence of enlarged nectar chambers harboring unusually high amounts of nectar for an asclepiad species, may be essential traits driving long-tongued pollinators to *S. grandiflora*.

In species of the subfamily Asclepiadoideae, corona, gynostegium, and pollinarium morphologies play a very important role in determining the functional mechanism of pollination, as well as the pollinators of a given species (Ollerton et al., 2003; Wiemer et al., 2012). Asclepiads present complex processes of synorganization that led to the development of unique structures (Endress, 1994), which have surprised naturalists since early observations of these plants (Brown, 1833; Sprengel, 1793). Even nowadays, despite the increasing number of studies about Asclepiadoideae pollination (see Ollerton et al., 2019 and references therein), the complexity of its floral morphology has been still revealing different pollination system and functional mechanisms (Cocucci et al., 2014; Heiduk et al., 2016; Pauw, 1998; Xiong et al., 2020). The mechanism of pollinaria concatenation observed in *S. grandiflora* may be an adaptation to export more pollinaria (Coombs et al., 2012). However, the concatenation could affect individual fitness, because it can reduce the reproductive performance of the proximal pollinaria by means of interference among the concatenated pollinaria (Cocucci et al., 2014).

Specialization for pollination by Lepidoptera, particularly hawkmoths, is very uncommon among Asclepiadoideae, in which primary

