Article

IMPROVED METHOD FOR MEASURING POLLEN DEPOSITION BY NECTAR-FEEDING BATS: A CASE STUDY USING *NICOTIANA OTOPHORA* GRISEB. (SOLANACEAE)

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Abstract—Pollen deposition experiments are widely used to study pollination systems in a variety of experimental conditions. However, pollen capture techniques available in the literature have not addressed the possibility of using the same experimental flowers multiple times. This study presents an optimized technique designed for quantifying repeated pollen depositions on the same flowers by floral visitors, using *Nicotiana otophora* Griseb. (Solanaceae) as a model system. The technique consists of attaching a pollen-trapping surface to the floral stigma, which is made of a thin sheet of transparent plastic covered with different adhesive materials tested (double-sided tape, silicone oil, and glycerin jelly). These materials facilitate the adherence of pollen transported by anthophilous bats and deposited onto the stigmatic surface during floral visits. The main advantage of this protocol is the rapid replacement of the pollen-capture surface, allowing the same flower to be reused multiple times and enabling the documentation of fine-scale temporal variation in pollen deposition during anthesis. To validate the methodology, the efficiency of the three adhesive materials was compared after a single visit per flower by two nectar-feeding bats, *Glossophaga soricina* or *Anoura caudifer* (Phyllostomidae). There were no significant differences in pollen capture capacity among the different adhesive materials or between the bat species, demonstrating the robustness of the method, independent of the material used and the floral visitor involved. In addition to providing direct evidence of effective pollination by bats in *N. otophora*, this methodological approach represents a simple, economical, and field-applicable alternative that does not alter the visitor's behaviour and is useful for comparative studies of pollination effectiveness.

Keywords—*Anoura caudifer*, *Glossophaga soricina*, Chiroptera, floral visit, improved method, pollination

INTRODUCTION

A pollinating agent is defined as a floral visitor that transfers pollen from the anthers of a flower to the receptive stigma of the same or a different flower of the same species, potentially having a beneficial effect on the sexual reproduction of plants through ovule fertilization and seed set (Abrol 2011; Willmer 2011; Freitas 2013). The estimation of the effectiveness of a floral visitor as a pollinator should consider the frequency of floral visits, the amount of pollen removed from the anthers, the deposition of conspecific pollen on the stigma, fruit set, and seed production (Willmer 2011; Freitas 2013).

One of the most frequently used methods to evaluate pollen deposition by a floral visitor is exposing the stigmas of virgin flowers to single visits and subsequently collecting them to count the number of pollen grains deposited on the stigma (Kearns & Inouye 1993). Recently, more novel techniques have been implemented, such as the placement of double-sided adhesive tape on the stigmas of flowers, which allowed the quantification of pollen deposition by bats and hummingbirds in 10 species of plants of the genus *Burmeistera* H. Karst. & Triana (Campanulaceae) (Muchhala 2006). Similarly, an adhesive tape ring rolled up to form a cylinder, has also been used to capture pollen deposition by bats, hummingbirds, sphingids, and other moths in *Aphelandra acanthus* Nees (Acanthaceae) flowers (Muchhala et al. 2008).

In this study, we tested a method useful to evaluate repeated pollen depositions on the same experimental flowers by floral visitors, based on methods proposed by Muchhala (2006) and Muchhala et al. (2008), inspired by the operation of Hirst-type volumetric traps (Hirst 1952), which are the most widely used monitoring method in palynological stations worldwide (Buters et al. 2018). These aerobiological particle traps contain a transparent, continuously moving tape inside, which is covered by an adhesive substance to trap airborne particles (Thibaudon et al. 2017). In order to test our proposed methodology, we selected *Nicotiana otophora* Griseb., one Solanaceae species distributed from central and southern Bolivia to northwestern Argentina (Goodspeed 1954). Our method involves using a transparent, flexible sheet of cellulose acetate, coated with adhesive elements that facilitate the adhesion of pollen carried by visited flowers and act as a structural support that optimizes sample recovery.

