Journal of Applied Entomology

WILEY

SPECIAL ISSUE The Neglected Pollinators: Understanding the Importance of Lesser-Known Insect Taxa in Pollination

JOURNAL OF APPLIED ENTOMOLOGY

ORIGINAL ARTICLE OPEN ACCESS

Nocturnal Lepidopterans as Essential Pollinators of *Aspidosperma pyrifolium* (Apocynaceae), a Keystone Tree in the Caatinga Dry Forest

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Received: 31 March 2025 | **Revised:** 22 July 2025 | **Accepted:** 30 October 2025

Funding: This work was supported by CAPES (Financial Code 001), CNPq (310508/2019-3), and FACEPE (BCT-0085-2.03/19; DCR-0031-2.03/21).

Keywords: Caatinga dry forest | hawkmoth-pollination | Noctuidae | nocturnal pollination | pereiro tree | settling moths

ABSTRACT

Pollination is a key ecosystem service, yet most studies focus on diurnal interactions, often overlooking nocturnal pollinators such as hawkmoths and settling moths. In arid environments, nocturnal pollination plays a crucial role in maintaining native plant species of high biocultural value, as seen in the Caatinga dry forest. In this study, we investigated the pollination biology of *Aspidosperma pyrifolium* (Apocynaceae), pereiro tree, a species of significant economic and cultural importance, recognized for its biotechnological potential and as a dominant keystone species in regenerating areas. We examined its reproductive phenology, floral biology, primary pollinators, and assessed its mating system, dependency on pollinators for reproductive success, and overall reproductive performance under natural conditions. Our results indicate that the flowering and pollination dynamics of *A. pyrifolium* are consistent with a well-adapted sphingophilous–phalenophilous system within the Caatinga dry forest. The species' reliance mainly on nocturnal lepidopterans for effective pollination (i.e., especially settling moths and hawkmoths) underscores the critical role of nocturnal pollinators in ensuring reproductive success. Furthermore, the presence of a late-acting self-incompatibility mechanism reinforces the importance of outcrossing. This study highlights the ecological significance of nocturnal lepidopterans pollination in sustaining species with essential cultural and environmental roles in semi-arid ecosystems. As these pollinators face increasing threats, understanding their interactions with key plant species becomes vital for conservation strategies and ecosystem resilience.

1 | Introduction

Pollination is a fundamental ecosystem service linked, among many other aspects, to the maintenance of plant species with biocultural importance for indigenous peoples and local communities (Avila Jr et al. 2025). However, most pollination studies focus on diurnal interactions, often overlooking the contributions of nocturnal pollinators (Macgregor and Scott-Brown 2020), among which Lepidoptera stand out, particularly hawkmoths (Sphingidae) and settling moths. Hawkmoths are recognised as highly effective pollinators that exert significant selective pressures on floral morphology through their long proboscides, which enable them to access nectar in flowers with long and narrow corolla tubes in a specialised manner (Johnson

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et al. 2017). Another noteworthy group is the settling moths, commonly also referred to as small moths or millers; these species belonging to families other than Sphingidae commonly fed while remaining perched on the corolla of the flowers (sensu San Blas and Devoto 2025). Among them, we can highlight the superfamily Noctuidea, one of the most diverse and ecologically successful animal lineages, and the most species-rich within Lepidoptera, comprising over 12,000 described species across at least 1150 genera (Kristensen 1999; Keegan et al. 2021; Atwater et al. 2025). While many species remain under revision or undescribed, recent systematic revisions split the former Noctuidae into two families: Noctuidae (trifine noctuids) and Erebidae (quadrifine noctuids, including Arctiinae and Lymantriinae) (Atwater et al. 2025). Adults from both groups provide important pollination services to native and cultivated plants (Walton et al. 2020; Ribas-Marques et al. 2022; San Blas and Devoto 2025).

Nocturnal pollination assumes particular importance in arid environments, where night blooming can confer crucial advantages, such as reducing floral evapotranspiration and minimizing pollinator exposure to high temperatures and low humidity (Borges et al. 2016). A striking example occurs in the Caatinga, the largest seasonally dry tropical forest in the Neotropics, renowned for its exceptionally high proportion of plants with nocturnal or crepuscular flowering (Machado and Lopes 2004; Quirino and Machado 2014; Domingos-Melo et al. 2023). In this context, nocturnal Lepidoptera play a crucial yet often underappreciated role in the pollination ecology of several bioculturally significant plant species in the Caatinga dry forest. This includes emblematic columnar cacti such as 'mandacaru' (*Cereus jamacaru*), 'caxacubri' (*Xiquexique tuberculatus*), 'xique-xique' (*X. gounellei*), and various species of 'facheiro' (*Pilosocereus* spp., Cactaceae) (Rocha et al. 2019; Albuquerque-Lima et al. 2023, 2024). In addition, common shrub species such as 'icó' (*Morisonia longifolia*) and 'feijão-bravo' (*M. flexuosa*, Capparaceae) (Costa et al. 2025), as well as iconic trees like 'amburana-de-cheiro' (*Amburana cearensis*, Fabaceae) (Barral et al. 2022), also exhibit pollination systems that involve nocturnal lepidopterans. A particularly compelling example is *Aspidosperma pyrifolium* Mart. & Zucc. (Apocynaceae), commonly referred to as 'pereiro' or 'pau-pereiro', a culturally and ecologically prominent species in the semi-arid region.

Aspidosperma pyrifolium holds substantial economic and cultural importance for local communities, being widely used in ethnobotanical practices and frequently studied for its biotechnological potential (Andrade-Júnior et al. 2020). Moreover, *A. pyrifolium* is among the 52 most valued plant species that account for over half of the region's ecosystem services, making it a strong candidate for biocultural restoration initiatives (Sena et al. 2022). Considered a keystone species in the Caatinga, *A. pyrifolium* plays a fundamental role in promoting ecological facilitation processes in this environment (Carrion et al. 2017). Additionally, it dominates the regenerative stratum in anthropogenic areas, demonstrating adaptive advantages that ensure its survival in highly arid and stressful environments. This resilience suggests its potential as a future dominant species, particularly under current global warming trends projected for the coming decades (Ramos et al. 2023).