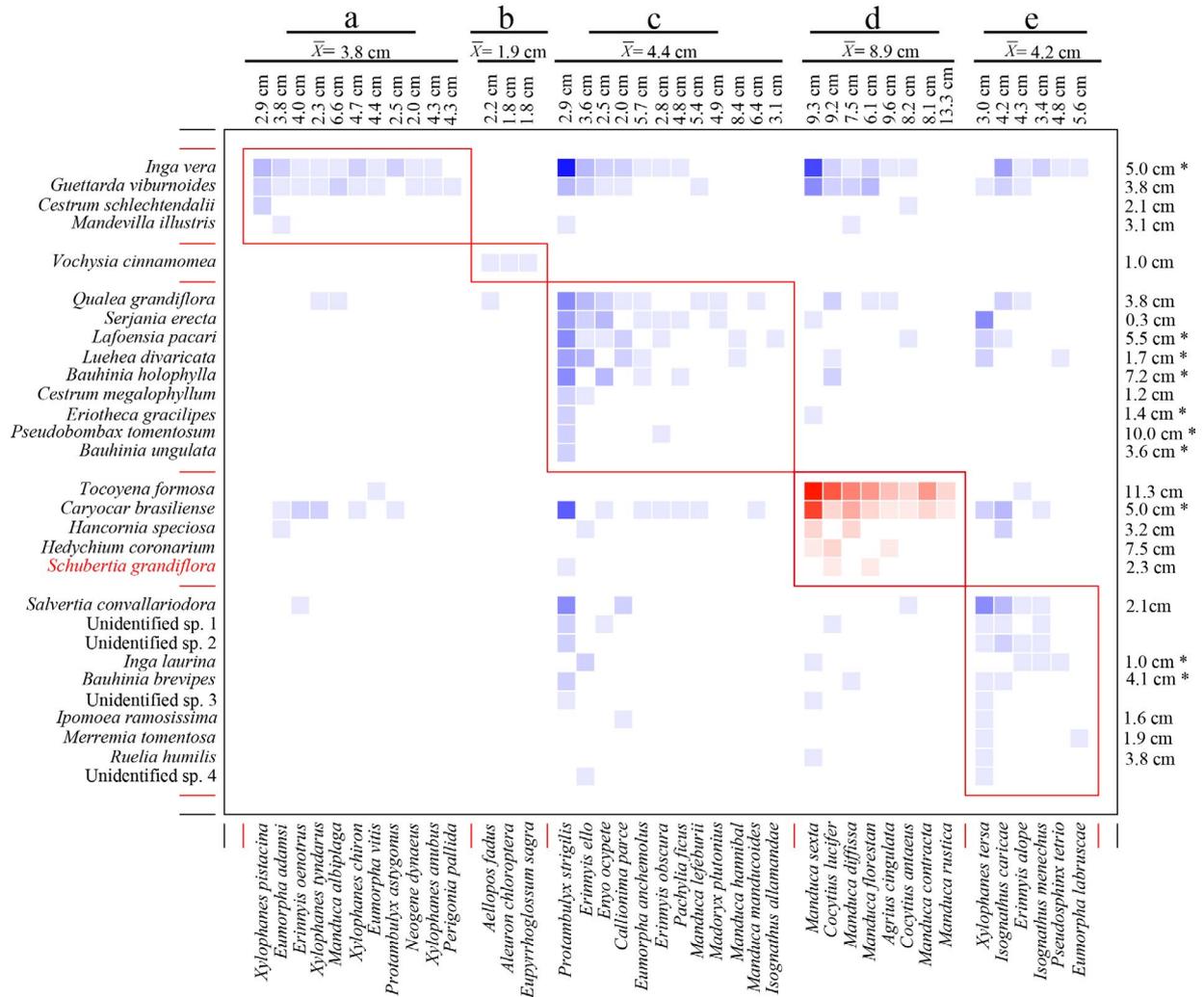
long-tongued hawkmoth pollination has never been described before (Ollerton et al., 2019). Possibly due to developmental constraints, the subfamily Asclepiadoideae lacks species with long corolla tubes typical of phenotypically and functionally specialized sphingophilous plants, as observed in less derived groups within the family Apocynaceae, such as Rauvolfioideae and Apocynoid grades (Ollerton et al., 2019). Besides *S. grandiflora*, occasional hawkmoth pollination has been recorded only in four species: *Asclepias syriaca* (Morse, 1982) and *A. incarnata* (Ivey et al., 2003) in North America; *Cryptostegia madagascariensis* in Madagascar (Walther, 1994) and *Pergularia daemia* in India (Bhatnagar, 1986). However, in spite of these records of short-tongued hawkmoths visiting and putatively pollinating a few generalist asclepiad species, *S. grandiflora* is the first asclepiad that has been shown to rely on the long-tongued hawkmoth pollinator guild. Notwithstanding this, we cannot rule out the possibility of other short- or medium-size-tongued hawkmoths to act as pollinators of *S. grandiflora* in other parts of its distribution in South America. As in our study sites in the Brazilian Cerrado, previous observations of this plant in Argentina have also reported both short- and long-tongued hawkmoth species visiting *S. grandiflora*, but with no information on actual pollination (Ollerton et al., 2019; Schulz, 1937). The short corolla tube of *S. grandiflora* does not impose any restriction to short-tongued moths from visiting the flowers and reaching the accumulated nectar. However, it is important to note that the evidence that only long-tongued hawkmoths act as actual pollinators of *S. grandiflora* in our study sites occurs even though this group of moths is less abundant and less species-rich than the short-tongued species occurring in the studied community (Amorim et al., 2009) and in the Cerrado biome as a whole (Camargo et al., 2018).

Despite the short-tube length, the amount of nectar produced by *S. grandiflora* flowers is unusual for the Asclepiadoideae subfamily, being as high as the amount of nectar produced by deep flowered specialized sphingophilous plants worldwide (Johnson et al., 2017). Nectar volume accumulated in *S. grandiflora* flower chambers is much higher than that observed in other asclepiad species, which is typically less than 1 $\mu$ l even after 24 hours accumulation (Ollerton et al., 2003), rendering a large amount of reward to the long-tongued moths. In hawkmoths there is a close relationship between body mass and tongue-length (Agosta & Janzen, 2005). Hence, long-tongued species also have a greater energetic demand than the short-tongued ones. Also, it is likely that the robust body size of the long-tongued hawkmoths may help these moths to remove the pollinarium and deposit the pollinium into the guide rail, which results in pollination and pollinaria concatenation. The need of the pollinator