Using *N. otophora* as a model system, we evaluated (1) whether three different adhesive materials (double-sided tape, silicone oil, and glycerin jelly) differ in their effectiveness in capturing pollen deposited by nectarivorous bats during a single floral visit; and (2) whether pollen deposition effectiveness varies depending on the bat species performing the floral visit. We expected that if the tested method were robust enough, differences among adhesive materials would be negligible and consistent across visitor species, and that the method would perform reliably across single-visit deposition events. The pollen-capture optimization technique we examined in this study allowed us to address a key gap in the biology of *N. otophora*, where floral traits and previous studies have suggested bat pollination (Vogel 1969; Nattero et al. 2003; Boero 2021), but direct evidence of effective pollen transfer was lacking. By combining methodological evaluation with single-visit measurements, our results provide the first direct evidence of pollen deposition on stigmas of *N. otophora* by anthophilous bats, demonstrating actual pollination events under natural conditions and the functional role of bats as legitimate pollinators of this species.

MATERIALS AND METHODS

STUDY SITE

This study was conducted in the Piquirenda Viejo, Aguaray Municipality, General San Martín Department, Salta Province (22°20'14.57"S; 63°50'41.42"W), located in northwestern Argentina. This area belongs to the Yungas ecoregion, which, together with the Paranaense Rainforest in the northeast, constitutes Argentina's subtropical forests (Brown et al. 2005; Rodríguez & Silva 2012) and the southern limit for the distribution of humid tropical forests, as well as for the interaction between nectarivorous bats and plants in the Neotropics (Brown et al. 2002; Boero 2021; Bracamonte 2022).

The study site represents a transitional forest slope moving toward mountain forest, characterized by a warm, sub-humid climate, with few winter frosts and summer rainfall of approximately 1,000 mm of average annual rainfall. It has been recognized by RELCOM (Red Latinoamericana y del Caribe para la Conservación de los Murciélagos; https://www.relcomlatinoamerica.net/) as an AICOM (Área de Importancia para la Conservación de los Murciélagos; Bracamonte 2022).

STUDY SPECIES

Nicotiana otophora is a woody shrub or subshrub that has flowers arranged in loose, simple, or sparsely branched panicles, which emerge at an inclined angle of up to 40 cm above the upper foliage, placing the flowers in an optimal position for exposure (Goodspeed 1954; Vogel 1969). The flowers are protogynous, featuring a campanulate calyx, and the corolla consists of a greenish tubular basal portion and a distally enlarged throat-shaped portion that is usually greenish, although it is often surrounded by a red or reddish-purple line near the corolla edge (Fig. 1). The corolla limb is retroflexed and necrotic at the apex, meaning it curves backward during a concave expansion, while the short, acuminate tips wilt prematurely. The stamens are notably exserted and dorsally arranged, with the filaments inserted at the base of the corolla and curved at their apical end; the style is slightly longer than the stamens (Goodspeed 1954; Cocucci & Hunziker 2005). Vogel (1969) suggested that the flowers of this species may be adapted to bat pollination, as they are relatively small, have a mask-like corolla shape, open at dusk, and remain open for several days and nights.

In northwestern Argentina, there are records of interactions of *N. otophora* with different animals considered as its floral visitors. In the Jujuy Province, Nattero et al. (2003) carried out video recordings on floral visits, which allowed them to record the presence of *N. otophora* pollen on the proboscis of six moth species (Sphingidae, Lepidoptera): *Manduca diffissa*, *M. tucumana*, *Euryglottis guttiventris*, *Eumorpha neuburgeri*, *Erinnyis ello*, and *Xylophanes pluto*. They also captured a specimen of *Anoura caudifer* (Phyllostomidae, Chiroptera) carrying pollen on its snout and forehead and could extract pollen