Previous community-level studies have suggested that *Aspidosperma pyrifolium* is pollinated by both diurnal and nocturnal Lepidoptera (Quirino and Machado 2014; Queiroz et al. 2021). However, detailed data on its pollination process and interactions with these insects remain unavailable. This study aims to explore key aspects, including its reproductive phenology, detailed floral biology encompassing the sequence of anthesis, morphological traits, and pollination mechanisms. Additionally, it seeks to identify the primary pollinators, assess the species' mating system, evaluate its reliance on pollinators for successful reproduction, and investigate its reproductive success under natural conditions. We aim to generate applied knowledge on the reproductive ecology of the species, providing a basis for future management and restoration initiatives. At the same time, we seek to demonstrate the key role of nocturnal Lepidoptera pollination in maintaining a species of high cultural, biotechnological, and ecological value.

2 | Materials and Methods

2.1 | Study Site

Fieldwork was conducted at the Private Natural Heritage Reserve (RPPN) Fazenda Almas (7°28′45″S, 36°54′18″W), located in the western 'Cariri' region of Paraíba State, northeastern Brazil. The RPPN Fazenda Almas is one of the largest protected remnants of Caatinga vegetation in Paraíba, encompassing 3505 ha. This area is characterised by arboreal-shrub vegetation (Andrade-Lima 1981) and marked xerophytic adaptations (Prado 2003), including deciduous foliage, succulence, dominance of shrubs and small trees, and discontinuity in canopy cover (Rodal and Sampaio 2002). The western Cariri, also referred to as 'Cariris Velhos', has a hot semi-arid climate (BSh) according to Köppen's classification. The region experiences well-defined rainy (March to June) and dry (July to February) seasons (Governo do Estado da Paraíba/Secretaria de Educação/Universidade Federal da Paraíba 1989). Rainfall patterns during the 2 years of observation (April 2007 to January 2009) reflected the region's characteristic irregularity. In the first year, total precipitation was 439.3 mm, with rainfall extending from January to September. In 2008, precipitation was more intense, exceeding 800 mm, with the highest monthly total recorded in March (366.8 mm) (AESA 2025).

2.2 | Phenology

We marked 15 individuals of *Aspidosperma pyrifolium* and monitored them monthly from April 2007 to January 2009. During each survey, the presence of open flowers and dehiscent fruits was recorded. Additionally, during the flowering period, we documented the timeline from the emergence of the first flower buds to the peak bloom and the complete absence of flowers in each individual.

2.3 | Floral Biology and Pollination Mechanism

Flowers were marked in the field to track anthesis events. The period of stigma receptivity was determined using a

potassium permanganate solution ($KMnO_4$ 0.25%) applied to the stigma of flowers ($n = 10$) at different stages of anthesis, and through hydrogen peroxide tests (Zeisler 1938; Domingos-Melo et al. 2018).

We described the inflorescence type and flower arrangement. For morphometric analysis, 30 flowers were fixed in 70% ethanol and taken to the laboratory for measurements under a stereomicroscope. The pollen/ovule ratio (P/O; Cruden 1977) was calculated based on direct counts of pollen grains and ovules per flower ($n = 30$). Pollen viability was assessed by counting pollen grains stained with a 2% acetocarmine solution (Radford et al. 1974). The stigma region was identified using fluorescence microscopy to analyse the penetration of pollen tubes into the stylar head in naturally pollinated flowers ($n = 10$—see details below in the item with the 'pollen tube development' protocol). Finally, we also recorded the fruit type and development, as well as the seed dispersal mode.

To analyse the pollination mechanism, we conducted manipulative experiments on fresh flowers. The number of pollen grains potentially deposited on insect proboscises during visits to *A. pyrifolium* flowers was estimated by counting pollen grains on nylon threads inserted into the floral tubes ($n = 10$) of previously bagged flowers. For each flower, five nylon threads were sequentially inserted and removed to simulate five visits as adapted from Moré et al. (2007) and Araújo et al. (2014). Since the successful release of the pollination mechanism significantly varies with the diameter of the nylon threads used (Moré et al. 2007), we selected threads with a diameter of 0.25 mm. This choice was based on previous findings suggesting that similar diameters are effective in triggering the pollination mechanism in *Mandevilla tenuifolia* (Araújo et al. 2014), an herbaceous species of Apocynaceae sympatric to *A. pyrifolium*, with similarly sized flowers and sharing some of the same pollinators.

Pollen grains on the threads were removed using glycerin jelly stained with basic fuchsin mounted on histological slides and counted under a stereomicroscope (Dafni 1992).

2.4 | Floral Visitors

Floral visitors were observed directly in the field on focal plants. Observations were conducted over 12 days in January and February 2008, totalling 98 h. Among them, 24 h of observation were carried out from late afternoon to early evening (14:00–18:00), coinciding with flower opening, and approximately 74 h were conducted at night. Observations focused on open flowers, without distinguishing between different days of the floral lifespan. During the day, the entire canopy was monitored, while at night, due to limited visibility, observations were restricted to lower branches. As a result, visitation frequencies are comparable within each time period but not between day and night sessions.

Visitors were photographed for behavioural analysis. Due to the high similarity in size and coloration among the different noctuid morphospecies, we were unable to achieve species-level distinction when recording visitation frequency. Therefore, the data were pooled and reported as settling moths. Data recorded included visitation times, frequency, and behaviour during visits.

Specifically, for the Lepidoptera, specimens of each recorded morphospecies were collected and analyzed for pollen deposition on their proboscises. For this, the proboscides were removed using tweezers and examined under a stereomicroscope. Once pollen grains were detected, they were collected using glycerin jelly, mounted on histological slides as described above, and examined under a light microscope to confirm whether they belonged to *A. pyrifolium*. Voucher specimens were mounted and deposited in the Laboratory of Floral and Reproductive Biology, Department of Botany, Federal University of Pernambuco. Floral visitor identification was conducted with the assistance of specialists.