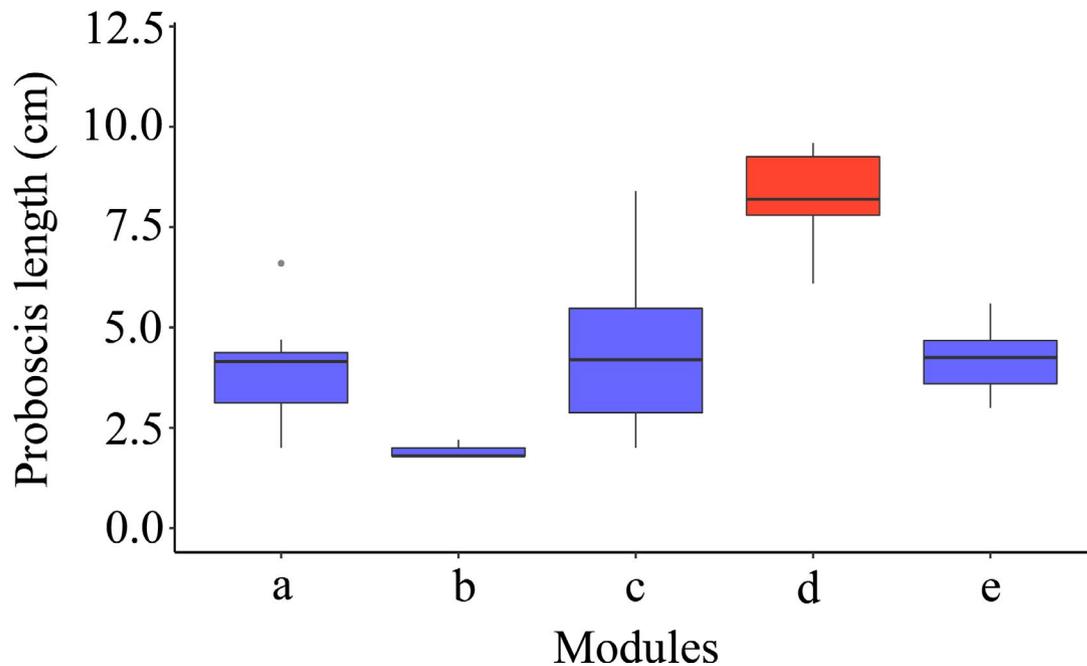
**FIGURE 4** Community-level analyses of hawkmoth-plant interactions. (a) Module conformation of the hawkmoth-plant interaction network. Numbers on the right side of each line and above the columns represent lengths of the flowers and hawkmoth proboscises, respectively. Asterisks represent plants with brush-flowers, of which flower length was assessed based on the size of the stamens and pistils. (b) Variation in the mean proboscis length of hawkmoths among the modules in the interaction network observed in a. Different colors in boxes represent significant differences between pair of modules by Tukey *post hoc* test ( $p < .0001$ ). Note that *Schubertia grandiflora* belongs to the module highlighted in red in a, which includes only long-tongued hawkmoths (red box in b) and the most phenotypically and functionally specialized hawkmoth-pollinated plants in the community

(a)

Modules



(b)



to exert a force in order to release the proboscis after it gets attached to the corpuscle, may suggest that long-tongued and heavier hawkmoths are less prone to get stuck to the flower, as reported earlier in the genus *Asclepias* (Frost, 1965; Robertson, 1928), and in the “mothcatcher” asclepiad *Araujia sericifera* (Coombes & Peter, 2010), though, we never recorded any moth stuck in *S. grandiflora* flowers. It is known, however, that the pollination mechanism of asclepiads can trap even the main pollinators, causing damage of their mouthparts (Shuttleworth & Johnson, 2009).

