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Short Communication

Male Emphorini (Hymenoptera: Apidae) bees use flowers of *Ipomoea carnea* (Convolvulaceae) as overnight resting sites

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Abstract: The present study describes the behavior of male Emphorini bees in exploiting Convolvulaceae flowers as sleeping-sheltering place. The observations occurred inadvertently during a field study in an Agroforestry System in the Caatinga region, near the banks of the São Francisco River in Petrolina, Pernambuco – NE-Brazil. Males were observed and collected in wilted flowers of *Ipomoea carnea* (Convolvulaceae) during the evening. The sampling revealed nine Emphorini bees, belonging to three oligolectic species specialized on Convolvulaceae. We propose that the infundibuliform morphology of *Ipomoea carnea* flowers and the varied timing of anthesis could influence the choice of these flowers as overnight resting places. Furthermore, we endorse the idea present in the literature that males of oligolectic bees generally engage in patrolling flowers utilized by females, thereby enhancing the chances of encountering potential mates, making these flowers rendezvous places. The observation of this behavior highlights the interdependence between bees and plants and raises the possibility that the loss of these flowers could negatively impact bees not only in a nutrition aspect but also regarding shelter and reproduction.
Keywords: *Caatinga; Ipomoea; Emphorini; floral visitor; Melitoma; Melitomella; oligolectic bees; rendezvous place.*

Machos de abelhas Emphorini (Hymenoptera: Apidae) utilizam flores de *Ipomoea carnea* (Convolvulaceae) como locais de pernoite

Resumo: O presente estudo documenta o comportamento de abelhas machos do grupo Emphorini em explorar flores da família Convolvulaceae como local para dormir e se abrigar. As observações foram realizadas inadvertidamente durante um estudo de campo em um Sistema Agroflorestal na região da Caatinga, próximo às margens do Rio São Francisco, em Petrolina, Pernambuco - Nordeste do Brasil. Machos foram observados e coletados em flores fechadas de *Ipomoea carnea* (Convolvulaceae) durante o entardecer. Em nossa amostragem, encontramos nove machos da tribo Emphorini, pertencentes a três espécies oligoléticas especializadas em Convolvulaceae. Propomos que a morfologia infundibuliforme das flores de *Ipomoea carnea* e os diferentes períodos de antese das flores podem influenciar a escolha dessas flores como locais de descanso durante à noite. Além disso, nossos dados endossam a ideia de que machos de abelhas oligoléticas geralmente patrulham flores utilizadas por fêmeas, aumentando assim as chances de encontrar possíveis parceiras, tornando essas flores locais de encontro para acasalamento. O registro desse comportamento destaca a interdependência entre abelhas e plantas e levanta a possibilidade de que a perda dessas flores possa impactar negativamente as abelhas não apenas em relação a sua nutrição, mas também em relação ao abrigo e reprodução.
Palavras-chave: *Caatinga; Ipomoea; Emphorini; visitante floral; Melitoma; Melitomella; abelhas oligoléticas; rendezvous place.*

Introduction

Flowers serve as ephemeral habitats for anthophilous organisms, ensuring a provision of resources – whether nutritional or otherwise – while also may acting as protective shelters (Raguso 2023). Among the biological interactions described between plants and animals, one of the most intriguing behaviors is the habit of sleeping inside flowers. This behavior has been recorded in many insect groups, such as bees, beetles, and flies (Pinheiro et al. 2017, Schlindwein & Martins 2000, Barahona-Segóvia et al. 2022**)**, and even in vertebrates as frogs (Cardoso & Gonçalves 2022). However, the use of flowers as sheltering-sleeping place is generally documented in occasional field observations, being rarely investigated systematically (see example in Welsford & Johnson 2012). As a result, there are gaps in understanding its meaning.

The behavior of male bees sleeping in flowers has been consistently documented in the literature, encompassing diverse records across various species, and being particularly prevalent among oligolectic ones – i.e. bees specialized in collecting pollen exclusively from a given specific plant taxa (Dodson 1966, Westrich 1989, Michener 2007, Milet-Pinheiro & Schlindwein 2010, Watts et al. 2013, Sabino et al. 2017). Moreover, reports indicate that male bees can engage in it individually, with each bee in a separate flower, or collectively, with several on the same flower, often forming a cluster of males (Pinheiro et al. 2017). It has been suggested that the number of males found in a flower is related to its morphology (Watts et al. 2013). Some proposed explanations for this male behavior in the aforementioned studies range from the simple use of the flower as shelter to a potential search for females interacting with flowers. In the first scenario, flowers would provide a non-nutritive reward, offering the male a place to spend the night with the assistance of a microclimate and hiding place, thereby eventually favoring the pollination. In the second hypothesis, males of oligolectic bees would tend to forage (and sleep) on plants used by females as exclusive pollen source. This behavior could ensure a higher probability to find mating partners. In this case, flowers can be defined as rendezvous places (i.e. locations where animals are likely to encounter mates - *sensu* Barrows 1976).

