

# Complex flowers and rare pollinators: Does ant pollination in *Ditassa* show a stable system in Asclepiadoideae (Apocynaceae)?

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Received: 23 August 2016 / Accepted: 28 January 2017 / Published online: 23 February 2017  
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**Abstract** If on one hand, ant pollination is rare, on the other Asclepiadoideae flowers are the most complex among Eudicots. Both themes are exciting in pollination biology. Although there are records of ants with the ability to remove the pollinarium on this subfamily, the role of these insects as pollinators is not yet known. Therefore, we investigated the interaction between flowers and ants, as well as the associated features in two species of Asclepiadoideae, *Ditassa capillaris* and *D. hastata*. The studied species were both visited by *Cephalotes inaequalis* and *Dorymyrmex thoracicus*. We analyzed the common traits of ant pollination of the plant species, recorded the number of visit by ants, and carried out experiments of selective pollination, developing an effectivity index. Both *Ditassa* species have common traits of ant pollination, such as small bright flowers with exposed floral resource. The pollinarium is clip-shaped and resistant to the metapleural gland, which ensures effective pollination. *Dorymyrmex thoracicus* is a very frequent but not very efficient pollinator, whereas *Cephalotes inaequalis* is more efficient though at a lower frequency. Therefore, the total contributions of both ants to the pollination of both *Ditassa* species are similar. The features of both *Ditassa* species which favor ant pollination,

include some synapomorphies within Asclepiadoideae. Furthermore, the efficiency of the ants as pollinators to the reproductive success of the plants was similar despite the different behaviors observed in both ant species. Hence, we suggest that the role of ants in pollination within this subfamily has been neglected.

**Keywords** Ant pollination · Asclepiadoideae · Metastelmatinae · Pollination effectivity · Semi-arid environments · Specialist/generalist system

## Introduction

Ant pollination is rare and restricted to a few species within different families of angiosperms (Peakall and Beattie 1991), generally because of the peculiar conditions of these insects, such as their small size, self-cleaning behavior and limited displacement (Faegri and Pijl 1979). However, the main concern is the interference in pollen germination by secretions of the metapleural gland, which is a part of the body of ants that produces antibiotic substances against fungi (Beattie et al. 1984, 1986; Wagner 2000). On the other hand, this pollination system has the advantage of low energetic cost (Hickman 1974). Moreover, ant pollination provides another advantage when winged pollinators are scarce in arid or alpine environments (see, e.g., Gómez et al. 1996; Puterbaugh 1998; Ibarra-Isassi and Sendoya 2016).

Despite these limitations and advantages, ant pollination sometimes evolved in different forms within angiosperms, including adjustments in flowers as well as in ants (Beattie 2006). Observations of evolutionary convergences of flowers allow us to know the conditions involved in the occurrence of the system, while understanding the features of its

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pollinators allows us to understand their performance (Fægri and Pijl 1979; Tucker 1997; Fenster et al. 2004; Fenster and Martén-Rodríguez 2007; Niet and Johnson 2012). Hence, the study of new records in more angiosperm families and comparisons with the interactions of the different ants with flowers will help us to comprehend this peculiar interaction.

For this purpose, we investigated two species of Asclepiadoideae (Apocynaceae) (the rupicolous *Ditassa hastata* and the climbing *D. capillaris*), both of which were visited by the ants *Dorymyrmex thoracicus* and *Cephalotes inaequalis*. Although there are more visitors to both plant species, such as other Hymenoptera (winged), Diptera and Lepidoptera, all of which have been confirmed as pollinators (Domingos-Melo et al., unpublished data), only ants were recorded on every day of our observations and were more frequent. Therefore, we chose to study these interactions in order to confirm the role of ants as legitimate pollinators, and examine how the floral traits and the natural history of the ants have influenced this system.

The flowers of the Asclepiadoideae are known as more specialized and functionally complex within Eudicots (Endress and Steiner-Gafner 1996). There are some records of probable ant pollination from the description of the removal of pollinaria, but without confirmation of the deposition of pollinia (Table 1). Taking into account the records of ant pollination in Orchidaceae, a family whose flowers are one of the more complex within Monocots, we would expect to find this system in Asclepiadoideae (Peakall and Beattie 1989; Dressler 1993; Mondragón-Palomino and Theiben 2008). Thus, to understand the interaction of the features of *Ditassa* species with their respective ants will show us an important perspective on the interaction of ants with complex flowers. Furthermore, some factors of the above-mentioned ant species that visit *Ditassa* species lead us to a question about how different morphological and behavioral features influence their role in pollination.

Therefore, we intend to characterize the interaction between plants and ants, addressing the follow questions. (1) Do *Ditassa* species have features that facilitate the pollination by ants? (2) How similar is the behavior of visits to *Ditassa* flowers by *Dorymyrmex thoracicus* and *Cephalotes inaequalis*? (3) Are those ant species pollinators? (4) What are the differences in performance and effectiveness between the two ant species? We expect that the two *Ditassa* species have similar features that are associated with pollination by ants, and although the ants have different foraging behaviors, both are effective pollinators.