The requirement of pollinators to exert a force to remove the pollinarium highlights that trait matching may still occur in this system (as in Córdoba & Cocucci, 2011), though it is not obvious as observed in phenotypically specialized plants typically occupying the long-tongued hawkmoth pollinator niche (e.g. Johnson & Raguso, 2016). The complexity of the floral morphology of asclepiads emphasizes the importance of studying multiple traits (e.g. Castellanos et al., 2004) to better understand the pollination mechanisms of this group of plants. But given the higher abundance and species richness of short-tongued hawkmoths in relation to the long-tongued species, what should be the advantage of the evolutionary specialization (*sensu* Fenster et al., 2004) of *S. grandiflora* in this small subset of pollinators? Long-tongued hawkmoths are long-living insects that can fly over large distances (as long as 15–20 km, see Amorim et al., 2014) or even migrate (Janzen, 1987), which favors pollen flow among different plant populations. Also, by offering amounts of nectar as large as plant species with long corolla tubes, *S. grandiflora* encompass the high foraging costs of the heavier and long-tongued hawkmoth species granting higher pollinator fidelity. Despite flower visitation by long-tongued hawkmoths can be quite infrequent (e.g., Amorim et al., 2014), these moths were reliable flower visitors of *S. grandiflora* over the entire period of study.

To conclude, in this study we have presented the first case of specialization in long-tongued hawkmoth pollination in an asclepiad species, and a specialization uncoupled from the usual long floral tubes observed in the sphingophily syndrome, and from the morphological match that rules hawkmoth-plant community-level interactions (Sazatornil et al., 2016). After our observations over more than one and a half decades of long-tongued hawkmoths consistently acting as primary pollinators (particularly *Manduca florestan*) of this short-tubed species, we conclude that *S. grandiflora* is functionally specialized on long-tongued hawkmoths, but the exact mechanism by which the more abundant short-tongued hawkmoths are prevented from acting as pollinators of this asclepiad species is not yet clear. Probably other specific attractants yet to be disclosed, such as flower scent or nectar chemistry, besides a large amount of nectar production, assure higher fidelity among large-sized hawkmoths.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

FWA did most of the fieldwork for more than 15 years, set up the study framework, and the quantitative analyses; SM described the flower morphology and provided the illustration drawings; PASV helped to describe flower morphology and to organize the data; JO provided the overview on Asclepiadoideae floral organization and pollination, and also reviewed English writing and style; PEO helped with the fieldwork, nectar measurements and visitors recording. FWA and PEO led the manuscript writing, but all authors collaborated with manuscript writing and organization.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.np5hqbzqv> (Amorim et al., 2022).

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## REFERENCES

- Agosta, S. J., & Janzen, D. H. (2005). Body size distributions of large Costa Rican dry forest moths and the underlying relationship between plant and pollinator morphology. *Oikos*, 108, 183–193. <https://doi.org/10.1111/j.0030-1299.2005.13504.x>
- Amorim, F. W. (2020). Are the New World hummingbird-hawkmoths functional equivalents of hummingbirds? *Ecology*, 00, e03161. <https://doi.org/10.1002/ecy.3161>
- Amorim, F. W., Avila, R. S. Jr, Camargo, A. J. A., Vieira, A. L., & Oliveira, P. E. (2009). A hawkmoth crossroads? Species richness, seasonality and biogeographical affinities of Sphingidae in a Brazilian Cerrado. *Journal of Biogeography*, 36, 662–674. <https://doi.org/10.1111/j.1365-2699.2008.02033.x>

