Brief Communication / Comunicación Breve

Nocturnal delivery: First record of orchid pollinia (Orchidales: Orchidaceae) on prominent moths (Lepidoptera: Notodontidae)

Entrega nocturna: Primer registro de polinios de orquídea (Orchidales: Orchidaceae) en polillas prominentes (Lepidoptera: Notodontidae)

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Abstract. Moths are significant pollinators and flower visitors globally, however their roles and mutualistic relationships with plants remain underexplored. This study highlights the novelty of the Nystaleinae *Nycterotis jacobensis* (Thiaucourt, 2008) as a potential orchid pollinator, evidenced by pollinia found on its proboscis. The specimen, collected in Utría National Natural Park, Colombia, would represent the first report of a prominent moth visiting orchids in this region. Additionally, we review recent observations of Dioptinae moths feeding on flowers, suggesting their potential role as pollinators. Our findings underscore the need for further research on the biology and ecology of Neotropical moths, particularly in ecosystems vulnerable to climate change. Future studies should focus on identifying the specific orchid species visited by *Nycterotis* and other Nystaleinae, as well as implementing diverse methodologies to assess the impact of these nocturnal pollinators on tropical ecosystem dynamics and their response to environmental changes.

Key words: Dioptinae; Neotropical; *Nycterotis*; Nystaleinae; pollen.

Resumen. Las polillas son polinizadores y visitantes de flores de gran importancia a nivel mundial, aunque sus roles y relaciones mutualistas con las plantas aún están poco explorados. Este estudio destaca la novedad de *Nycterotis jacobensis* (Thiaucourt, 2008) de la subfamilia Nystaleinae como polinizador potencial de orquídeas, evidenciado por la presencia de polinios en su probóscide. El espécimen, recolectado en el Parque Nacional Natural Utría, Colombia, representaría el primer caso publicado de una polilla prominente polinizando orquídeas en esta región. Además, revisamos observaciones recientes de polillas Dioptinae alimentándose de flores, lo que sugiere su posible papel como polinizadores. Nuestros hallazgos subrayan la necesidad de más investigación sobre la biología y ecología de las polillas neotropicales, particularmente en ecosistemas vulnerables al cambio climático. Los estudios futuros deberían centrarse en identificar las especies específicas de orquídeas polinizadas por *Nycterotis* y otros miembros de Nystaleinae, así como en implementar metodologías diversas para evaluar el impacto de estos polinizadores nocturnos en la dinámica de los ecosistemas tropicales y su respuesta a los cambios ambientales.

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Palabras clave: Dioptinae; Neotropical; *Nycterotis*; Nystaleinae; polen.

Moths are globally recognized as important pollinators and flower visitors. According to Krenn (2010) and Wardhaugh (2015), 90% of butterflies and moths visit flowers. In Brazil's Cerrado, 11% of plants are pollinated by moths, and in Costa Rica, an estimated 16% of flowering trees rely on moth pollination (Bawa *et al.* 1985; Oliveira *et al.* 2004). Similarly, in the U.K., one-third of Noctuidae species and one-tenth of Geometridae species have been identified as flower visitors (Norris 1936; Anderson *et al.* 2023). Despite their significance, many fundamental aspects of their roles as pollinators, flower visitors, and their mutualistic relationships with plants remain poorly understood, largely due to the lack of natural history and biological information about these organisms and their interactions (MacGregor *et al.* 2019; Van Zandt *et al.* 2020).

Among insect-plant interactions, moths' role in orchid pollination is quite well-known. Charles Darwin famously predicted the existence of a then unknown hawk moth as the likely candidate for feeding on (and thereby pollinating) the particularly long-spurred Madagascan orchid *Angraecum sesquipedal* Thouars (Arditti *et al.* 2012). This is just one case of moth-orchid coevolution and other orchid species are also known to be almost entirely or exclusively visited by moths, such as *Dendrophylax lindenii* (Lindl.) Bentham ex Rolfe which is visited by sphingids and *Platanthera chlorantha* (Custer) Rchb by *Sphinx pinastri* Linnaeus, 1758 (Danaher *et al.* 2020; Steen *et al.* 2019), and several species have been identified as effective pollinators (Xion *et al.* 2020). Orchids employ various reproductive strategies, including specialized structures known as pollinia— masses of pollen grains clumped together into specific structures, and their shape, size, and orientation are of taxonomic importance (Wan-Ting *et al.* 2023). Pollinia have been found on different moth families during their foraging behaviors, such as on the heads of Noctuidae and Sphingidae, and on the tarsi of Drepanidae (Graham 2010; Fox *et al.* 2013; Van Zandt *et al.* 2020).