A notorious group distinguished by the prevalence of oligolectic bees is the Emphorini tribe, Apidae (Sipes & Tepedino 2005). To the best of our knowledge, there are only a couple of publications with anecdotal mentions of flower-sleeping behavior among bees of this tribe, but lacking proper descriptions. They are *Ptilothrix fructifera* on *Platyopuntia brunneogemmia*, Cactaceae (Schlindwein & Wittmann 1997), and *Melitoma segmentaria* on *Ipomoea* sp., Convolvulaceae (Cortopassi-Laurino et al. 2018). Given the limited understanding of numerous flower-insect interactions, many of these relationships may vanish before we unravel the mechanisms and reasons behind them (Cardoso et al. 2011). In this study, we present a novel and naturalistic report of such a phenomenon, detailing a group of bees from the Emphorini tribe sleeping inside the flowers of *Ipomoea carnea* (Convolvulaceae).

Material and Methods

The observation occurred opportunistically during a field study as part of a broader sampling effort focused on the comprehensive study of pollinators in the Caatinga seasonally dry tropical forest. The study site is an Agroforestry System along the banks of the São Francisco River, situated in an area of the municipality of Petrolina (9°21'27.2"S 40°21'03.8"W), Northeast Brazil (Figure 1A, B). The site conserves preserved areas of riparian forest (Figure 1C), an environment that has been shown to be crucial for the maintenance of bee fauna in the region (Mariano et al. 2024). Furthermore, this location employs permaculture practices, with native Caatinga areas preserved near different crops maintained through a family farming system (Figure 1D).

The plants in which the observations were performed belong to the Convolvulaceae family, specifically the species *Ipomoea carnea* Jacq (Figure 1E). In Brazil, there are 18 genera and 340 species of Convolvulaceae, distributed partially in open vegetations such as Cerrado and Caatinga (Paz & Pigozzo 2013). Among the main morphological characteristics of *I. carnea* flowers are their funnel-shaped structure, pink coloration, and floral resources (nectar and/or pollen) that tend to attract a diverse range of flower visitors (Paz et al. 2013, Martins et al. 2020). Another factor contributing to this diversity of visitors is the pattern of flower anthesis in this species; the flowers can open in the morning, afternoon, or night, each period attracting specific visitors (Paz et al. 2013). Therefore, at dusk, it is possible to find both recently opened flowers (Figure 2A) and flowers that have just wilted (Figure 2B).

Bee individuals were captured between 17h00 and 18h00 of May 26 2023 through active search on seven *Ipomoea carnea* plants. A total of 33 flowers were examined, including 21 with the tubular part upright and the distal part with the corolla closed, forming a protective enclosure. The closed flowers were opened to search for pollinators, revealing the presence of bees sleeping inside (Figure 2C). The collected bee individuals were euthanized with ethyl acetate, transported to the Laboratory of Ecological Interactions and Semiochemicals at the University of Pernambuco, prepared, and sent for identification by an expert.

Results and Discussion

Throughout our sampling, a total of nine male individuals from the Emphorini tribe were found, representing three different species: *Melitoma segmentaria* (Fabricius 1804) (n = 7), *M. osmiodes* (Ducke 1908) (n = 1) and *Melitomella grisescens* (Ducke 1907) (n = 1). While none of the open flowers contained bees, 43% of the closed ones were occupied by one male individual. Concerning the collected species, *M. segmentaria* had previously been reported overnighting in *Ipomoea* sp. (Convolvulaceae) in Bertioga, São Paulo - Brazil (Cortopassi-Laurino et al. 2018). Bertioga is located approximately 1,700 km from our study site and is situated within the Atlantic Forest, which represents a completely different biome. Thus, this observation may suggest that the sleeping behavior of Emphorini in *Ipomoea* spp. flowers is a more intricate relationship than currently reported. This makes sense, since *Melitomella grisescens* and *Melitoma segmentaria* (and probably also *M. osmiodes*) are oligolectic bees specialized on Convolvulaceae (Pick and Schlindwein 2011). The female bees of these species consistently use a small set of Convolvulaceae species as pollen source, suggesting that males might similarly choose the same species for overnight stays and find females in next day. If this indeed occurs, flowers of *Ipomoea* spp. could be considered rendezvous places for Emphorini. It is possible that this process went unnoticed, since the male bees were consistently found inside closed flowers, and observing this behavior