- Amorim, F. W., Galetto, L., & Sazima, M. (2013). Beyond the pollination syndrome: nectar ecology and the role of diurnal and nocturnal pollinators in the reproductive success of *Inga sessilis* (Fabaceae). *Plant Biology*, 15, 317–327.
- Amorim, F. W., Wyatt, G. E., & Sazima, M. (2014). Low abundance of long-tongued pollinators leads to pollen limitation in four specialized hawkmoth-pollinated plants in the Atlantic Rain forest, Brazil. *Naturwissenschaften*, 101, 893–905. <https://doi.org/10.1007/s00114-014-1230-y>
- Amorim, F. W., Marino, S., & Sanz-Veiga, P. A., Ollerton, J., & Oliveira, P. E. (2022). Dataset for: Short flowers for long tongues: Functional specialization in a nocturnal pollination network of an asclepiad in long-tongued hawkmoths (Biotropica). *Dryad Digital Repository*. <https://doi.org/10.5061/dryad.np5hqzvzq>
- Beckett, S. J. (2016). Improved community detection in weighted bipartite networks. *Royal Society Open Science*, 3, 140536. <https://doi.org/10.1098/rsos.140536>
- Bhatnagar, S. (1986). On insect adaptations for pollination in some asclepiads of Central India. In: R. P. Kapil (ed.), *Pollination biology – an analysis* (pp. 37–57). New Delhi, India: Inter-India Publications.
- Brown, R. (1833). On the organs and mode of fecundation in Orchideae and Asclepiadeae. *Transactions of the Linnean Society of London*, 16, 685–738.
- Camargo, A. J. A., Camargo, W. R. F., Corrêa, D. C. V., Vilela, M. F., & Amorim, F. W. (2018). *Mariposas polinizadoras do Cerrado: identificação, importância e conservação*. Embrapa Cerrados, Planaltina, Distrito Federal, Brazil.
- Castellanos, M. C., Wilson, P., & Thomson, J. D. (2004). 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, 17, 876–885. <https://doi.org/10.1111/j.1420-9101.2004.00729.x>
- Cocucci, A. A., Marino, S., Baranzelli, M., Wiemer, A. P., & Sêrsic, A. (2014). The buck in the milkweed: evidence of male–male interference among pollinaria on pollinators. *New Phytologist*, 203, 280–286. <https://doi.org/10.1111/nph.12766>
- Coombs, G., Dold, A. P., Brassine, E. I., & Peter, C. I. (2012). Large pollen loads of a South African asclepiad do not interfere with the foraging behaviour or efficiency of pollinating honey bees. *Naturwissenschaften*, 99, 545–552. <https://doi.org/10.1007/s00114-012-0932-2>
- Coombs, G., & Peter, C. I. (2010). The invasive 'mothcatcher' (*Araujia sericifera* Brot.; Asclepiadoideae) co-opts native honeybees as its primary pollinator in South Africa. *AoB PLANTS*, 2010, plq021.
- Córdoba, S. A., & Cocucci, A. A. (2011). Flower power: its association with bee power and floral functional morphology in papilionate legumes. *Annals of Botany*, 108, 919–931. <https://doi.org/10.1093/aob/mcr196>
- Darwin, C. (1862). *The various contrivances by which British and foreign orchids are fertilized by insects, and on the good effects of intercrossing*, 1st ed. John Murray.
- Dormann, C. F., & Strauss, R. (2014). A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution*, 5, 90–98. <https://doi.org/10.1111/2041-210X.12139>
- Drewniak, M. E., Briscoe, A. D., Cocucci, A. A., Beccacece, H. M., Zapata, A. I., & Moré, M. (2020). From the butterfly's point of view: learned colour association determines differential pollination of two co-occurring mock verbains by *Agraulis vanillae* (Nymphalidae). *Biological Journal of the Linnean Society*, 130, 715–725. <https://doi.org/10.1093/biolinnean/blaa066>
- Endress, P. K. (1990). Patterns of floral construction in ontogeny and phylogeny. *Biological Journal of the Linnean Society*, 39, 153–175. <https://doi.org/10.1111/j.1095-8312.1990.tb00509.x>
- Endress, P. K. (1994). *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press.