The role of prominent moths as pollinators or flower visitors remains underexplored. The Notodontidae, with over 4,700 species and 21 subfamilies (Prada-Lara *et al.* 2023; St Laurent *et al., in review*), are still relatively poorly studied in their biology, ecology, and natural history. Few species have been studied in depth, being smaller and less well-studied than the large charismatic Sphingidae, it is no surprise that the nocturnal habits of notodontids have avoided scrutiny. The only known adult feeding behavior in this family is observed in the Dioptinae which feed on flowers, puddling in Pygaerinae, Dudusinae, which are tear/mucus drinkers, and a record of an *Hemiceras* sp. feeding on orchids (however no deposition of pollinia into stigmatic cavities were observed) (Miller 1991; Prada-Lara *et al.* 2023; Rech *et al.* 2010). However, the outlook for the development of Notodontidae life history research is beginning to change. With the contributions of community science, which is generating new information at an extraordinary rate, and newly collected material, we are gaining fresh insights into these moths. In the present work, we state the novelty of the Nystaleinae *Nycterotis jacobensis* (Thiaucourt, 2008) as a possible orchid pollinator due to the presence of pollinia on the proboscis. We also review recent sightings of Dioptinae feeding on flowers and their potential role as pollinators. These discoveries promise to enhance our understanding of the evolutionary biology and ecology of Neotropical moths, addressing previous knowledge gaps and contributing to broader ecological research.

The examined moth in this study was found deposited in the Notodontidae section of the "Museo Javeriano de Historia Natural" (MPUJ) in Bogotá, which is the Colombian collection with the highest number of prominent moths (Prada-Lara *et al.* 2023). To identify the specimen, we dissected the genitalia following the standard techniques proposed by Miller (1987) and followed the work by Thiaucourt (2008). The photographic records of

the Dioptinae were obtained from iNaturalist (https://www.inaturalist.org/) with the corresponding permissions of the authors.

Images were taken with a Fujifilm Finepix S8300 camera and with a stereomicroscope ZEISS StereoDiscovery V20. Plates were arranged using Adobe Photoshop Creative Cloud 2024.

To document orchid occurrences specific to the collected moth (Utría National Natural Park (PNN Utría)), we examined records deposited in the main herbaria of Colombia, including Herbario Pontificia Universidad Javeriana (HPUJ), Colección Herbario Federico Medem (FMB), Herbario Jardín Botánico de Bogotá (JBB), Herbario Universidad del Cauca (CAUP), Herbario Joaquín Antonio de Medellín (JBM), Herbario José Cuatrecasas Arumí (VALLE), Herbario Forestal Universidad Distrital Francisco José de Caldas (UDBC), and others. In addition to herbarium records, we accessed digital data from repositories such as the Global Biodiversity Information Facility (GBIF) and Tropicos, along with field observations obtained from iNaturalist (https://www.inaturalist.org/).

The genus *Nycterotis* Felder, 1874 is currently composed of 41 species, distributed from the USA to Argentina. This genus is one of the most diverse within the Nystaleinae, exhibiting significant morphological variation that requires genitalia dissections for accurate species-level identification (Prada-Lara *et al. in prep*). Seven species have been recorded in Colombia (Prada-Lara *et al.* 2023), and this study presents a new distribution record for *N. jacobensis*, previously not recorded for the country.

Examined material. 1 male. COLOMBIA: Chocó, PNN Utría, Puente Esterogrande, Segunda Estación. N 06°01'08.8'' W 77°20'44.8''. 15 ago-14 sept 2017. 6 m. L. Prada & S. Vargas. MPUJ_ENT0047838.

According to Fox *et al*. (2013), finding a specimen with pollinia is the most convincing evidence that a species acts as a pollinator; however, it is necessary to develop studies of the frequency of visits and seed production in the field to confirm this role. *Nycterotis jacobensis* was found carrying four pollinia on its proboscis (Fig. 1), confirming it as a potential orchid pollinator—the first known instance of a Nystaleinae pollinating these remarkable plants.

Additionally, this moth was collected in Utría National Natural Park, Chocó (PNN Utría), a region renowned for its high rates of endemism and biodiversity (Velásquez *et al.* 2006). To date, 169 orchid species have been recorded in the Chocó department, with *Epidendrum* L. being the most abundant and species-rich genus (Ledezma *et al.* 2006). However, an official orchid species list for the park has not yet been created.

Our search into orchid records for PNN Utría revealed only one herbarium specimen corresponding to the species *Vanilla dressleri* Soto Arenas (Vanilloideae). Additionally, we identified 11 observations from iNaturalist that include the following species: *Brassavola nodosa* (L.) Lindl. (Epidendroideae), *Epidendrum nocturnum* Jacq. (Epidendroideae), *Eriopsis biloba* Lindl. (Epidendroideae), *Elleanthus capitatus* (Poepp. & Endl.) Rchb.f. (Epidendroideae), *Maxillaria fulgens* (Rchb.f.) L.O.Williams (Epidendroideae), *Vanilla rivassi* Molineros, R.T.González, Flanagan & J.T.Otero (Vanilloideae), and *Polystachia* sp. (Epidendroideae).