## Materials and methods

### Studied site

The field activities were conducted in the National Park of Catimbau (PARNA Catimbau), Pernambuco – Brazil, which displays a semiarid climate, BSh according Köppen scale (Peel et al. 2007). The annual average temperature and rainfall are 25 °C and 1095.9 mm, respectively (Andrade et al. 2004). We carried out this study in semi-deciduous shrub Caatinga areas, where we found populations of *D. hastata* on sandstone outcrops (37°14'27.9"S, 8°35'3.8"W), on which individuals reached a maximum of 60 cm high and generally occurred inside slits in the rock. The *D. capillaris* population was found in a shrub-tree Caatinga area (37°14'59.4"S, 8°32'1.0"W), where branches of individual shrubs were above the canopy and reached up to 2 m high.

### Species characterization

*Ditassa hastata* and *D. capillaris* occur in Caatinga and Cerrado areas. They are included in the subfamily Asclepiadoideae, within the Metastelmatinae clade, which

**Table 1** Records of the removal of the pollinarium by ants (without confirmation of the deposition of pollinia) in the flowers of Asclepiadoideae (Apocynaceae)

Plant species	Ant species	Location	Reference
<i>Asclepias curassavica</i>	<i>Monomorium</i> sp	India	(Chaturvedi and Pant 1986)
<i>Asclepias exaltata</i>	<i>Lasius niger</i>	EUA	(Betz et al. 1994)
<i>Asclepias syriaca</i>	<i>Formica subsericea</i>	EUA	(Kephart 1979)
<i>Ditassa banksii</i>	Undetermined	Brazil	(Koschnitzke 2015)
<i>Ditassa rothundifolia</i>	<i>Cephalotes</i> sp	Brazil	(A. Domingos-Melo, UFPE, Pernambuco – Brazil, unpublished)
<i>Gomphocarpus physocarpus</i>	<i>Formicidae</i> sp	South Africa	(Coombs et al. 2009)
	<i>Iridomyrmex purpureus</i>	Australia	(Forster 1994)
	<i>Polyrachis</i> spp		
<i>Leptadenia pyrotechnica</i>	<i>Crematogaster</i> sp	Pakistan	(Ali 1994)
<i>Leptadenia reticulata</i>	<i>Crematogaster</i> sp	India	(Pant et al. 1982)

exhibits great floral diversity with the diversity center in South America (Rapini 2002). The main features of the Asclepiadoideae are flowers with three exclusive structures: the corona (a third sterile whorl), the gynostegium (made up of the complex fusion between the androecium and the gynoecium) and the pollinarium (a structure with two pollinia attached to the corpusculum by a caudicle). In the gynostegium, the lateral edge of the anthers touch each other, forming guide rails with stigmatic chambers inside formed by nectar-producing filamentous tissue. The pollinia are deposited within the stigmatic chamber, where they germinate and emit the pollen tube. The pollination in this group occurs when the pollinia is inserted into the inner guide rail. For this, the corpusculum has a slit, which is continuous with the guide rail. Then, when some floral visitor drinks nectar, a part of its body becomes stuck in the guide rail (Fig. 1a) and, to remove it, it has to move along of the guide rail and then by the slit of the corpusculum. At this moment, the corpusculum becomes detached and sticks onto the body of the pollinator carrying with it the pollinarium (Fig. 1b). When the pollinator visits another flower, the same process occurs, but now a pollinium gets inserted into the inner guide rail (Fig. 1c). This mechanism is known for many species of this subfamily (Endress and Steiner-Gafner 1996; Wiemer et al. 2011; Vieira et al. 2012).

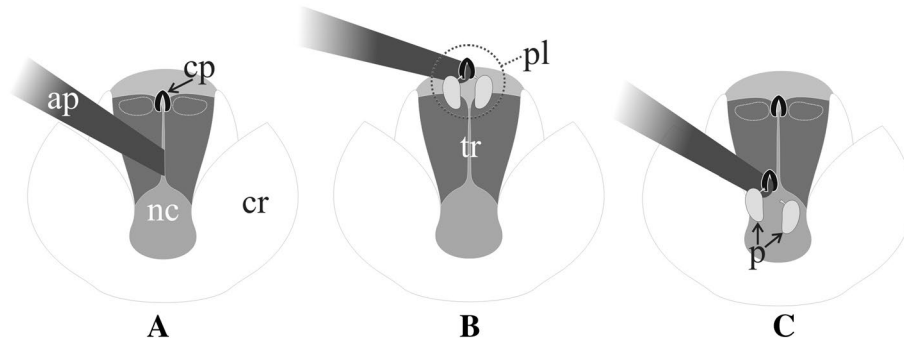
The ants studied here are from different taxonomic and functional groups, but both are generalist consumers, living in different plant strata. *Dorymyrmex thoracicus* belongs to the subfamily Dolichoderinae and its main features are dominance and invader behavior (Andersen 1995). The genus *Dorymyrmex* is known as dominant because of its good use of thermic and metabolic power, and its intense activities and large colonies (Lach et al. 2010). *Dorymyrmex thoracicus* is a small ant associated with litter and soil, being a suitable environment for nesting (Vasconcelos et al. 2006). On the other hand, *Cephalotes* belongs to the subfamily Myrmecinae, a generalist group, which

does not monopolize resources (Lach et al. 2010). This genus is essentially arboreal, nesting in timber, but it can be also found on litter (Vasconcelos et al. 2006). It is larger than *Dorymyrmex* and has a dorsoventrally flattened body, which helps it to maneuver in free fall (Yanoviak et al. 2005).