Figure 1. Flower of *Nicotiana otophora* with the pollen-capturing surface attached to the stigma. Note the parafilm paper used to isolate the stigmatic surface and allow the placement of the pollen-capturing surface. Scale = 10 mm

from the gut after dissection. Additionally, in the Salta Province, floral visits by bees, bumblebees, moths, hummingbirds, and bats were recorded through videos and direct observations (Boero 2021; Hoyos-Díaz et al. unpublished data). Moreover, according to data obtained from individuals sampled in an artificial roost, the pollen of this species was an important component of the diet of *Glossophaga soricina* (Phyllostomidae, Chiroptera; Boero 2021). Together, these results demonstrate that bats visit *N. otophora* flowers to take advantage of nectar and/or pollen as food, probably functioning as effective pollinators.

Pollen deposition

Between October 30th and November 13th, 2023, and from October 2nd to 7th, 2024, we recorded pollen deposition by anthophilous bats following a single floral visit, using freshly opened flowers of *N. otophora* (see details in the following section). For this purpose, we wrapped the flower stigma with parafilm paper to isolate the moist stigmatic surface, following the method described by Muchhala (2006) and Muchhala et al. (2008). Subsequently, we placed a pollen-capturing surface, consisting of a thin, squared transparent plastic sheet measuring 4 x 4 mm. The sheet has double-sided tape on one side, allowing it to be placed on the stigma, leaving the other side exposed to floral visitors (Fig. 1).

We examined the effectiveness of the proposed technique for recording pollen deposited by bats during floral visits by comparing three pollen-capture materials placed on the plastic sheet, using six to nine individuals: (1) Plastic sheets with double-sided tape on both sides were installed, a method for capturing pollen from floral visitors that has been employed in various investigations (Muchhala 2006; Muchhala 2007; Muchhala & Potts 2007; Muchhala et al. 2008; Muchhala & Thomson 2009; Muchhala & Thomson 2010; Caballero-Martínez et al. 2012; Cárdenas Cajamarca 2017; Lagomarsino & Muchhala 2019; Moreira-Hernández 2022), wich is based on the tape's adhesive capacity to capture pollen grains during the floral visit. (2) A thin layer of silicone oil was applied to the installed plastic sheet using a brush, an inert substance used in several Hirst-type aerobiological particle traps (Hirst 1952; Thibaudon et al. 2017). (3) A small amount of glycerin jelly with basic fuchsin (Beattie 1971) was applied on the plastic sheet, a substance used in palynological preparations to capture and visualize pollen grains.

Following a single floral visit to each experimental flower, we mounted the plastic sheets with double-sided tape and the silicone oil on microscopic slides, positioning the pollen-capturing surface between the slide and the cover slip. For each sample obtained, we applied a small amount of glycerin jelly stained with basic fuchsin onto the coverslip, which we melted by briefly holding it near the flame of a lighter, taking care to avoid the formation of bubbles. Then, we positioned the plastic sheet on the molten jelly, ensuring that the side exposed to floral visitors was in direct contact with the glycerin jelly. Clear nail polish was used to seal the preparation. For the mounting of the capturing surfaces to which glycerin jelly with basic fuchsin was applied, we removed the stigmatic surface from the flower and, using an alcohol-sterilized palynological needle, transferred the portion of jelly from the sheet to a slide. The jelly was melted by bringing the slide close to the flame of a lighter and subsequently covered with a cover slip, sealing the preparation with previously melted liquid paraffin.

To identify pollen grains present in the samples, palynological slides were prepared from *N. otophora* and other plant species with nocturnal anthesis in the study area to serve as a reference collection. The samples from the different trials were examined under an optical microscope (Carl Zeiss Primo Star) and the presence of pollen was recorded as positive if at least three pollen grains were found. The observed pollen was identified and quantified using the scanning technique following Girón Vanderhuck (1995). During the examination of the samples prepared with the plastic sheets, we used a higher light intensity to compensate for the reduced transparency caused by the adhesive tape. This tape, which was used to secure the sheet, was kept in place during the mounting process.