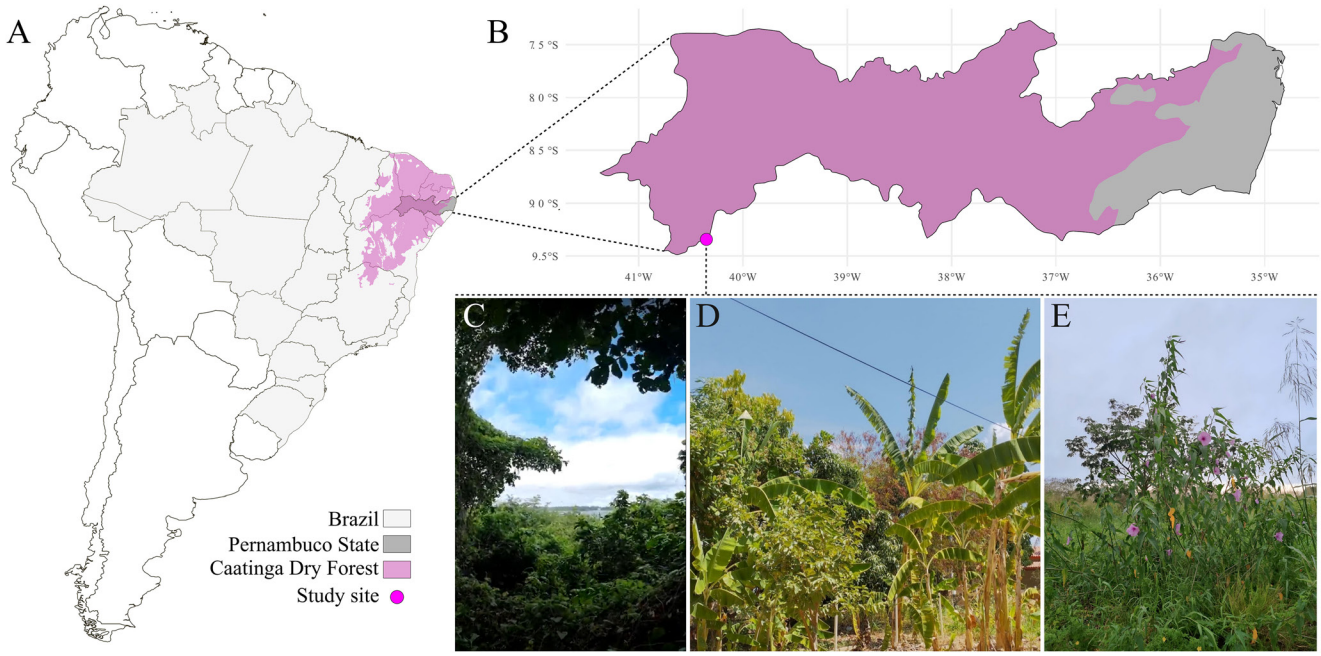

Figure 1. Study site and studied species. (A) Map of South America, highlighting the geopolitical division of Brazil and the Caatinga Dry Forest. (B) The study site is pinpointed in the state of Pernambuco. Photographs of Agroforestry System: (C) Preserved riparian forest area, adjacent to (D) diverse crops, and (E) *Ipomoea carnea* (Convolvulaceae) in an open field area.

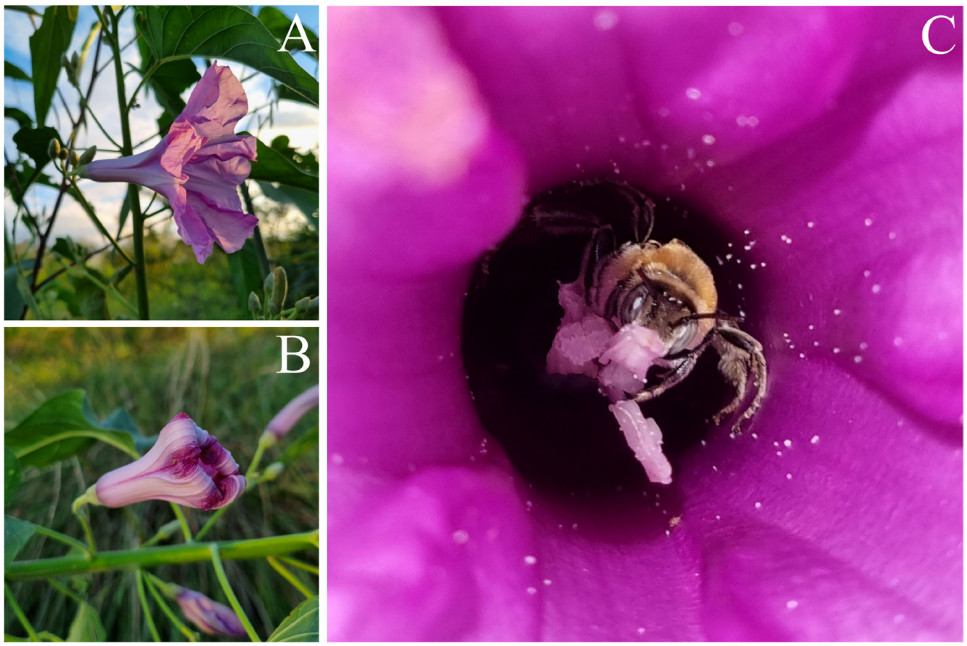

Figure 2. Interaction between *Melitoma segmentaria* bees (Apidae) and *Ipomoea carnea* (Convolvulaceae). (A) Lateral view of *I. carnea* at the onset of floral anthesis, photo taken at dusk; (B) Lateral view of *I. carnea* during closure, at the same moment as the previous photo; (C) Close-up frontal view of *I. carnea* flower with a male bee taking shelter inside.