### Attributes associated with ant pollination

We analyzed the following publications, which describe important traits concerning pollination by ants: (1) the first classification of the attributes that guarantee pollination by ants, which was proposed by Hickman (1974); (2) the pollination syndrome by ants described by Faegri and Pijl (1979); (3) the list of features of alpine species and those living in arid environments which are pollinated by ants (Gómez et al. 1996); and (4) a review made by Beattie (2006). Furthermore, we listed the attributes which are associated with this pollination system. From this, we verified whether both *Ditassa* species resemble other plants pollinated by ants. Finally, we analyzed the majority of these attributes of the *Ditassa* species in the field and the laboratory looking at flowers using stereomicroscopy.

We analyzed the effect of the metapleural gland of the two ants on the germination of pollinia of both *Ditassa* species. Once the flowers had opened, at 0300 hours, we bagged floral buds the previous night, then collected the opened flowers during the morning. We put these flowers in plastic boxes with Agar–Agar. At the same time, we captured both species of ants in the field and carried them alive in polystyrene boxes to the laboratory, where we carried out manual self-pollination as follows. We removed the pollinia of both *Ditassa* species and rubbed them against the body of the live ants. Then, we placed the pollinia in the stigmatic chamber of its respective species. We performed repetitions of these proceedings using 15 flowers of each species and an individual of the associated ant species for each



**Fig. 1** Sequence of removal and deposit of pollinium in *Ditassa*: one of the five pollination units of the flower of *D. capillaris*. **a** First, a part of the animal (tongue, palpus, leg) is inserted into the guide rail; **b** when the animal moves that part up, the corpusculum is removed,

which drags the pollinia; **c** in another visit, the pollinium detaches itself from the corpusculum and remains on the guide rail. (*ap* animal part, *nc* nectar chamber, *cr* corona, *cp* corpusculum, *gr* guide rail, *pl* pollinarium, *p* pollinium)

flower. We carried out manual self-pollination in 20 flowers without rubbing the pollinia against the body of the ants for control purposes. After 2 days, we analyzed how many pollen tubes had reached the ovules using fluorescence microscopy (Martin and Glover 2007).

### Behavior of visitors

We described the behavior of the ants on the flowers through detailed focal observations. For this, we analyzed the access to the flowers by the respective ant species and the interactions between floral morphology and ant behavior. Furthermore, we quantified the number and duration of visits, which allowed us to compare the behavior between the different species. We carried out the following procedures. When the ant visited a flower, we followed it for 15 min and recorded the number of flowers visited and the duration of each visit. We performed repetitions of these proceedings every 15 min for each plant species ( $n=35$  for *Dorymyrmex thoracicus* in *Ditassa hastata*;  $n=20$  for *Cephalotes inaequalis* in *D. capillaris*). Hence, we obtained the number of visits/hour, the mean time of the stay of the ant on the flower, and the total duration of visits/hour for each pairing of ant and plant species.

### The role of ants as pollinators

We estimated the intensity of ant activity on each plant and the deposited/removed pollinia ratio to verify the role of the ants as pollinators. We considered the ants as pollinators if they showed continuous activity on the plants and the ability to remove and deposit the pollinia.

Additionally, we monitored the intensity of ant activity on each plant by recording the times when there were ants foraging on the plant and by quantifying the number of individual ants which simultaneously visited the same plant. In the first scenario, we monitored both *Ditassa* species from dawn to dusk, recording the presence or absence of ants per hour. In the second, we measured length (A), width (B) and thickness (C) of an isolated branch and estimate its volume (Vol) according to the parallelogram formula ( $A \times B \times C = \text{Vol}$ ), which varied from 4000 to 6000 cm<sup>3</sup>. Then, we collected and quantified the ants on the branch and divided their number by the volume of the branch. Finally, we quantified the number of pollinia that were removed by these ants.

We carried out experiments of selective pollination to quantify the number of pollinia that were deposited. For both plant species, we bagged floral buds of five different individuals with voile bags during the night ( $n=20/\text{sp.}$ ). On the subsequent morning, after the floral opening, we inserted a live ant into the bag, being the respective pollinator species of each plant. Before we inserted the ant into

the bag, we verified that it was not carrying any pollinia adhering to its body. After 1 h, we collected the flowers and verified how many pollinia had been removed or deposited. Then, we obtained the ratio of pollinia deposition by dividing the number of deposited pollinia by the number of removed pollinia.

### Data analyses

We applied Student's *t* test to compare the number of visits/hour, the mean time of a visit and the total time of visits per hour between the ant species. We used the average and frequency test to generate an index of intensity and the effectivity of ant activity to compare the role of both ant species as pollinators. Also, we applied the Mann–Whitney test on the data of the sampled ants on the branch of each *Ditassa* species, as well as in the index generated by them. We used the  $\chi^2$  test to compare the ratio of pollinia deposition from the experiments of selective pollination among the *Ditassa* species.