The information obtained about local orchids and the morphology of the four pollinia joined by a sticky viscidium observed on the moth, allowed us to determine that they are typical of the Epidendroideae subfamily (Johnson & Edwards 2000; Singer *et al*. 2008). However, higher resolution analysis is needed to determine the specific genera or species accurately.

Many Neotropical Notodontidae, as noted by Weller (1989), possess a well-developed proboscis and are proficient fliers. This observation is confirmed here since *N. jacobensis* was collected in the mangrove ecosystem of this National Park (which spans 33 hectares), and at its maximum tidal limit, this ecosystem transitions into tropical rainforest, where orchids and other floral food sources are likely found.

Among the 21 subfamilies of Notodontidae, Nystaleinae is one of the subfamilies that is known to exhibit nectar-feeding habits (*Hemiceras* recorded by Rech *et al*. 2010, and the formerly subfamily Dioptinae, which was recently synonymized with Nystaleinae, is known to feed on flowers (Miller 1991; St Laurent *et al. in review*)), supporting the role as potential pollinators of orchids proposed by the presence of pollinia on *Nycterotis*, and photographic records of *Poresta lanassa* (Druce, 1890) and *Didugua argentilinea* Druce, 1891 feeding on flowers (Fig. 2). Many genera of "Dioptinae" are largely or entirely diurnal and several genera have been observed visiting and drinking nectar on flowers (Miller 1991, 2009) (Fig. 2).

It is urgently necessary to commence studies that allow us to understand the biology and ecology of these species, especially those in ecosystems most vulnerable to climate change, like Utría National Natural Park, which may be in particular impacted by sea level rise. Assessing the influence of a pollinator species on a plant species can be accomplished through various methods, such as direct observation, querying large community science databases like iNaturalist (https://www.inaturalist.org/), examining collected moths for adhered pollen, using floral traps, and implicitly by analyzing proboscis morphology (Van Zandt *et al.* 2020). These strategies are crucial and should begin to be used in different research studies, considering that plants pollinated by moths exhibit greater gene flow, longer-distance dispersal, and higher quality pollination compared to plants pollinated by butterflies (Macgregor *et al.* 2015; Anderson *et al.* 2023).

Figure 1. *Nycterotis jacobensis.* **A-F.** Proboscis with pollinia. Notice the proboscis is uncoiled and extended above the head. **G.** Male genitalia. **H.** VIII tergite (left above), sternite (right above) and aedeagus (below). **I.** Pollinia. Scale: 1 mm. / **Figura 1.** *Nycterotis jacobensis.* **A-F.** Proboscis con pollinia. Note que la proboscis está desenrollada y extendida sobre la cabeza. **G.** Genitalia masculina. **H.** tergito y esternito VIII arriba), y aedeago (abajo). **I.** Pollinia. Escala: 1 mm.

The identification of *N. jacobensis* as a potential orchid pollinator in Utría National Natural Park, Chocó, marks an advancement in our understanding of the ecology of prominent moths and their role in pollination. However, what we know is superficial since the specific orchid species to which the pollen belongs could not be identified, limiting the full comprehension of this interaction. Future research should focus on identifying the orchids pollinated by *Nycterotis* and other members of the Nystaleinae, as well as studying the biology and ecology of these moths in various ecosystems. Additionally, it is crucial to implement diverse methods for studying pollinators and their plants to comprehensively assess the impact of these organisms as providers of nocturnal pollination services, their role on tropical ecosystem dynamics, and their response to climate change.

Figure 2. Nystaleinae as flower visitors. **A-C.** *Josia* sp. **D.** *Lyces* sp. **E.** *Polypoetes* sp. **F-I.** *Scea* sp. **J.** *Didugua* sp. **K-L.** Male and female of *Poresta lanassa*. (Photographs authors (in order): Leonardo Leiva, Jason Headly, Lucas Fornero, Elizabeth Krüger, Roberto Cyrino, Carlo Brescia, Erik Oberg, Jordana, Josh Vandermeulen, Juan Cruzado Cortés, and Ricardo Moyano). / **Figura 2.** Visitantes florales de Nystaleinae. **A-C.** *Josia* sp. **D.** *Lyces* sp. **E.** *Polypoetes* sp. **F-I.** *Scea* sp. **J.** *Didugua* sp. **K-L.** Macho y hembra de *Poresta lanassa*. (autores de las fotografías (en orden): Leonardo Leiva, Jason Headly, Lucas Fornero, Elizabeth Krüger, Roberto Cyrino, Carlo Brescia, Erik Oberg, Jordana, Josh Vandermeulen, Juan Cruzado Cortés, and Ricardo Moyano).

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Author Contributions

LP: Conceptualization, methodology, investigation, writing original draft, writing review & editing, visualization. **RST:** Conceptualization, writing original draft, writing review & editing, visualization. **MR:** Methodology, investigation, resources, writing original draft. **GF:** Validation, resources, writing original draft, writing review & editing, visualization, supervision.

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