exclusively in open flowers may result in undersampling. After all, it was not possible to confirm the presence of sleeping bees in flowers without opening them.

During the observations, bees were found inside the tubular part of wilted flowers of *I. carnea*, hanging on the reproductive structures. This situation compares with other records where males of oligolectic bees are often observed sleeping on flowers with both dish-like and tubular shapes (Watts et al. 2013, Westrich 1989). We suggest that the selection of *I. carnea* flowers by Emphorini males might be linked to the flower's ability to shelter the individual inside due to the protection offered by the final portion of the infundibulum, which remains turgid during the night even when the corolla ends completely close.

Another interesting aspect that makes this interaction possible is the timing of anthesis of *I. carnea*. For some of the most abundant

Convolvulaceae species visited by Emphorini bees in the Caatinga, such as *Ipomoea bahiensis*, *I. nil*, and *Merremia aegyptia* (Pick and Schlindwein 2011), flower anthesis occurs synchronously in the early morning and close around noon or in the mid-afternoon, when male bees are often still active (Kill & Ranga 2000, Maimoni-Rodella 2007, Gimenes et al. 2021). As a result, the access of male bees to the flowers is no longer possible in the late afternoon. Furthermore, even when considering the fact that the Caatinga dry forest harbors a significant number of species with crepuscular and nocturnal flowers, including certain bat-pollinated *Ipomoea* species (Domingos-Melo et al. 2023), bee sheltering remains unfeasible in such species. Since nectar-feeding bats can be voracious consumers of insects (Herrera et al. 2001), they pose a risk for the bees to occupy. Thus, the presence of *I. carnea* flowers with anthesis occurring during the afternoon and closing at dusk seems to be a key factor for male Emphorini bees to use them as overnight shelters.

Finally, as regards to the potential role of overnight sheltering from the plant perspective (e.g. pollination efficiency), our observations revealed both pollen deposition on the bees' bodies and pollen transfer to the stigma. Thus, it is plausible to suggest that Emphorini males could contribute to pollination by sleeping in the flowers. However, experimental studies with other bee groups have found that this behavior has little contribution to either seed production or pollen dispersal (Welsford & Johnson 2012). Indeed, plants providing both nourishment and mating opportunities for pollinators tend to allocate a greater portion of their cross-fertilization assurance budget to advertisement compared to plants offering only food rewards, implying that the flower's role as rendezvous place contributes to its own evolution (Fishman & Hadany 2013). Therefore, the consequences of the interaction between Emphorini males and *I. carnea* for the plant's reproductive biology still need further investigation.

Conclusion

This study documents a poorly-described behavior of male Emphorini bees sleeping in flowers of *Ipomoea carnea.* Males of oligolectic species associated with Convolvulaceae seem to regularly use *Ipomoea spp*. flowers as overnight resting sites, and a potential place for finding females. We hypothesize that the loss of these flowers would threaten the aforementioned bee species, not only in terms of their nutritional source but also as a shelter and rendezvous place. From the plant perspective, several characteristics of *I. carnea* flowers are important to establish this interaction. Firstly, the funnel-shaped morphology of the flowers ensures that wilted flowers form a casing capable of protecting the males. Secondly, the presence of different times of anthesis, including flowers that begin wilting precisely at dusk, when males are seeking shelter. Third, the fact that *I. carnea* flowers are profitable in terms of resources, offering both nectar and pollen. Furthermore, several hypotheses arise from these findings, including the role of floral signaling. It seems highly likely that bees can distinguish which flowers are starting to wilt from those that are opening within the same timeframe, suggesting that the flowers of *I. carnea* may have different floral volatile compounds according to their timing of anthesis, and that bees can use these signals while searching for an overnight place.

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Andressa Stefany Santos Gomes: conceptualization; methodology; data curation; writing – original draft.

Paulo Milet-Pinheiro: conceptualization; methodology; writing – review & editing.

Arthur Domingos-Melo: conceptualization; methodology; data curation; writing – original draft; writing – review & editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics

This study was conducted following the guidelines for field research within private areas in the state of Pernambuco.

Data Availability

The data presented in this work are available at https://doi.org/10.7910/DVN/KPMHC4.

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