FLORAL VISITORS

To record floral visits, a Minolta MN4K20NV camcorder with night vision capability (IR Night Vision Mode) was installed on a tripod, positioned approximately 2 m from individuals with flowers that opened that same night. Using the camcorder screen, we observed the flowers to record floral visits. When a floral visit occurred, we immediately removed the pollen-capturing surface and mounted it onto a slide, noting the visit time for the floral visitor, who would later be identified from the recording. When possible, we reused the same flower to record new pollen depositions, replacing the capturing surface after each visit. While reuse of a flower was possible in most cases, specific situations limited this procedure. These situations included physical damage to the floral style or accidental detachment of the flower as a result of repeated handling during the installation and removal of the pollen-collecting surfaces. We acknowledge floral damage from handling as an inherent constraint of the method, which depends largely on the fragility of the reproductive structures of the species.

For the correct identification of bat species in the recordings, we used snout size and uropatagium shape and size as diagnostic criteria. The nectarivorous species are distinguished from each other because *A. caudifer* has a reduced uropatagium and the longest snout, compared to *G. soricina*, which has a well-developed uropatagium and a comparatively shorter snout (Barquez & Díaz 2020). Frugivorous phyllostomid bat species that could potentially consume floral resources in northwestern Argentina (*Artibeus planirostris*, *Sturnira lilium*, *Sturnira erythromos*, *Sturnira oporaphilum*, and *Pygoderma bilabiatum*; Barquez et al. 1999), have short faces and larger bodies, which differentiates them from the two nectarivorous bat species in the study location.

STATISTICAL ANALYSIS

To evaluate the effects of adhesive type of pollen-capturing surfaces (double-sided tape, silicone oil, and glycerin jelly) and bat species (*G. soricina* and *A. caudifer*) on the number of pollen grains deposited on the adherent surface, we fitted Generalized Linear Models (GLM) that included the interaction between the two factors. The time elapsed from sunset was incorporated as a continuous covariate to assess the potential temporal variation in pollen deposition throughout the night. The temporal frame was determined by considering the visit time and the evening twilight time obtained from the Servicio de Hidrografía Naval de Argentina (https://www.hidro.gov.ar/observatorio/Astronomia.asp). Due to the overdispersion of the count data, a negative binomial error distribution was used. All analyses were performed using R version 4.4.2 (R Core Team 2024).

RESULTS

FLORAL VISITS

All floral visits observed (61) on *Nicotiana otophora* were performed by nectarivorous bats, *Anoura caudifer* and *Glossophaga soricina* (Fig. 2A, B). In the video recordings, the bats were observed approaching the flowers from different positions; they positioned themselves in front of the flowers and introduced their snouts into the corolla, accessing the nectar and simultaneously contacting the anthers and stigma. Each floral visit lasted less than one second (1 sec) and during the visit the bats maintained sustained flight. On occasions, it was observed that at the end of the visit the bats slightly dropped before beginning their withdrawal flight (Supplementary Video).

POLLEN DEPOSITION

We recorded a single floral visit on each of 61 pollen-capturing surfaces, representing the three trials evaluated. Of these, 21 corresponded to surfaces with adhesive tape, 20 to surfaces with silicone oil, and 20 to surfaces with glycerin jelly. The analysis of pollen presence on the capturing surfaces indicates that pollen exclusively from *N. otophora* was recovered in all three treatments. The percentage of samples with pollen presence was high for all three treatments: 100% for surfaces with adhesive tape, 80% for those with silicone oil, and 95% for surfaces with glycerin jelly.