2.5 | Mating System and Reproductive Success of the Population

To assess the presence of pre-zygotic self-incompatibility mechanisms (whether self-pollen is inhibited at the stigma surface or during pollen tube growth within the style, Gibbs 2014) we monitored pollen tube development following controlled manual pollinations. Prior to anthesis, we bagged flower buds using voil mesh bags. Upon anthesis, we applied two controlled pollination treatments: (i) self-pollination ($n = 10$ flowers/individuals) and (ii) cross-pollination ($n = 10$ flowers/individuals). To avoid interference among treatments, we pollinated only one flower per inflorescence. We deposited pollen onto the stigma using 0.25 mm nylon threads (see details above in Section 2.3). For self-pollination, we used pollen from the same flower, whereas for cross-pollination, we sourced pollen from different individuals located at least 50 m apart. After pollination, we kept all flowers bagged and collected pistils at 24, 48, and 72 h. We preserved the pistils in 50% FAA, stained them with aniline blue, and examined them under a fluorescence microscope following the protocol of Martin (1959).

To analyse the reproductive system, we conducted pollination experiments on flowers of different *A. pyrifolium* individuals ($n = 15$). Spontaneous self-pollination was assessed by observing fruit set in inflorescences ($n = 45$) bagged since the bud stage up to complete senescence of all flowers without any manipulation. Fruit formation was also examined after manual pollinations conducted in the same manner as described above for the pollen tube growth experiment (self-pollination, $n = 55$; cross-pollination, $n = 75$). To quantify natural pollination, flowers ($n = 250$) under natural conditions were marked and monitored for fruit set.

To characterise the overall reproductive success of the population, we quantified fruit set per flower and seed set per ovule. The average number of flowers produced per individual ($n = 10$) was estimated by multiplying the number of flowers per inflorescence ($n = 40$), the number of inflorescences on secondary branches ($n = 20$) approximately 1 m long, and the number of secondary branches per individual ($n = 10$). The average number of fruits per individual was counted in 100 individuals from five subpopulations. The flower-to-fruit ratio was estimated by dividing the average number of flowers per individual by the average number of fruits per individual. The seed-to-ovule ratio (S/O) was calculated

by dividing the average number of mature seeds per fruit ($n = 100$) by the number of ovules per flower ($n = 30$) (Wiens 1984).

3 | Results

3.1 | Reproductive Phenology

Both flowering and fruiting of *Aspidosperma pyrifolium* exhibited significant seasonal patterns, as indicated by circular statistics (Table 1; Figure 1). The flowering period of the studied population lasted approximately three months, from early November to late February (Figure 1A), with the highest number of individuals in bloom recorded in December and January. The period from bud dormancy break to floral senescence in each monitored individual lasted about 15 days, with peak flowering occurring over a five- to seven-day period. Fruiting began one month after the first flowering event, in November/December (Figure 1C), when small, developing fruits were first observed, with seed dispersal beginning between August and September.

3.2 | Floral Biology and Pollination Mechanism

Regarding the anthesis sequence, young floral buds of *Aspidosperma pyrifolium* are light green in colour, turning

TABLE 1 | Circular statistics of reproductive phenology of *Aspidosperma pyrifolium* (Apocynaceae) in a Caatinga dry forest area—Almas Farm, Paraíba, Brazil.

Phenofase presence	*n*	Min.	1st qu.	Median	Mean	3rd qu.	Max	Rayleigh test	*p*
Flower	28	−1.05	−0.52	0.00	−0.22	0.00	0.52	0.90	> 0.001
Fruit	136	0.00	1.57	2.62	2.65	−2.62	−0.52	0.24	0.004