In relation to the index of effectivity, we estimated the number of deposited pollinia by each ant species for each individual of its respective plant species, considering the values of the effectivity of pollination and the activity of the ants. Initially, we estimated the amount of deposited pollinia by each ant ( $P_D$ ), considering the mean of removed pollinia by the ant from each individual of plant ( $P_F$ ) and the ratio removed/deposited of pollinia ( $R_D$ ). In this regard, we considered the value of  $P_D$  ( $P_D = P_F \times R_D$ ) as a constant for each ant species, which was obtained through the experiments of selective pollination. Then, we calculated a value for the daily activity of the ants ( $A_D$ ), considering the number of ants/m<sup>3</sup> of branch ( $F_M$ ) which foraged on each individual plant, and the number of hours of daily activity ( $H_A$ ). Furthermore, we considered the value of  $A_D$  ( $A = H_A \times F_M$ ) as another constant for each ant species. Finally, we obtained a value for the total deposition of pollinia on each individual plant ( $D_T$ ), considering both the amount of deposited pollinia by each ant ( $P_D$ ) and the daily activity of the ants ( $A_D$ ), through the formula  $D_T = P_D \times A_D$ . Hence, the mean total deposition of pollinia by each ant species was an estimate of the total pollination made on 1 day on 1 m<sup>3</sup> of branch.

## Results

### Attributes associated with ant pollination

The attributes associated with ant pollination found in the literature, which exhibited discrepancies with the traits observed in the two *Ditassa* species, are shown in Table 2. Most attributes found in *Ditassa* meet the requirements of

**Table 2** Comparison of the traits associated with ant pollination and those of *Ditassa hastata* and *D. capillaris* in Caatinga sites of National Park of Catimbau, Pernambuco – Brazil

	Ant-pollination species <sup>a</sup>	<i>D. hastata</i>	<i>D. capillaris</i>
Place of occurrence	It usually occurs in arid places or at high altitudes where other pollinators are scarce	= It occurs in dry forest with little rain and high temperatures	
Habit, size and distribution	Habits that allow proximity to the ground (herbaceous or prostrate) and plants of a few centimeters can optimize access by ants. Furthermore, it is expected that the clustered distribution requires little displacement of these insects for the occurrence of cross-pollination	= Underbrush rupicolous = Close to 0.5 m in height and 0.7 m in diameter ≠ Occurs isolated in rock fissures	≠ Bindweed of canopy ≠ Branches reach to more than 2 m high = Branches of different individuals intertwine
Amount of flowers and flowering synchrony	Opens a few flowers daily in one individual plant, but generally has strong flowering synchrony in a population. This system avoids geitonogamy	≠ The same plant has hundreds of flowers open simultaneously = Individuals blooming simultaneously	
Floral insertion	Very long stems can hinder access by ants, so sessile flowers or short stems are preferable	= Pendulous flowers with short stems (reach 1 cm)	
Floral size and color	Flowers pollinated by ants are usually inconspicuous, with light color and small size (only a few millimeters). The small size allows the ants to access the pollination mechanism	= Corolla 4–6 mm in diameter = Light flowers, slightly greenish	
Floral shape and symmetry	Flowers with more open shapes, e.g., dish-shaped, facilitate access by ants; moreover actinomorphic symmetry allows access from either side	= Floral-shaped dish = Actinomorphic symmetry	
Nectar	The nectar should be exposed, accessible to ants, and occurs in small quantities, dissuading other visitors	= Little nectar (unmeasurable quantity) = Nectar partially exposed between the corona elements	
Pollen and ovules	It is expected that low amounts of pollen grains discourage clean-up behavior of the ants. This condition demands uniovulads flowers or with few ovules. Alternatively, the pollen grains could be gathered in pollinia, and in this case there is no limitation on the number of ovules	= Pollen gathered in five pollinaria with two pollinia each, not requiring limitation on the number of ovules	

= same as expected; ≠ different from expected

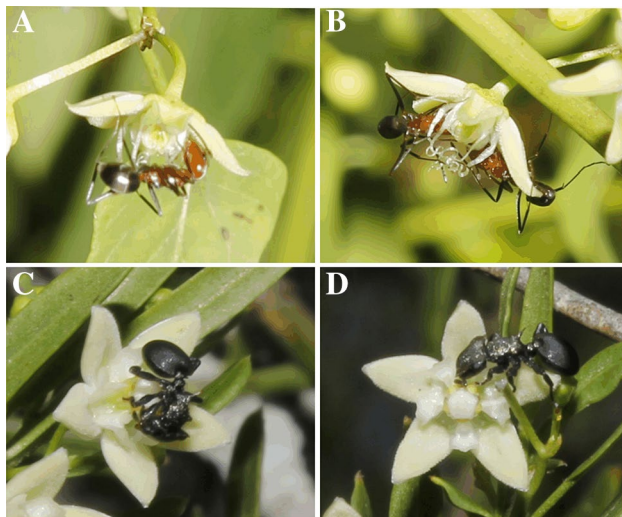
<sup>a</sup>List of attributes elaborated based on Hickman (1974), Faegri & Pijl (1979), Peakall and Beattie (1991), Gomes et al. (1996), e Beattie (2006)