The number of pollen grains deposited by anthophilous bats showed high variation among samples for both bat species, ranging from visits without pollen deposition to visits with more than a thousand pollen grains deposited on a single capturing surface (Table 1). The largest single-visit depositions were recorded on silicone oil surfaces: 3,488 grains by *A. caudifer* and 1,806 grains by *G. soricina*. Although silicone oil showed the lowest overall frequency of pollen deposition, primarily due to a lower pollen detection rate during *A. caudifer* visits (57.1%), it recorded the highest individual pollen deposition. In contrast, double-sided tape and glycerin jelly provided more consistent results for both bat species, with high frequencies of pollen detection (91.6–100%). Overall, pollen deposition was detected in a high proportion of samples across all treatments (57.1–100%), supporting the reliability of the proposed pollen-capture surfaces for monitoring single-visit deposition events.

GLM with a negative binomial error distribution showed no significant effects in the number of pollen grains captured among the three types of pollen-capturing surfaces (Likelihood Ratio Test, $\chi^2 = 2.06$, $df = 2$, $P = 0.356$), with estimated means of 553 ± 184 SE for silicone oil, 339 ± 112 SE for double-sided tape, and 275 ± 94 SE for glycerin jelly. No significant differences were detected between bat species ($P = 0.647$) nor in the interaction between surface type and bat species ($P = 0.77$). Time elapsed since sunset also had no effect on pollen deposition ($P = 0.826$). These results indicate that the three proposed variants of adherent surface are robust enough and that both species of visiting bats seem to be equally effective pollen vectors for *N. otophora* in the study area.

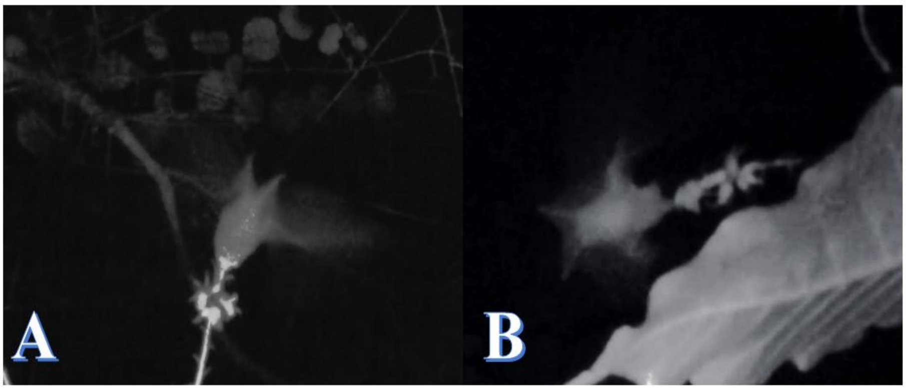

Figure 2. Floral visits to *Nicotiana otophora* by the nectarivorous bats *Anoura caudifer* (A) and *Glossophaga soricina* (B).

Table 1. Number (*N*) and percentage (%) of samples with positive pollen registration, mean, standard deviation (SD) and range of number of *Nicotiana otophora* pollen grains deposited by anthophilous bats (*Glossophaga soricina* and *Anoura caudifer*) on the pollen-capturing surfaces in the three evaluated treatments. Generalized Linear Models (GLM) with a negative binomial distribution showed no significant differences in pollen deposition among treatments (*P* = 0.356), between bat species (*P* = 0.647), or due to visit timing (*P* = 0.826).

	Double-sided tape (*N* = 21)			Silicone oil (*N* = 20)			Glycerin jelly (*N* = 20)		
Species	N (%)	Mean (± SD)	Range	N (%)	Mean (± SD)	Range	N (%)	Mean (± SD)	Range
Glossophaga soricina	10 (100 %)	376 (± 364.5)	24 - 1076	13 (92.3 %)	491.9 (± 543.2)	0 - 1806	8 (100%)	209. 3 (± 250.5)	8 - 638
Anoura caudifer	11 (100 %)	330.1 (± 258.4)	8 - 915	7 (57.1 %)	591.1 (± 1297.5)	0 - 3488	12 (91.6 %)	343.6 (± 452.7)	0 - 1311