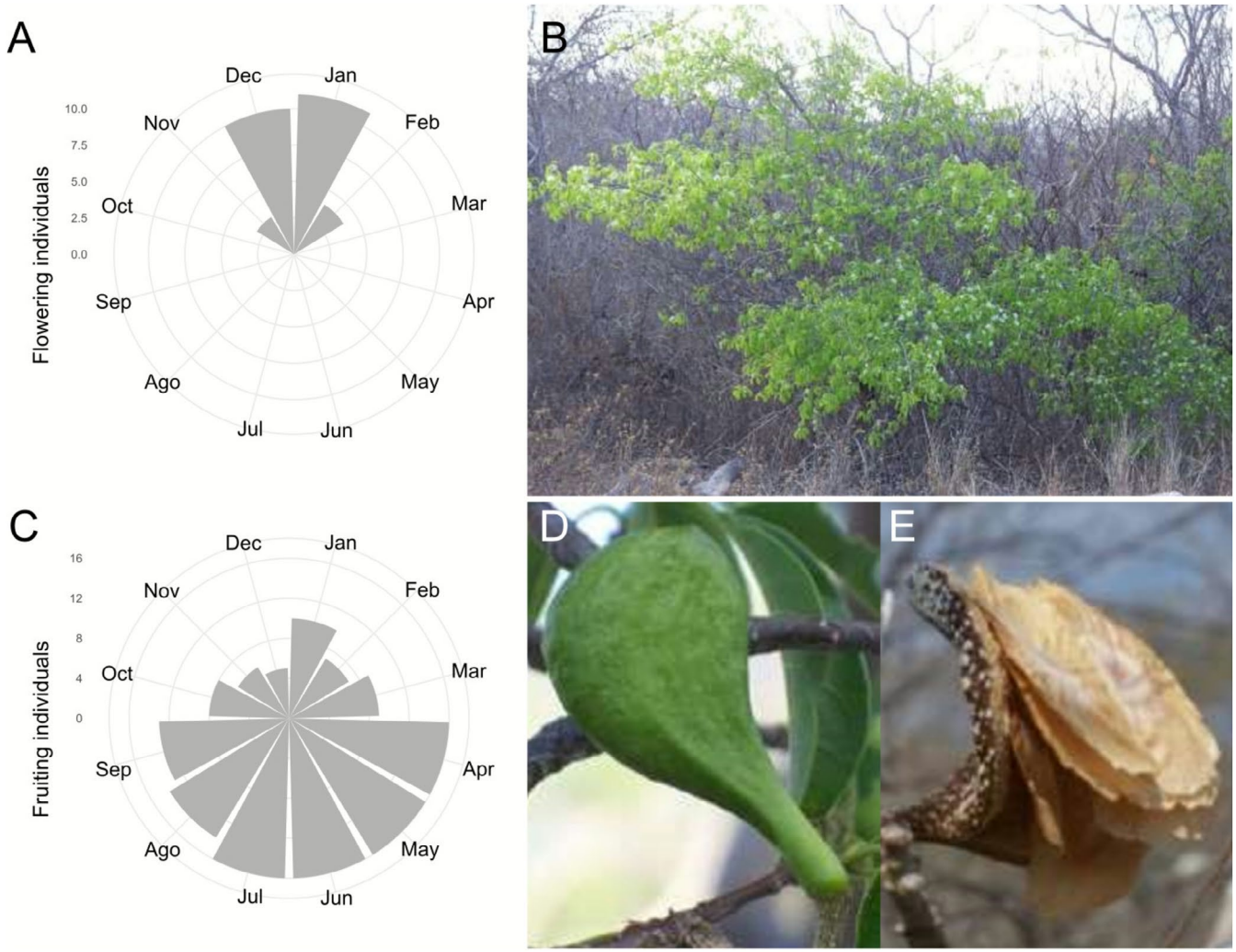


FIGURE 1 | Reproductive phenology of *Aspidosperma pyrifolium* (Apocynaceae) in a Caatinga Dry Forest Area—Almas Farm, Paraíba, Brazil. (A) Circular barplot showing flowering seasonality, (B) example of an individual with mass flowering, (C) circular barplot showing fruiting seasonality, (D) fruit in maturation and (E) seed dispersal. [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 2 | Floral anthesis sequence in *Aspidosperma pyrifolium* (Apocynaceae). (A) Inflorescence with flowers at the beginning of anthesis (15:00–16:00 h); flower 24 h (B) and 48 h (C) after the start of anthesis, arrow indicating the style head after corolla abscission. [Colour figure can be viewed at wileyonlinelibrary.com]

whitish just before opening during pre-anthesis (Figure 2A,B). Floral opening starts around 15:00 and occurs synchronously at dusk, with gradual expansion of the corolla lobes, completing by early evening, around 18:00. During this period, the corolla lobes emit a strong sweet scent, most noticeable between 16:30 and 18:00, persisting throughout the night and detectable from long distances. Anther dehiscence occurs early, observed even in pre-anthesis buds. The stigma remains receptive throughout the flower's life, with functional viability extending approximately one day after petal abscission. Anthesis can last up to five days, though approximately 72% of flowers senesce between the second and fourth days. Senescence is marked by petal fall, leaving only the calyx and female reproductive structures (Figure 2C). After petal abscission, the style head initially exhibits a greenish hue, gradually darkening to brown and desiccating, a process that takes two–three days.

Concerning floral morphology, *A. pyrifolium* inflorescences are cymose, with flowers presenting varied orientations, including vertical (faucial orientation upward or downward), pendulous, or horizontal. The flowers are small, actinomorphic, and hermaphroditic, with a hypocrateriform corolla consisting of five white, free lobes and a short greenish floral tube (Table 2). The androecium comprises five epipetalous stamens, with dorsifixed anthers, free from the style head, arranged to form a cone beneath the faucial region of the corolla and above the style head (Figure 3A). Pollen is stored in the space between the anther cone (Figure 3B). The gynoecium consists of the style head (housing the stigma), style, and a bicarpellate, dialicarpellate, superior ovary (Figure 3A). The style head is simple and claviform (Figure 3C), with two distinct regions: a larger, hairy region covered in a sticky substance that facilitates pollen grain adhesion and pollen tube development (Figure 3D); and a smaller, apical portion with two smooth protrusions, where no pollen grains or developing pollen tubes are observed. Each flower of *A. pyrifolium* produces an average of 1630.2 pollen grains and 20.66 ovules, resulting in a pollen/ovule (P/O) ratio of 78.5. The fruit is a capsule (Figure 2C), typically resulting from the development of a single flower per inflorescence. Although both carpels often initiate development, only one results in fruit. The seeds are winged and dispersed by wind.

Regarding the pollination mechanism, access to the floral tube occurs through the pentagonal corolla throat, where the flower's fertile structures are located. Simulations revealed that pollinators can remove large quantities of pollen on the proboscis during their first visit (Figure 3E). Consequently, five visits are sufficient to remove nearly half of the total pollen stock.

3.3 | Floral Visitors

3.3.1 | Frequence of Visits

During the late afternoon 786 floral visits were recorded (Figure 4H). More than half of these visits were made by noctuid moths (53.31%). Other visits were made by butterflies *Urbanus*

TABLE 2 | Quantifying flower characteristics of *Aspidosperma pyrifolium* (Apocynaceae), considering inflorescence, floral morphometry, pollen and ovule production, in a Caatinga dry forest area—Almas Farm, Paraíba, Brazil.