pollination by ants. Despite the fact that we did not make a formal test of the consistency of this information, we observed that most floral traits confirmed the expectations for ant pollination. Therefore, each species reached approximately 80% of similarity to the expected attributes. The data, regarding the effect of the secretion of the metapleural gland of ants, indicated the resistance of the pollinia of *Ditassa capillaris* to the ant *Cephalotes inaequalis*, as well as of *D. hastata* to the ant *Dorymyrmex thoracicus*. For all sampled flowers of both species, we observed pollen tubes reaching the ovaries, even after friction of the pollinia on the body of ants.

### Records of visiting behavior

An individual ant of the species *Dorymyrmex thoracicus* patrolled branches of *Ditassa hastata* by rapidly approaching several flowers and visiting the most intact to drink



**Fig. 2** Ant visiting behavior on the flowers of two plant species in a Caatinga area in National Park of Catimbau, Pernambuco, northeastern Brazil. **a, b** *Dorymyrmex thoracicus* on flowers of *Ditassa hastata* assuming different positions: **a** Leaning on the corona appendices with its legs on gynostegium and petals, **b** Leaning on the petals and accessing the nectar chamber ahead. **c, d** *Cephalotes inaequalis* visiting *D. capillaris* flowers and assuming different positions: **c** Leaning on the stigma head resting its legs on the gynostegium and petals, and **d** Leaning on petals and accessing the nectar chamber by the side

**Table 3** Quantification of the number and duration of visits that one ant can do in *Ditassa* flowers in Caatinga sites of National Park of Catimbau, Pernambuco – Brazil

	<i>Dorymyrmex thoracicus</i> (n=35)	<i>Cephalotes inaequalis</i> (n=20)	P value <sup>a</sup> (gl=53)
Number of visits/hour	46 ± 15	37 ± 11	0.036
Duration of visits	40 ± 22 s	57 ± 16 s	0.001
Total time of visitation in 1 h	26:54 ± 9:54 min	33:12 ± 7:30 min	0.030

<sup>a</sup>Results of comparison of the behavior of the ants using *t* test with  $\alpha=0.05$

nectar. Access to the nectar was granted when the ants leant their body against the corona appendices, resting their legs on the gynostegium and petals, and inserting their mouth apparatus into the nectar chamber (Fig. 2a), or leaned on the petals facing the guide rail (Fig. 2b). In the first case, only the legs could remove or deposit pollinia. In the second case, the antennae played that role, as they are thin and long enough to access the guide rail. In both cases, the ant circled behind the calyx and moved among the petals to access the five cavities among the corona lobes, where the nectar was accumulated.

An individual of the species *Cephalotes inaequalis* slowly patrolled the branches of *Ditassa capillaris*. The visits occurred with ants leaning against the head of the stigma, resting their legs on the gynostegium, and inserting their mouth apparatus in the nectar chamber (Fig. 2c). In that position, the pollinia could be deposited on their legs, mouth apparatus, and ventral parts on their body, which removed the pollinia by friction, even without passing through the guide rail. To access the five cavities where the nectar was accumulated, the ants spun their body around the axis of the flower. Alternatively, the ants accessed the nectar chamber diagonally by leaning on the petals (Fig. 2d).

In addition to the descriptions above, the tests detected differences in behavior between the two ant species (Table 3). The species *Dorymyrmex thoracicus* made shorter visits and *Cephalotes inaequalis* made fewer but longer visits. In general, the former species spent less time visiting the flowers than the latter. We did not observe antagonistic interactions with other flower visitors (bees, wasps, flies, and mosquitoes) in any of the two species.

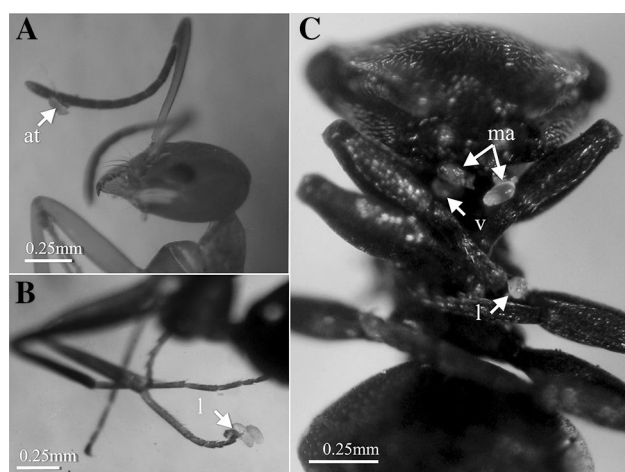
### Role of the ants as pollinators

*Dorymyrmex thoracicus* was always active from sunrise (0600 hours) to sunset (1800 hours), for a total of 12 h per day. The activity of *Cephalotes inaequalis*, however, was restricted to 0900–1700 hours, a total of 8 h per day. There was a difference between the species in the number of ants active on the branches:  $286 \pm 98$  ants/m<sup>3</sup> of branch in *Ditassa hastata*, and only  $63 \pm 21$  ants/m<sup>3</sup> of branch in *D. capillaris* ( $U=0$ ;  $Z(U)=2.7386$ ;  $p=0.0062$ ).