- Endress, P. K. (2016). Development and evolution of extreme synorganization in angiosperm flowers and diversity: a comparison of Apocynaceae and Orchidaceae. *Annals of Botany*, 117, 749–767. <https://doi.org/10.1093/aob/mcv119>
- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, 35, 375–403. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132347>
- Fishbein, M. (2001). Evolutionary innovation and diversification in the flowers of the Asclepiadaceae. *Annals of the Missouri Botanical Garden*, 88, 603–623.
- Frost, S. W. (1965). Insect and pollinia. *Ecology*, 46, 556–558.
- Heiduk, A., Brake, I., von Tschirnhaus, M., Göhl, M., Jürgens, A., Johnson, S. D., Meve, U., & Dötterl, S. (2016). *Ceropegia sandersonii* mimics attacked honeybees to attract kleptoparasitic flies for pollination. *Current Biology*, 26, 2787–2793.
- Ivey, C. T., Martinez, P., & Wyatt, R. (2003). Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae). *American Journal of Botany*, 90, 214–225.
- Janzen, D. H. (1987). How moths pass the dry season in a Costa Rican dry forest. *Insect Science and Its Applications*, 8, 489–500. <https://doi.org/10.1017/S1742758400022530>
- Johnson, S. D., Balducci, M. G., Bijl, A., Castañeda-Zárate, M., Cozien, R. J., Ortman, C. R., & van der Niet, T. (2020). From dusk till dawn: camera traps reveal the diel patterns of flower feeding by hawkmoths. *Ecological Entomology*, 45, 751–755. <https://doi.org/10.1111/een.12827>
- Johnson, S. D., Moré, M., Amorim, F. W., Haber, W. A., Frankie, G. W., Stanley, D. A., Cocucci, A. A., & Raguso, R. A. (2017). The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. *Functional Ecology*, 31, 101–115. <https://doi.org/10.1111/1365-2435.12753>
- Johnson, S. D., & Raguso, R. A. (2016). The long-tongued hawkmoth pollinator niche for native and invasive plants in Africa. *Annals of Botany*, 117, 25–36. <https://doi.org/10.1093/aob/mcv137>
- Lautenschleger, A., Vizentin-Bugoni, J., Cavalheiro, L. B., & Iserhard, C. A. (2020). Morphological matching and phenological overlap promote niche partitioning and shape a mutualistic plant–hawkmoth network. *Ecological Entomology*, 46, 292–300. <https://doi.org/10.1111/een.12961>
- Maruyama, P. K., Sonne, J., Vizentin-Bugoni, J., Martín González, A. M., Zanata, T. B., Abrahamczyk, S., Alarcón, R., Araujo, A. C., Araújo, F. P., Baquero, A. C., Chávez-González, E., Coelho, A. G., Cotton, P. A., Dehling, D. M., Fischer, E., Kohler, G., Lara, C., Las-Casas, F. M. G., Machado, A. O., ... Dalsgaard, B. O. (2018). Functional diversity mediates macroecological variation in plant–hummingbird interaction networks. *Global Ecology and Biogeography*, 27, 1186–1199. <https://doi.org/10.1111/geb.12776>
- Mochizuki, K., Furukawa, S., & Kawakita, A. (2017). Pollinia transfer on moth legs in *Hoya carnosae* (Apocynaceae). *American Journal of Botany*, 104, 953–960.
- Morse, D. H. (1982). The turnover of milkweed pollinia on bumble bees, and implications for outcrossing. *Oecologia*, 53, 187–196. <https://doi.org/10.1007/BF00545662>
- Nilsson, L. A. (1988). The evolution of flowers with deep corolla tubes. *Nature*, 334, 147–149. <https://doi.org/10.1038/334147a0>
- Nilsson, L. A. (1998). Deep flowers for long tongues. *Trends in Ecology and Evolution*, 13, 259–260. [https://doi.org/10.1016/S0169-5347\(98\)01359-7](https://doi.org/10.1016/S0169-5347(98)01359-7)
- Ollerton, J., Johnson, S. D., Cranmer, L., & Kellie, S. (2003). The pollination ecology of an assemblage of grassland asclepiads in South Africa. *Annals of Botany*, 92, 807–834. <https://doi.org/10.1093/aob/mcg206>
- Ollerton, J., Killick, A., Lamborn, E., Watts, S., & Whiston, M. (2007). Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, 56, 717–728. <https://doi.org/10.2307/25065855>
- Ollerton, J., & Liede, S. (1997). Pollination systems in the Asclepiadaceae: a survey and preliminary analysis. *Biological Journal of the Linnean*