Floral structures	*N*	Variation range	Mean ± SD
Flowers/buds per inflorescence	30	6.0–48.0	24.55 ± 8.80
Open flowers per day	30	2.0–7.0	3.76 ± 1.83
Length of corola lobes (mm)	90	7.0–22.0	13.22 ± 3.70
Length of floral tube (mm)	30	4.0–5.0	4.76 ± 0.41
Width of fauce (mm)	30	0.41–0.73	0.54 ± 0.08
Length anthers (mm)	30	0.88–1.38	1.11 ± 0.15
Style head (mm)	30	0.59–0.80	0.69 ± 0.06
Style (mm)	30	3.1–4.30	3.90 ± 0.20
Space between anther cone and style head (mm)	20	0.14–0.41	0.25 ± 0.08
Pollen grains per flower	30	1261–1882	1630.2 ± 159.80
Pollen viability (%)	30	92–100	97.95 ± 1.79
Ovules per flower	30	18–23	20.66 ± 2.40
Ratio P/O	30	54.82–94.77	78.50 ± 10.85

sp. (Hesperiidae), 1.39%, and *Ascia monuste* (Pieridae), 35.88%, with additional infrequent visits by *Cogia calchas* (Hesperiidae), though quantification was not possible. In this same period of time, visits by bees (*Apis mellifera*, *Trigona spinipes*, and *Xylocopa grisescens*—Apidae) were also observed, collectively accounting for 9.41% of visits.

During the night, a total of 221 visits were registered, of which 120 were hawkmoth and 101 were moths belonging to at least five different morphospecies (Figure 4I). The most frequent hawkmoth was *Erinnyis ello* (39.81% of visits; Figure 4A), and the least frequent was *Callionima grisescens* (14.02% of visits). The peak of visits occurred early in the evening, decreasing until around 22:00, when it stabilized. *C. grisescens* exhibited fluctuating visits throughout the night, with small peaks at 20:00 and 01:00.

3.3.2 | Floral Visits Behaviour

Both butterflies and noctuid moths (diurnal and nocturnal) landed on the corolla lobes and moved throughout the inflorescence, inserting their proboscis into the floral tube accessing the pollination mechanism, and repeating this behaviour across multiple inflorescences of the same individual. Each visit to a flower was brief (1–2 s), but they could last several minutes on a single individual, a behaviour that favours geitonogamy (Figure 4B–D). In concern to the bees, *Trigona spinipes* was observed cutting floral buds and pillaging their pollen, while the long-tongued bee *X. grisescens* appears capable of accessing the pollination mechanism, acting as a legitimate pollinator.

The hawkmoths (*E. ello* and *C. grisescens*) typically hovered while foraging, inserting their proboscides into the floral tube without landing and accessing the pollination mechanism. They visited only a few flowers per individual and consistently moved between different plants, a foraging pattern that favours cross-pollination.

3.3.3 | Pollen Transport on Lepidoptera Proboscides

Although a precise quantification of pollen loads was not possible, analysis of the proboscides revealed that both noctuids and the two hawkmoth species carried substantial amounts of pollen on these structures (Figure 4E–G), indicating a strong potential for pollen transfer despite the lack of formal comparison between groups.

3.4 | Mating System and Reproductive Success of the Population

Pollen tubes were observed contacting ovules within 24 h of manual pollination, originating from both self-pollen and exogenous pollen. Regarding the reproductive system, in a pollinator exclusion experiment, no fruit set occurred in flowers of *A. pyrifolium*, nor in those subjected to manual self-pollination. However, fruits were formed in the cross-pollination treatment, albeit at a low rate (17.33%). Flowers marked for natural pollination did not produce fruits.

The estimated number of flowers produced per individual was high (76,513.00 ± 114,955.00) but highly variable (8452.00–458,640.00), reflecting the size variability of individuals within the population, as the sample included both tall (up to 8 m) and short (1–3 m) individuals. The average fruit production per individual was extremely low (4.03 ± 9.66),