Both ants acted as pollinators, as they removed and deposited pollinaria with different parts of their bodies (Fig. 3). The rate of transported pollinia/ant in *Dorymyrmex thoracicus* was  $1/25 \pm 1/11$ , a lot smaller than in *Cephalotes inaequalis*, which showed  $1/2 \pm 1/1$  transported pollinia/ant ( $U=0$ ;  $Z(U)=2.7386$ ;  $p=0.0062$ ). Regarding the capacity for depositing pollinia, *Dorymyrmex thoracicus* deposited one-third of the pollinia it removed and *Cephalotes inaequalis* one-fourth. We did not observe a significant difference in  $m^3$  deposition rate between the species ( $G=0.14$ ;  $df=2$ ;  $p=0.93$ ).

In terms of the action of the ants on the plants during the day, we found that *Dorymyrmex thoracicus* foraged more on *D. hastata* than *Cephalotes inaequalis* on

*D. capillaris* ( $U=0$ ;  $Z(U)=2.7386$ ;  $p=0.0062$ ), which is almost a seven-fold difference (Fig. 4a). However, in terms of the efficiency of the removal and deposition of pollinia, we observed a different situation: *Cephalotes inaequalis* is 6.5 times more efficient than *Dorymyrmex thoracicus* (Fig. 4b) ( $U=0$ ;  $Z(U)=2.7386$ ;  $p=0.0062$ ). Finally, when we considered ant action and efficiency together, both species showed similar results ( $U=3.50$ ;  $Z(U)=0.2739$ ;  $p=0.7842$ ). Thus, each species contributes similarly to the pollination of its respective plant (Fig. 4c): *Dorymyrmex thoracicus* deposited  $66 \pm 49$  pollinia per day on flowers of *D. hastata* and *Cephalotes inaequalis* deposited  $59 \pm 22$  pollinia per day on flowers of *D. capillaris*.



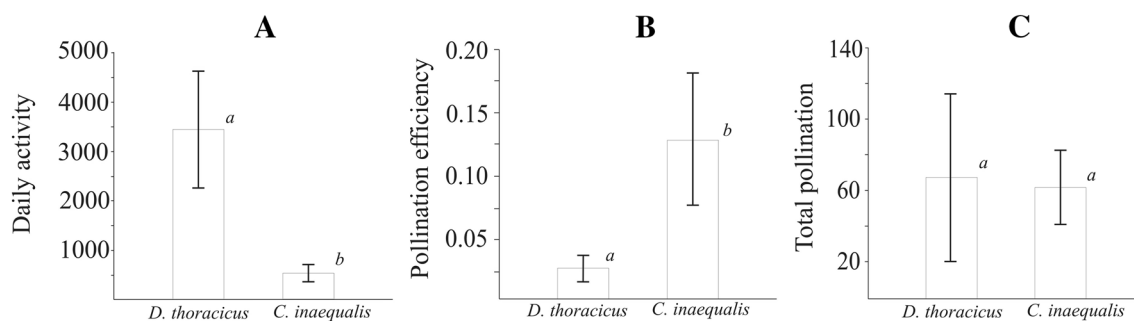
**Fig. 3** Sites of pollinia deposition on the body of ants in a Caatinga area in National Park of Catimbau, Pernambuco, northeastern Brazil. **a,b** Pollinia of *Ditassa hastata* on the antenna (*at*) and leg (*l*) of the ant *Dorymyrmex thoracicus*. **c** Pollinia of *D. capillaris* adhering to the mouth apparatus (*ma*), ventral region (*v*) and leg (*l*) of the ant *Cephalotes inaequalis*

## Discussion

As expected, most of the characteristics of both *Ditassa* species were similar to those expected for ant pollination, including those related to the metapleural gland. Not only the visiting behavior of ants but also their activity and efficiency differed between species. However, the final contribution given by each ant species to its plant was similar. Here, we discuss the implications of the attributes of Asclepiadoideae, in particular Metastelmatinae (the clade to which *Ditassa* belongs) to ant pollination. We advance the knowledge of how the biology of each ant species affects its interaction with flowers and compare our results to expectations for pollinating ants. Finally, we explore the effect of the ants, which can differ markedly between species, in spite of their similar contribution to pollination.