- Society*, 62, 593–610. <https://doi.org/10.1111/j.1095-8312.1997.tb00324.x>
- Ollerton, J., & Liede, S. (2003). Corona structure in *Cynanchum*: linking morphology to function. *Ecotropica*, 09, 107–112.
- Ollerton, J., Liede-Schumann, S., Endress, M. E., Meve, U., Rech, A. R., Shuttleworth, A., Keller, H. A., Fishbein, M., Alvarado-Cárdenas, L. O., Amorim, F. W., Bernhardt, P., Celep, F., Chirango, Y., Chiriboga-Arroyo, F., Civeyrel, L., Cocucci, A., Cranmer, L., da Silva-Batista, I. C., de Jager, L., ... Quirino, Z. (2019). The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study. *Annals of Botany*, 123, 311–325. <https://doi.org/10.1093/aob/mcy127>
- Pauw, A. (1998). Pollen transfer on bird's tongues. *Nature*, 394, 731–732. <https://doi.org/10.1038/29421>
- Phillips, R. D., Peakall, R., van der Niet, T., & Johnson, S. D. (2020). Niche perspectives on plant-pollinator interactions. *Trends in Plant Science*, 25, 779–793. <https://doi.org/10.1016/j.tplants.2020.03.009>
- Queiroz, J. A., Diniz, U. M., Vázquez, D. P., Quirino, Z. M., Santos, F. A. R., Mello, M. A. R., & Machado, I. C. (2021). Bats and hawkmoths form mixed modules with flowering plants in a nocturnal interaction network. *Biotropica*, 53, 596–607. <https://doi.org/10.1111/btp.12902>
- Robertson, C. (1928). Insect relation of certain asclepiads. *Botanical Gazette*, 12, 244–250.
- Sazatornil, F. D., Moré, M., Benitez-Vieyra, S., Cocucci, A. A., Kitching, I. J., Schlumpberger, B. O., Oliveira, P. E., Sazima, M., & Amorim, F. W. (2016). Beyond neutral and forbidden links: morphological matches and the assembly of mutualistic hawkmoth-plant networks. *Journal of Animal Ecology*, 85, 1586–1594. <https://doi.org/10.1111/1365-2656.12509>
- Schulz, A. G. (1937). Las Asclepiadáceas del territorio del Chaco (Argentina). *Lilloa*, 1, 347–391.
- Shuttleworth, A., & Johnson, S. D. (2009). Palp-faction: an African milkweed dismembers its wasp pollinators. *Environmental Entomology*, 38, 741–747. <https://doi.org/10.1603/022.038.0326>
- Soteras, F., Pisano, M. A. R., Bariles, J. B., Moré, M., & Cocucci, A. A. (2020). Phenotypic selection mosaic for flower length influenced by geographically varying hawkmoth pollinator proboscis length and abiotic environment. *New Phytologist*, 225, 985–998. <https://doi.org/10.1111/nph.16192>
- Sprengel, C. K. (1793). *Das entdeckte geheimnis der natur im bau und in der befruchtung der blumen*. Friedrich Vieweg dem aeltern.
- Walther, R. (1994). *Pollenfrucht als Indikator fuer Ressourcennutzung und Einnischung bei Madagassischen Schwaermern (Lepidoptera)*. Ph.D. dissertation, Friedrich-Alexander University.
- Wasserthal, L. T. (1997). The pollinators of the malagasy star orchids *Angraceum sesquipedale*, *A. sororium* and *A. compactum* and the evolution of extremely long spurs by pollinator shift. *Botanica Acta*, 110, 343–359.
- Wiemer, A. P., Sérsic, A. N., Marino, S., Simões, A. O., & Cocucci, A. A. (2012). Functional morphology and wasp pollination of two South American asclepiads (Asclepiadoideae-Apocynaceae). *Annals of Botany*, 109, 77–93. <https://doi.org/10.1093/aob/mcr268>
- Xiong, W., Ollerton, J., Liede-Schumann, S., Zhao, W., Jiang, Q., Sun, H., Liao, W., & You, W. (2020). Specialized cockroach pollination in the rare and endangered plant *Vincetoxicum hainanense* (Apocynaceae, Asclepiadoideae) in China. *American Journal of Botany*, 107, 1355–1365.

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