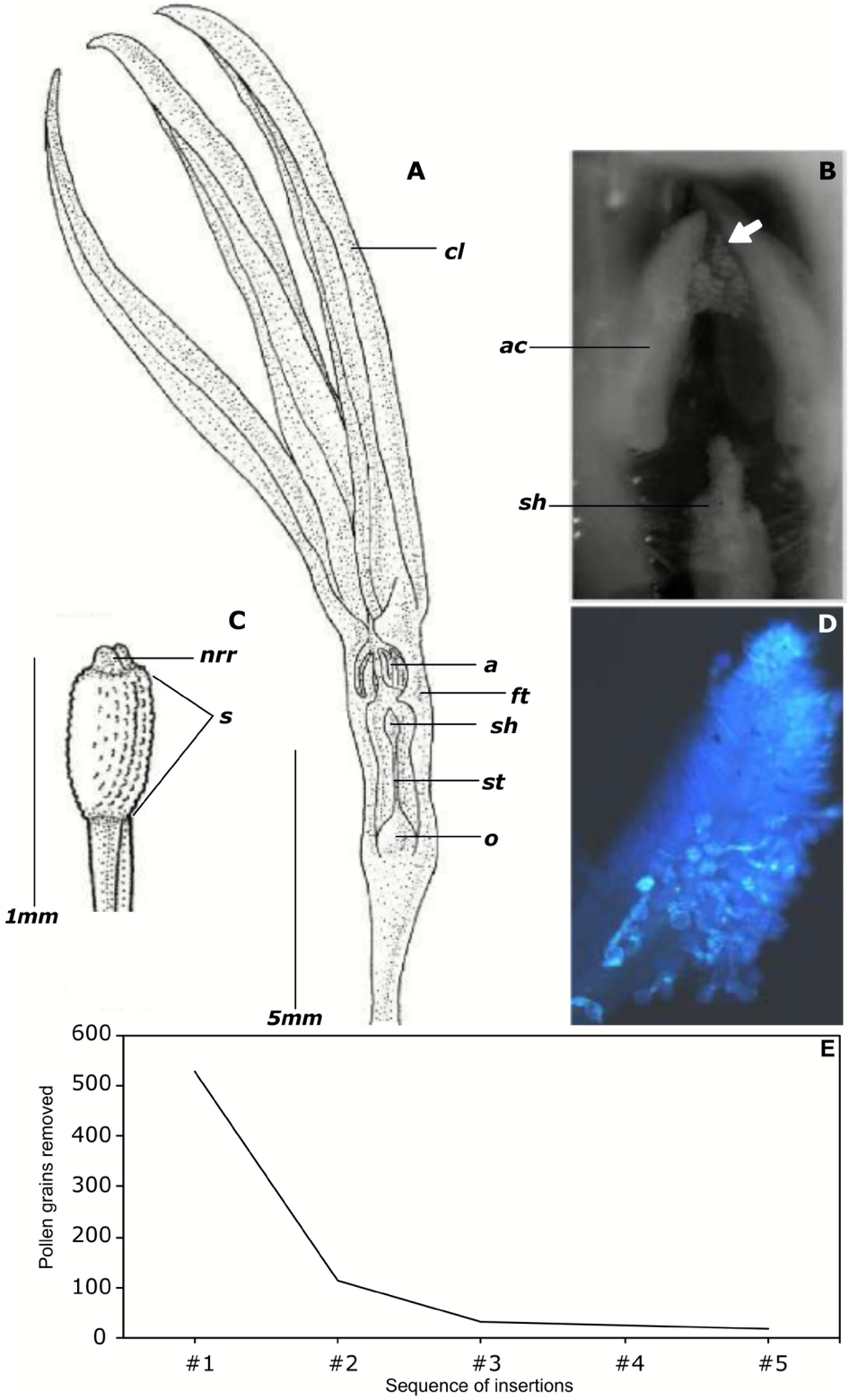


FIGURE 3 | Floral structure of *Aspidosperma pyrifolium* (Apocynaceae). (A) Internal organisation of the floral reproductive structures: a = anther, ft. = floral tube, Cl = corolla lobes, o = ovary, sh = style head, st = style. (B) Detail of the anther cone and style head within the floral tube: Ac = anther cone, sh = style head, arrow indicating the site of pollen presentation. (C) Style head: Nrr = non-receptive region of the style head, s = stigma. (D) Pollen tube development (stigma) within the style head under fluorescence microscopy (10× magnification). (E) Pollen removed from flowers after simulated visits using nylon threads. [Colour figure can be viewed at wileyonlinelibrary.com]

with no fruits recorded in 58% of the monitored individuals ($n = 100$). This resulted in a high flower/fruit ratio (18,985.00), with an average of 7.43 (±1.66) seeds per fruit, yielding a seed/ovule ratio of 0.37, indicating that when pollination occurs, approximately 37.1% of the ovules in the flower are fertilised.

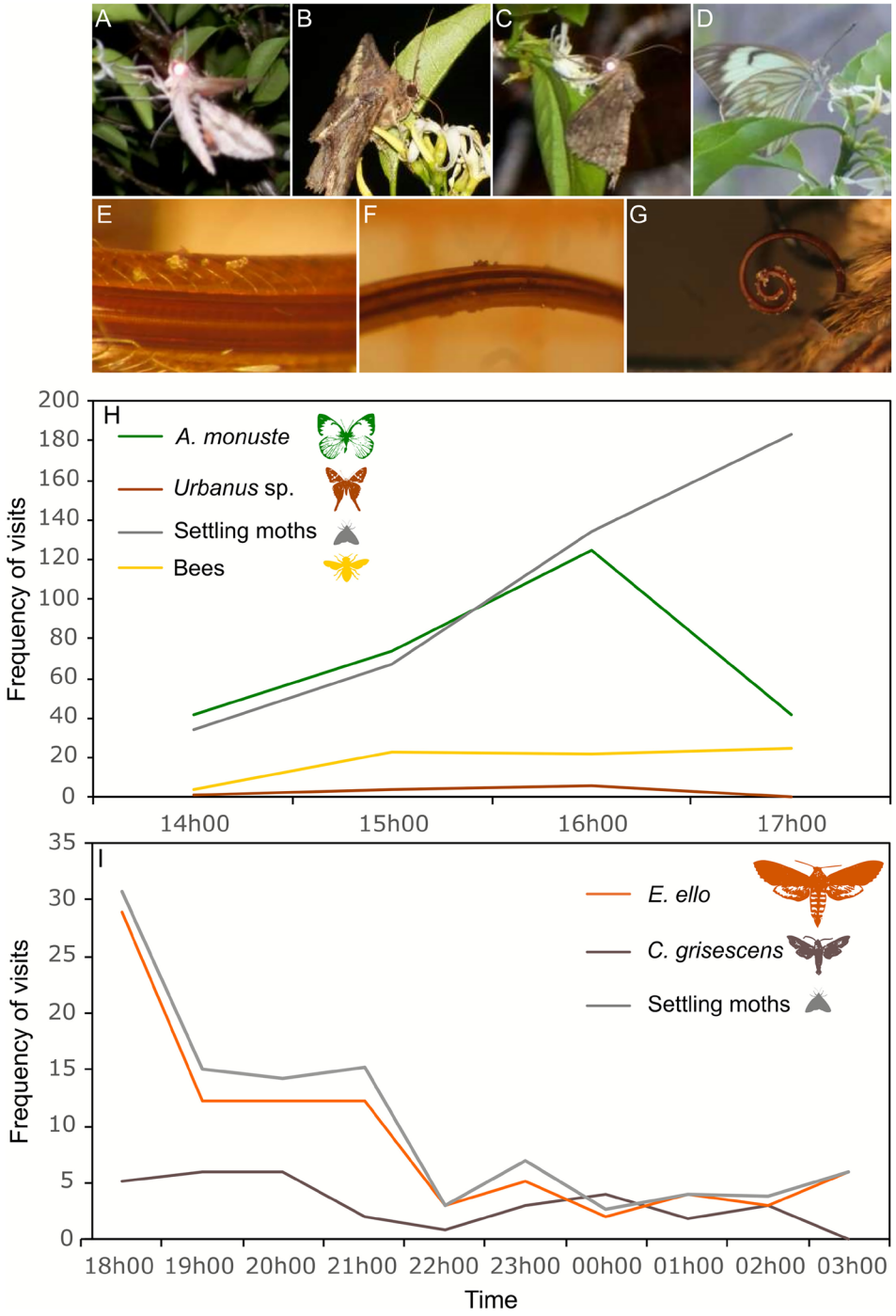


FIGURE 4 | Interaction between *Aspidosperma pyrifolium* (Apocynaceae) and its lepidopteran pollinators in a Caatinga Dry Forest area—Almas Farm, Paraíba, Brazil. Floral visits by (A) the hawkmoth *Erinnyis ello* , (B) a morphospecies of Erebidae, (C) a morphospecies of Noctuidae, and (D) the butterfly *Ascia monuste* . Pollen grains deposited on the proboscis of hawkmoths (E) *E. ello* , (F) *Callionima grisescens* and (G) the butterfly *Urbanus* sp. (H) Frequency of visits documented during the afternoon and evening. (I) Frequency of visits documented during the nocturnal period. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

