

Notes and Comments

Nocturnal ant integrates generalist pollination system in the Caatinga dry forest

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Ant pollination is an uncommon system that has evolved independently in different Angiosperm families (Beattie, 2006). This interaction has been associated with arid environments because plants need produce floral resources and ants are ubiquitous in arid environments, even when other pollinators become scarce (Domingos-Melo et al., 2017; Leal et al., 2017; Oliveira et al., 2017). Notwithstanding, many interactions between plants and ants in arid environments may shift to the night (e.g. herbivory or consumption of extrafloral nectar), likely due to the influence of climatic factors or in response to resource competition (Dattilo et al., 2014; Leal et al., 2017). This nocturnal condition likely extends to ant pollination. In fact, interactions with nocturnal vectors (e.g., bats, beetles, hawkmoths, small insects, and non-flying vertebrates) are important pollination strategy in dry environments, since nighttime interactions are not impeded by high temperatures and low humidity (Borges et al., 2016).

Recently, was described in two *Ditassa* species (Asclepiadoideae, Apocynaceae) a generalist pollination systems including groups as wasps, bees, flies and the first case ant pollination in the Caatinga (Domingos-Melo et al., 2017). This dry forest in northeastern Brazil has low annual precipitation and a water deficit for most of the year, but there, ants are a highly diverse and conspicuous group (Machado et al., 1997; Leal et al., 2017; Oliveira et al., 2017). Despite the pollinator ants recorded at the time being diurnal, it is estimated that more than 22% of pollination systems in the Caatinga plant species are nocturnal (Machado and Lopes, 2004). In this context, new field records have revealed that *Ditassa hastata* Decne is visited by the nocturnal ant *Camponotus palleescens* (Mayr, 1887), Formicidae.

Many *Camponotus* species present nocturnal activity, which can be explained by self-regulation of the circadian rhythm of the colony itself and the availability of resources (Mildner and Roces, 2017). Additionally, its species are characterized by a vast food repertoire that includes the consumption of floral nectar, and some of them have been exceptionally registered as pollinators

in some ecosystems, including a South African asclepiad, *Eustegia minuta* (Romero 2002; De Vega et al., 2009; Lach et al., 2010; Chirango et al. 2019; Del-Claro et al., 2019; Delnevo et al., 2020). Here, we report how the visitation frequency of *Camponotus palleescens* varies throughout the night and estimate its contribution to pollination.

The fieldwork was conducted in the Catimbau National Park (8.61667°S, 37.15°W), Pernambuco, Brazil. There, the annual average temperature is 25°C and average rainfall is 1095.9 mm (Andrade et al., 2004). Most of the park has quartzolic sandy soils where individuals of *Ditassa hastata* can occur on sandstone outcrops (Domingos-Melo et al., 2017). We performed focal observations during five consecutive nights from 17:30 to 2:30 (45 hours total). At each observation, we recorded the number of ants that visited flowers in 1-hour periods. We run a Kruskal-Wallis test with pairwise comparisons using Wilcoxon rank sum test to assess the differences in the frequency of visits between intervals. We measured each visit's duration (n=60) and we randomly collected 73 ants to quantify the presence of attached pollinaria and pollinia.

At each visit, *Camponotus palleescens* travels on the branches of *D. hastata* searching for nectar in the flowers. Visit time was $61 \pm 56s$, during which ants generally attempted to access all nectariferous chambers, accessing the mechanism of pollination with its front appendages (Figure 1A). We recorded a total of 205 visits (41 ± 8 visits per night). Ants arrived on the branches after 17:30h, but visits intensified only after 19:00h; as the night advances, ants reduce their activity, and visits become scarcer until stopping at 2:00h ($\chi^2=32.63$; $df=8$; $p<0.0001$ - Figure 1B). This visit frequency, characterized by a few foraging individuals at nightfall followed by a substantial increase, aligns with the group recruitment common to the genus *Camponotus* (Lach, 2010). In this behavior, successful foraging recruits a group of several nestmates for the resource (Lach et al., 2010; Mildner and Roces, 2017).