Discussion

Our proposed methodological approach for optimizing pollen deposition recording techniques developed by Muchhala (2006) and Muchhala et al. (2008) represents simple, economical, and practical methods for application under field conditions. These methods apparently do not alter the behaviour of bats visiting the flowers of *Nicotiana otophora*, allowing for effective assessment and quantification of pollen deposition events. Their main benefit is that they allow the utilization of a single experimental flower multiple times during anthesis, due to the possibility for replacement of the surface used for pollen capture.

Pollen deposition methods used until now have allowed the comparison among groups of functional visitors categorized by their activity (Caballero-Martínez et al. 2012; Lagomarsino & Muchhala 2019) and pollen deposition events of different floral visitors that could act as pollinators (Muchhala 2006; Muchhala et al. 2008; Cárdenas Cajamarca 2017). Similarly, they enabled the investigation of questions related to the mechanical fit between the corolla and the pollinator (Muchhala 2007), the divergence in the exertion length of the floral structures of sympatric species and their relationship with competition for pollinators (Muchhala & Potts 2007), as well as the mechanical efficiency in pollen deposition between the fur of bats and the plumage of hummingbirds (Muchhala & Thomson 2010), quantifying deposition in extreme floral morphologies (Muchhala & Thomson 2009) and interspecific pollen transfer in recent neotropical radiations (Moreira-Hernández 2022).

In addition, previous research has shown that pollen deposition by bats can vary throughout the night, depending on changes in nectar production and bat foraging behaviour during floral anthesis (Domingos-Melo et al. 2023). Because our proposed method allows for rapid replacement of the pollen-capturing surface, it is well-suited for recording this temporal variation at such a fine scale within a single flower. This highlights its value in studies aimed at understanding the pollen deposition dynamics during successive visits within the same anthesis period, opening the possibility for its use to address a broad spectrum of the research questions, including the above-mentioned ones.

The proposed method is best suited for flowers with large and accessible stigmas that allow to be wrapped in parafilm without obstructing the corolla or altering the floral geometry. Among the categories of flowers visited by bats (Fleming et al. 2009), "shaving-brush" or "stamen ball" types (e.g., Malvaceae and Lythraceae) constitute ideal candidates only when their stigmas offer a large and exposed contact surface. Cup-shaped, tubular or bell-shaped flowers could be adequate if their size and the stigma morphology allow the pollen-capture surface to be placed correctly. Finally, in pollination experiments with inflorescences, all the flowers with receptive stigmas should be treated to evaluate pollen deposition per reproductive unit. Overall, we recommend conducting preliminary tests to assess whether the stigma size and morphology allow for proper installation of the pollen-capture system without altering the behaviour of pollinators.

We recognize that the proposed pollen-capture method with its three variants has dimensional limitations and uncertainty persists regarding whether the capturing surfaces retain more or less pollen than the flower's stigma, due to factors such as the adherence of the element used (adhesive tape, glycerin jelly with basic fuchsin, silicone oil) or the size of the capturing surface. However, the robustness of the technique lies in its reliability for comparative purposes, supported by the fact that no significant differences were detected in the number of pollen grains deposited by nectar-feeding bats on the different adhesives used on the pollen-capturing surfaces, nor was there an effect of the timing of the visit (time elapsed since sunset).