The flowering of *Aspidosperma pyrifolium* is annual, characterised by mass flowering (sensu Newstrom et al. 1994; Gentry 1974) during a single episode restricted to the dry season in the Caatinga dry forest. Sphingophilous plants flowering in the dry season typically receive fewer visits compared to those blooming during the rainy season (Haber and Frankie 1989). Mass flowering, however, enhances visual display, odour emission, and floral resource advertisement, which can increase the attraction of floral visitors (Bawa 1974; Albuquerque-Lima et al. 2020). Indeed larger displays are known to attract more pollinators and typically receive higher visitation rates (van der Kooi et al. 2023), which may enhance opportunities for cross-pollination. This possibility is supported by the fact that only a single flower per inflorescence of *A. pyrifolium* typically sets fruit, reinforcing the idea that the production

of multiple flowers per individual primarily functions to enhance floral display. This strategy could be particularly advantageous, given the expected reduced availability of pollinators during the dry season (Duarte Júnior and Schlindwein 2005). Similar flowering patterns have been reported in other Apocynaceae pollinated by hawkmoths (Haber 1984; Herrera 1991; Lin and Bernardello 1999; Amorim et al. 2022; Pereira et al. 2022).

The floral structure of *A. pyrifolium* represents the simplest organisational level within Apocynaceae, typical of the subfamilies Plumerioideae or Rauvolfioideae (Schick 1982; Fallen 1986; Endress and Bruyns 2000). This simplicity is evidenced by two primary characteristics: (1) the absence of morphological and functional differentiation in the style head, which is uniformly covered by a secretory epidermis, and (2) the presence of simple, non-specialised anthers that are free from the style head and lack sclerenchymatic tissue on their dorsal region. This structural simplicity permits pollen tube germination along the entire style head, rendering the entire structure receptive (Schick 1982; Fallen 1986). However, *A. pyrifolium* is a sister lineage to the other species of *Aspidosperma* sect. *Aspidosperma* and is distinguished by unique floral traits; it has lanceolate corolla lobes, whereas other species exhibit oval, oblong, or ovate lobes, its stigma head is elliptical rather than oblong or globose, and it is pubescent, unlike the glabrous stigma head found in the other species (Teixeira et al. 2024). These floral particularities may significantly influence the interaction of *A. pyrifolium* with its pollinators compared to other species in the section and need further investigation. Indeed, hawkmoths show an innate preference for lanceolate corolla lobes over rounded forms with similar internal diameters (Kaczorowski et al. 2012), and corolla curvature facilitates nectar location (Campos et al. 2015). In turn, stigma shape and surface texture in complex flowers may reflect pollinator-mediated selection (Kriebel et al. 2022), although this has not yet been recorded for hawkmoths.

In *A. pyrifolium*, pollen grains are presented outside the pollen sacs, aggregated within the cone formed by the anthers. This facilitates pollen capture and deposition onto the proboscis of floral visitors, as commonly described for Apocynaceae (Schick 1982; Fallen 1986; Lopes and Machado 1999; Darrault and Schlindwein 2005; Araújo et al. 2011; Ollerton et al. 2019). However, unlike typical mechanisms, pollen deposition in *A. pyrifolium* occurs as the proboscis passes through the anther cone, rather than upon contact with the apical region of the style head during proboscis retraction. This basal mechanism ensures efficient pollen deposition on the visitors' proboscis.

The low P/O ratio estimated for *A. pyrifolium* aligns with trends commonly observed in outcrossing Apocynaceae (Herrera 1991; Torres and Galetto 1998; Lin and Bernardello 1999; Lopes and Machado 1999; Darrault and Schlindwein 2005; Araújo et al. 2011). Reduced P/O ratios have been reported for species such as *Hancornia speciosa* (77.3) (Darrault and Schlindwein 2005) and *Allamanda blanchetii* (168.4) (Araújo et al. 2011). This trend likely reflects the specialised pollination apparatus of these plants, which ensures high pollen deposition efficiency on visitors' proboscises.

The flowers of *A. pyrifolium* exhibit traits associated with two primary pollination syndromes, phalaenophily and sphingophily (Faegri and van der Pijl 1979), including a hypocrateriform structure, whitish corolla, nocturnal anthesis, and a sweet scent. Other traits, such as small flower size, clustered inflorescences, and minimal nectar production, suggest adaptations to noctuid moth pollination (Silberbauer-Gottsberger and Gottsberger 1988; Primo et al. 2025). According to Haber and Frankie (1989), sphingophilous flowers with short tubes, predominant during the dry season in Costa Rica, are accessible to both short- and long-tongued hawkmoths, whereas long-tubed flowers restrict access to specialised, long-tongued hawkmoths. While both settling moths and hawkmoths acted as pollinators, hawkmoths are likely the more effective contributors to cross-pollination. This is because settling moths often visited multiple flowers on the same individual, increasing geitonogamy, while hawkmoths tended to visit fewer flowers per plant and frequently moved between individuals, thereby promoting outcrossing. The presence *of A. pyrifolium* pollen on most floral visitors' proboscises reinforces the idea that pollen flow can be effectively mediated by a broad spectrum of pollinators.