In our sample, we recorded 8% of the *C. palleescens* individuals carrying pollinaria in their legs (6 individuals with one pollinarium each). Two of these pollinaria were

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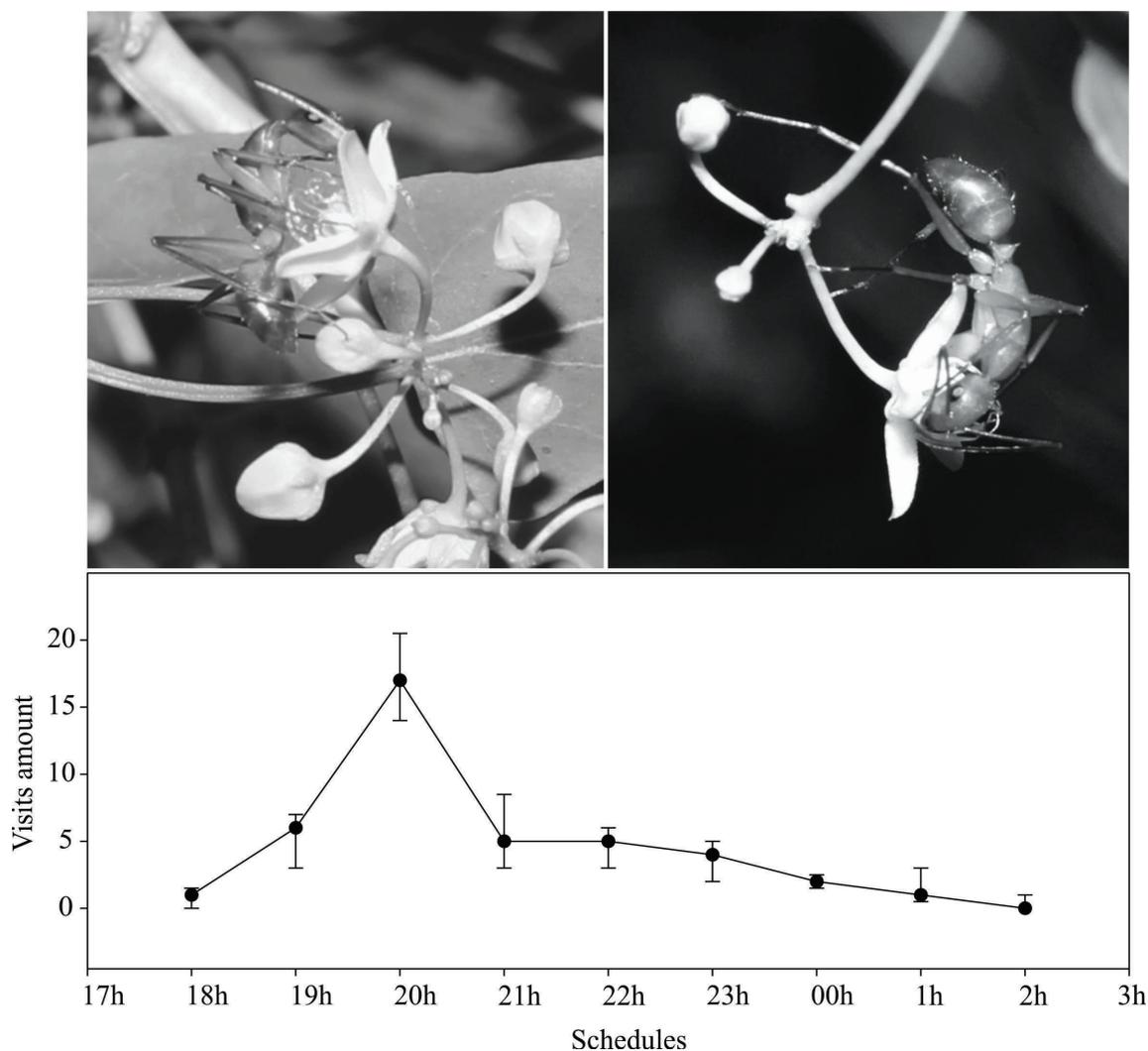


Figure 1. Visit behaviour of *Camponotus pallescens* on *Ditassa hastata* flowers in the Catimbau National Park, Pernambuco, Brazil. (A-B) *C. pallescens* taking nectar in different positions and (C) its visit frequency to *D. hastata* flowers throughout the night. (Black dots indicate median and error bars indicate first and third quartiles; Different letters indicate statistically significant difference from Kruskal-Wallis test with pairwise comparisons using Wilcoxon rank sum test with $\alpha=0.05$)

complete with two polliniums; one had a single remaining pollinium, and three had no pollinia (suggesting that seven pollinia could have been deposited). Despite this small contribution of *Camponotus pallescens* as a pollinator for *Ditassa hastata*, it addresses the absence of other nocturnal pollinators in this species. Since in most asclepiads anthesis occurs both day and night, the contribution of nocturnal pollinators may be important (Bertin & Willson, 1980; Domingos-Melo et al., 2019). In fact, even pollinators that play a secondary role in reproductive success are important for maintaining a species' resilience to environmental changes (Waser and Ollerton, 2006). The Caatinga is subject to climatic oscillations that can generate episodes of severe drought, resulting in ants' expanding their search for flowers (Santos et al., 2014; Leal et al. 2017). In this scenario, we understand that including nocturnal ants among the pollinator set could help increase pollination success.

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