Our proposed method provides an effective and reliable option for examining pollen deposition by nectar-feeding bats on *N. otophora*. The three adhesive treatments tested performed satisfactorily, with a high frequency of pollen deposition events observed across all materials (80–100%), although variation was observed, such as the lower proportion of pollen present in *A. caudifer* on silicone oil surface (57.1%). Conversely, this bat species deposited the highest pollen in a single visit (> 3,000 grains). Despite this evident variability, statistical analyses showed no significant systematic differences between the methods. Taken together, our results suggest that a high proportion of visits by bats to *N. otophora* result in pollen deposition, with nectarivorous bats depositing pollen in at least half of their visits across all treatments. Furthermore, considering the observed range of pollen grains deposited per visit (0–3,488) in relation to the estimated number of ovules per flower in *N. otophora* (840–9600; Hoyos-Díaz et al., unpublished data), our findings suggest that bats can deposit sufficient pollen to fertilize a substantial proportion of ovules per flower, particularly when multiple visits occur during a flower's lifespan. These findings confirm that both *G. soricina* and *A. caudifer* are highly effective pollinators, transferring substantial and comparable pollen loads that are critical for the reproductive success of *N. otophora* in the study area.

We can assure that the recovered pollen was deposited exclusively by bats during their single visit. This is guaranteed by our experimental design: we used protogynous flowers of *N. otophora* that opened on the same night of the experiment, thereby preventing any prior pollen deposition by other floral visitors. This pollen could originate from flowers of other individuals (cross pollen) or from other flowers of the same individual (geitonogamous pollen). It is also relevant to note that the presence and the high quantity of conspecific pollen recorded on the capturing surfaces after a single floral visit performed by *G. soricina* and *A. caudifer*, confirm that both bat species play an important role as effective pollinators of *N. otophora*, emphasizing that the absence of significant differences between the species suggests that both bats contribute equally to the reproductive success of this plant, providing direct evidence that confirms predictions based on floral morphology (Vogel 1969) and previous records of pollen contact (Nattero et al. 2003; Boero 2021). Furthermore, although this technique was tested to evaluate anthophilous bats, it could also be used to examine the performance of other floral visitors of *N. otophora* (e.g., hawkmoths), as combining pollen deposition data with visitation frequency would allow for a more accurate designation of the relative importance of each visitor group as functional pollinators of this species.

The high percentage of conspecific pollen in the samples examined could be explained by the position of the anthers and stigma of *N. otophora*, which occupy a similar position on the flower, located dorsally and projecting slightly beyond the corolla margin. This allows the reproductive floral parts to come into contact with the same regions of the bat's body during a floral visit. This interpretation is supported by video analyses that identify the nape of the neck as a potential contact site for the anthers (Boero 2021) and by records at the study site of *N. otophora* pollen on the forehead and dorsal region of an *A. caudifer* individual captured with mist nets (Hoyos-Díaz et al., unpublished data), coinciding with records in northwestern Argentina of pollen on the snout and forehead of *A. caudifer* (Nattero et al. 2003).

The use of plastic sheets as platform for adhesive materials to capture pollen during floral visits represents a practical and accessible refinement of existing pollen capture protocols, with demonstrated reliability across three adhesive materials and two pollinator species. Its key advantage, the ability to sample pollen repeatedly across multiple visits by replacing the capturing surface, opens new possibilities for fine-scale temporal studies of pollination dynamics. We encourage its testing and adaptation in other plant-pollinator systems with floral morphologies compatible with the method's requirements. In addition to its methodological contributions, this study provides the first direct evidence of pollen deposition by nectarivorous bats on *N. otophora*, confirming the ecological role of *G. soricina* and *A. caudifer* as pollinators of this plant at the southernmost margin of their Neotropical distribution.

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AUTHOR CONTRIBUTION

All authors conceived the study. JMHD collected the field and laboratory data, analysed the data, and drafted the initial version of the manuscript. All authors contributed substantially to the manuscript and gave final approval for publication.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

GENERATIVE AI DISCLOSURE STATEMENT

The authors declare that no generative AI or AI-assisted technologies were utilized at any stage of the research or manuscript development.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I: Supplementary Video - Floral visits by the nectarivorous bats *Anoura caudifer* and *Glossophaga soricina* (Phyllostomidae) to *Nicotiana otophora* in Salta Province, northwestern Argentina.

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