While moths and hawkmoths were the primary nocturnal visitors, diurnal visits by bees and butterflies were also recorded in high frequency. Similar dual-pollinator systems have been reported in other Apocynaceae species, where nocturnal pollinators are the main agents of pollen transfer, and diurnal visitors play a less significant role (Haber and Frankie 1989; Araújo et al. 2011; Ollerton et al. 2019). We suggest that moths and hawkmoths are the most frequent visitors to *Aspidosperma pyrifolium* flowers. The high frequency of hawkmoth visits during nocturnal hours strongly supports their role as the most effective pollinators of this species. The morphological and behavioural traits of hawkmoths (such as their long proboscises, swift flight, and hovering capabilities) align closely with the floral traits of *A. pyrifolium*. Similar patterns have been observed in other sphingophilous Apocynaceae (Haber 1984; Herrera 1991; Darrault and Schlindwein 2005; Araújo et al. 2011; Ollerton et al. 2019). Future studies that effectively compare the reproductive success resulting from diurnal and nocturnal visitation may play a key role in uncovering the relative contribution of these pollinators with different activity periods (Kendall and Nicholson 2025; Shibata and Kudo 2023).

Pollen tube development along the pistil and their contact with ovules in self-pollinated flowers, without subsequent fruit formation, suggests the presence of a late-acting self-incompatibility mechanism at the ovule level (Gibbs 2014), which seems to occur in *A. pyrifolium*. Within Apocynaceae, both self-compatibility (Torres and Galetto 1998; Löhne et al. 2004; Herrera 1991; Raju et al. 2005) and self-incompatibility (Araújo et al. 2011; Waddington 1976; Lipow and Wyatt 1999; Darrault and Schlindwein 2005; Haber 1984; Lopes and Machado 1999) have been documented in similar proportions. For the genus *Aspidosperma*, self-compatibility has been reported in *A. quebracho-blanco* (Lin and Bernardello 1999), while late-acting self-incompatibility has been observed in *A. macrocarpon* (Gibbs 1990). Pollination experiments confirm that *A. pyrifolium* is an obligate outcrossing species, with nocturnal moth-mediated pollination essential for fertilisation. This highlights its significant reliance on pollinators, as demonstrated in other Apocynaceae species (e.g., Waddington 1976; Haber 1984; Herrera 1991;

Darrault and Schlindwein 2005). The observed late-acting self-incompatibility mechanism in *A. pyrifolium* is consistent with reports from other Apocynaceae species, where incompatibility occurs at the ovule level after pollen tube germination and growth (Waddington 1976; Gibbs 1990; Lipow and Wyatt 1999). This mechanism is thought to prevent inbreeding depression by ensuring outcrossing while maintaining reproductive assurance in environments with unpredictable pollinator availability (Holsinger 1991; Gibbs 2014).

Our results highlight the essential contribution of nocturnal pollinators to the reproduction of ecologically and culturally significant plants in semi-arid ecosystems and underscore the urgent need for greater recognition of these often-overlooked ecological interactions. Considering its important biocultural value and its role as a keystone species, understanding the patterns described above may be instrumental for the conservation and management of *A. pyrifolium*. In this context, studies on *Cereus jamacaru* (Cactaceae) have shown that the management of sphingophilous species can alter floral traits, enhance resource availability, and increase reproductive success through more efficient pollination, while it may also introduce genetic barriers between managed and wild populations due to reduced pollinator sharing (Lima-Nascimento et al. 2024, 2025). These findings highlight the need for a cautious, ecologically informed approach when managing *A. pyrifolium*, which may be subject to similar dynamics. Furthermore, given the wide geographic distribution of *A. pyrifolium* (Brazilian Flora Group 2025), future studies are needed to determine whether the pollination patterns identified in a single population are consistent across the species' range.

To date, few studies have proved the fundamental role of nocturnal settling moths in the reproduction of native Caatinga plants (Domingos-Melo et al. 2019; Barral et al. 2022), in contrast to the prevailing focus on hawkmoth pollinators in the literature (San Blas and Devoto 2025). A deeper understanding of the ecological role of settling moths is strongly recommended as nocturnal pollination faces unique vulnerabilities (Macgregor and Scott-Brown 2020), which must be carefully considered in conservation strategies targeting species such as *A. pyrifolium*. Given the increasing pressures on pollinator populations in the Caatinga (Leal et al. 2017), conservation efforts should prioritize the protection of both hawkmoths and settling moths, along with their habitats, to safeguard the reproductive success of nocturnally pollinated species with biocultural importance.

5 | Conclusion

The flowering and pollination dynamics of *Aspidosperma pyrifolium* reflect a well-adapted phalaenophilous and sphingophilous system within the context of the Caatinga dry forest. The species' reliance on hawkmoths for effective pollination, which is complemented by the action of settling moths, highlights the critical role of nocturnal pollinators in maintaining reproductive success. The presence of a late-acting self-incompatibility mechanism further emphasizes the importance of outcrossing in this species. Future studies should explore the genetic basis of self-incompatibility in *A. pyrifolium* and investigate its potential implications for population structure and genetic diversity. Additionally, given the increasing pressures on pollinator populations in the Caatinga, conservation efforts should prioritize the preservation of hawkmoth and settling moth species and their habitats to ensure the continued reproductive success of nocturnal Lepidoptera-pollinated plants in this ecosystem.

Author Contributions

Joel A. Queiroz: conducted fieldwork, drafted the initial manuscript. **Arthur Domingos-Melo:** performed the statistical analyses, reviewed and refined the final version. **Zelma G. M. Quirino:** conducted fieldwork provided supervision and critical revisions. **Isabel C. S. Machado:** secured funding, supervised the study, contributed to the review process.

Acknowledgements

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Financial Code 001); Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for the Research Productivity Fellowship to I.C.S.M. (310508/2019-3) and PELD-Caatinga for financial support for fieldwork; and Fundação de Amparo à Ciência e Tecnologia de Pernambuco - FACEPE for postdoctoral fellowships to A.D.-M. (BCT-0085-2.03/19; DCR-0031-2.03/21). The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used is presented directly in the article and is openly available at https://doi.org/10.5281/zenodo.15786962.

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