### Attributes of *Ditassa* species that allow ant pollination

Because *D. rhothundifolia* is characterized by its low height and a distribution in rocky outcrops, this species allows



**Fig. 4** Interaction between *Dorymyrmex thoracicus* and *Ditassa hastata*, and between *Cephalotes inaequalis* and *D. capillaris* in terms of activity, efficiency, and estimated number of pollinated flowers. The values shown in the plots were calculated by weighting the values of activity and efficiency, where: **a** Daily activity represents the amount of ants/ $m^3$  on branches, weighted by the hours of activ-

ity/day; **b** Pollination efficiency represents the average of pollinia transported per ant weighted by the ratio pollinia deposit/removal; **c** Total pollination is an estimate of the total value of pollinia deposited per  $m^3$  of branch in a day of ant activity, resulting from the weighting between activity and efficiency. Different letters indicate significant differences detected by a Mann–Whitney test ( $\alpha=0.05$ )

terricolous ants to access its flowers, whereas *D. capillaris*, which is a climbing species, instead permits arboricolous ants. In ant pollination, the limited mobility of these insects is overcome by small flowers and clustered plants, allowing easy access and movement among flowering individuals (Hickman 1974). Pollen flow is limited in *Ditassa* due to the large number of flowers per individual and the secluded habit of *D. hastata*, which favors geitonogamy. This condition is not consistent with what Hickman (1974) suggested for the system, such as, for instance, high flowering synchrony with few open flowers per individual. However, as the two *Ditassa* species are self-compatible, but not autogamous (Domingos-Melo et al., unpublished), ants contribute to the reproduction of those plants (Peakall and Beattie 1989; Vega et al. 2009). Although we could not quantify it, cross-pollination should be considered, as different *D. hastata* individuals can occur in the same outcrop and individual *D. capillaris* can intertwine in the canopy.

The inconspicuous floral traits observed in *Ditassa* (light colors, small size, and easy access to limited resources) are diagnostic of pollination by ants, but also by wasps, flies, and several other small insects (Ollerton et al. 2003; Shuttleworth and Johnson 2012; Wiemer et al. 2011; Faegri and Pijl 1979; Proctor et al. 1996; Bawa et al. 1985; Machado and Lopes 2004). The presence of other winged insect pollinators together with ants, which are cursorial pollinators, is a common condition in this type of system (Beattie 2006). Winged pollinators of Asclepiadoideae are usually flies, wasps, and small bees (Peakall and Beattie 1991; Vega et al. 2009; Wyatt 1981). Indeed, both *Ditassa* species are pollinated by those animals, in particular flies in *D. hastata* and wasps in *D. capillaris* (Domingos-Melo et al., unpublished).

We particularly believe that this situation corroborates the generalist phenotype assumed for many Asclepiadoideae flowers (Ollerton et al. 2007; Wolff et al. 2008). Ant pollination is above all an ecological condition and not the result of a specialized pollination system (Gómez et al. 1996). It is the difficulty of attracting winged pollinators that determines how much the flowers are restricted to ant pollination. This difficulty may be related to a low availability of nectar or because the plant occurs in adverse sites, e.g., arid or alpine (Hickman 1974; Gómez et al. 1996; Puterbaugh 1998; Rico-Gray 1989). As *Ditassa* species only partially exhibit those conditions, their plants do not depend exclusively on ants.

Another important characteristic of *Ditassa* flowers that ensures pollination by ants is their specialized and precise system of pollinarium and guide rails. The efficiency of the system is essential, as the self-cleaning behavior of ants limits the amount of pollen grains carried and, consequently, the number of ovules that can be fertilized (Hickman 1974). In Asclepiadoideae, however,

a single pollinium has the potential to ensure the formation of a fruit with several ovules (Vieira et al. 2012). Additionally, the deposition of pollinia in this subfamily depends on the passage of the caudicle through the guide rails, which increases the precision of this system. In orchids, the mechanism of pollination by worker ants is similar, but it is also simpler and generates losses due to the deposition of pollinia on branches and adjacent plants (Peakall and Beattie 1989).

The efficiency of ant pollination in *Ditassa* is increased by the resistance of its pollinia to the secretion of the ants' metapleural gland. Other species of different plant families also deal with this problem. Some of those plants deposit pollen grains on the body of ants far from those glands, others tolerate the loss of part of the pollen grains, and there are also those that develop resistance (Hull and Beattie 1988; Gómez et al. 1996; Ramsey 1995). In the specific case of pollen gathered in pollinia, in addition to the resistance of the grains, there is an effect of the mechanical protection provided by the surrounding pellicle (Peakall and Beattie 1989, 1991).

### The possibility of ant pollination in Asclepiadoideae

The *Ditassa* species studied here have several attributes related to ant pollination. When considering how much we can expand those data, it is worth taking into account that many Asclepiadoideae are pollinated by bees and wasps (Ollerton and Sigrid 1997). Therefore, it is not surprising that ants, which are also hymenopterans, can be pollinators (Beattie 2006). However, there are also cases of ants that play a different role, as they are unable to trigger the proper pollination mechanism, or that repel the legitimate pollinators of Asclepiadoideae (Ollerton 1999).

As for *Ditassa*, other Asclepiadoideae can be exposed to arboricolous or terricolous ants, as most species are twining and grow as small decumbent shrubs or climbing plants (Silva et al. 2008). Open corollas are very common in the subfamily and the mechanism of pollinarium and guide rails are present in all species (Wolff et al. 2008; Rapini 2012). It is also possible to expect that the resistance of the pollinia to the metapleural gland persists in the subfamily. Specifically, in South America, the presence of the subfamily in Cerrado and Caatinga areas (which have arid or semi-arid climates) or in Restinga (a tropical forest area comparable to those found in deserts or Mediterranean habitats) increases the chance of interaction with ants (Rico-Gray 1989; Peel et al. 2007; Ibarra-Isassi and Sendoya 2016). Based on this evidence, ant pollination should probably be much more common in Asclepiadoideae than previously thought.



## Understanding different ant behaviors

Different visiting behaviors in ants can be discussed in the light of differences between species in their natural history. The large size of *Cephalotes inaequalis* provides a better adjustment to flowers and its slow movements seems to optimize access to guide rails, which was not observed, on the other hand, in *Dorymyrmex thoracicus*. Additionally, the presence of appendices on the corona of *D. hastata* hinders closer contact, keeps *Dorymyrmex thoracicus* away, and makes it move more on the flower. Differences in visit duration can be especially related to the speed of drinking nectar by the species of ants. *Dorymyrmex* belongs to the Dolicoderinae, a subfamily that shows a better performance in sucking liquids, as it usually deals with unpredictable resources (Davidson et al. 2004). The genus *Cephalotes*, nevertheless, is usually associated with insects and extrafloral nectaries, which have a slow secretion and consequently demand a lower suction performance (Andrade and Urbani 1999).

We verified that both ant species are active and efficient enough to be considered legitimate pollinators. In *Dorymyrmex thoracicus*, the larger number of ants per plant can be explained by its dominant behavior and mass-recruiting pattern (Torres-Contreras and Vasquez 2007). In addition, longer daily activity time in *Dorymyrmex thoracicus* is related to its capacity to use all thermal and metabolic energy (Torres-Contreras and Vasquez 2004). On the other hand, *Cephalotes inaequalis* was not markedly active, which we believe to be associated with their lack of recruitment, as they act alone guided by signals from the colony (Gordon 2012). There are examples where pollen consumer *Cephalotes* can impact the plants negatively, but it does not seem to occur in *Ditassa capillaris*, because this ant is a very efficient pollinator (Byk and Del-Claro 2010). We attributed the difference in the number of pollinia removed by ants to differences in their visiting behavior. However, the similar deposition rates should result from a precision in the pollination mechanism of Asclepiadoideae, which we have previously discussed.

We noticed that different ant species behaved differently on the flowers, but they made similar contributions to their pollination (in spite of the different implications described below). This result corroborated the possibility that different ant species might be pollinators of Asclepiadoideae. It also again brings to light the question of whether this system is more common in the group than previously thought. Such a situation also calls upon one of the most basic and instigating issues in ecology, which is the fact that different species can have similar success, even following opposite strategies within the conflicting demands to which they are subjected.

## An apparently stable system

Regarding the stability and success of the studied ant–plant system, we believe it to be successful, in spite of the possibility of some loss to one or the other side. One possible loss is related to high endogamy rate promoted by ants. However, plant populations can remain stable regardless of crossing rates, and autogamy is usually a derived evolutionary advantage (Solbrig et al. 1977, 1988; Lloyd 1992). Another problem is that ants can alter nectar quality as they transport yeast, which consumes part of the sugars (Vega and Herrera 2013). This decrease in sugar content can reduce visits by winged pollinators, but it is known that a few crossing events are enough to maintain the genetic cohesion of a population (Loveless and Hamrick 1984). Therefore, despite occurring at lower frequencies, the activity of winged pollinators might suffice. Moreover, although we do not have data on the relative contribution of ants in contrast with other pollinators, it is important to consider that in an ant pollination system is possible to maintain the reproductive fitness even with the exclusion of winged pollinators (Ibarra-Isassi and Sendoya 2016).

In turn, some arguments in favor of the maintenance of ant pollination in *Ditassa* are based on the requirements proposed by Beattie (2006): ants do not damage the reproductive organs, they are frequent and abundant, they trigger the pollination mechanism, they do not damage the pollen with metapleural secretions, and they build perennial nests. Hence, we believe that there are no selective pressures towards avoiding ants as flower visitors. This is the first step toward keeping the system stable.

## Conclusions

We conclude that the generalist habit and phenotype of *Ditassa* flowers contribute to ant pollination. Likewise, the mechanism of guide rails and pollinaria, which is peculiar to Asclepiadoideae, and the resistance of pollinia to the metapleural gland, might contribute strongly to the establishment of this system. These conditions comprise synapomorphic traits found in the group. Additionally, although ants differ markedly from one to another in their natural history, affecting their visiting behavior, activity, and efficiency, they can make similar contributions to pollination. This called our attention to the potential of different ant species as pollinators and indicated adaptation to some trade-offs. Finally, we believe that there is enough evidence in plants and ants to hypothesize the existence of more cases of ant pollination in Asclepiadoideae, being the next step necessary for the elucidation of the relative contribution of these insects to the detriment of other pollinators in these systems.

**Acknowledgements** We thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by master scholarship of ADM; to Dr. Diego Sotero Pinangé for reviewing our English; to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a research grant to ICM and for financial support through Programa Ecológico de Longa Duração (PELD/Catimbu).

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