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First record of the occurrence and abundance of phorids (Diptera: Phoridae) associated with leaf-cutting ants of the genus *Atta* (Hymenoptera: Formicidae) in the municipality of Cruz das Almas-BA

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Abstracts

Leaf-cutting ants of the genus *Atta* are considered one of the main forest and agricultural pests. Currently, chemical control using granulated baits is considered the most efficient, however, with the inadequate adoption of management it can harm non-target organisms and the agro-ecosystem. Phorid parasitoids have been studied so that they can be used as an alternative management strategy for leaf-cutting ants. The use of phorids as part of the management strategy in biological control, despite being still very incipient, may in the future become a safer alternative for the control of leaf-cutting ants. Thus, the objective was to report the occurrence of parasitoids of the family Phoridae associated with leaf-cutting ants of the genus *Atta* in the municipality of Cruz das Almas. During the months of April to July, three environments were studied, being: pasture area, eucalyptus plantation and a fragment of Atlantic Forest. In each of these environments, three colonies of two species of ants *Atta sexdens sexdens* and *Atta laevigata* were selected. Each collection consisted of six hours of observation, divided into eight periods of 45 minutes. Observations were made on the trails and scouts consecutively, always starting with the scout. A total of 112 phorids belonging to two genera were collected, being 64 to genus *Eibesfeldtphora* and 48 the genus *Myrmosicarius*.

Introduction

The family Phoridae belonging to the order Diptera, represent a group of insects with a great diversity of lifestyle during its immature phase, being mostly parasitoids (Bragança, 2016). Most of these parasitoids stand out for attacking ants of the genus *Atta* and *Acromyrmex*, known as leaf-cutting ants. Ants of these genera harm diverse cultures of importance, both forest and agricultural at any stage of development (Zanetti, 2000).

Phorid parasitoids stand out among the natural enemies of leaf-cutting ants, making use of workers as a host for their immature stage. Adults oviposit specific parts of ants' bodies. Thus, the larvae feed on the internal content of the parts where the oviposition occurred, culminating in the death of the ant. The geographical distribution, both of hosts and parasitoids, occurs throughout the American continent, where 28 species of ants are parasitized by at least 70 species of phorids (Bragança, 2011).

In the case of phorids of the genera *Eibesfeldtphora* and *Myrmosicarius*, the preferred place for oviposition is the head, where the larvae feed on the ant's head mass. For this reason, they are called decapitating flies (Porter & Powes, 2018; Porter, 1997). These flies generally attack ants in their tracks, causing a great impact on the behavior of the anthill (Bragança et al., 2003). The mere presence of these flies on the trails leads to the interruption of the cutting activity of the plant material, as the workers flee to the nest in order to prevent the action of the parasitoids. Mortality can occur from 1 to 6% in ants, in addition to having a significant impact on changing the behavior of all anthills (Bragança, 2011).

Parasitoids can be species specific or attack more than one ant species (Uribe et al., 2014). Environmental variables such as temperature and humidity, in addition to biotic

factors, such as ant traffic, seem to be limiting factors for the performance of these parasitoids. Studies show that some species prefer to oviposit in environmental conditions with high temperatures and low humidity. Such conditions, biotic or abiotic, are responsible for the successful performance of these parasitoids and the development of their immature phase (Calcaterra et al., 2005; Bragança et al., 2008).

The discovery of these adverse effects on the work of ants demonstrates a possible contribution of these parasitoids to the management of leaf-cutting ants. However, taking into account the economic importance of these pest insects, the objective of the work is to report the occurrence of phorids (parasitoids) in species of ants of the genus *Atta* in the municipality of Cruz das Almas-BA, evaluating the influence of abiotic factors (temperature and relative humidity and difference in living) and biotics (ants traffic in scouts and trails) in the activity of these parasitoids.

Materials and methods

Study area

The study was conducted in the municipality of Cruz das Almas-BA, with Latitude 12º 40 '12" S, Longitude 39º 06' 07" W and Altitude of 220 m, located 120 km from the capital Salvador (Inmet, 2019). The region's climate predominates high temperature and relative humidity according to the Köppen-Geiger classification (1936), varying between 17 to 38 ° C and average annual precipitation of 1.136 mm. The vegetation is classified as forest dense ombrophilous, having a high diversity in plant species, and the soils are classified as Yellow Latosol and Yellow Argisol with a sandy-clay-sandy texture (Ibge,2016).

Selection and characterization of areas

Three areas with different vegetation were selected, the first being a pasture area with no shading. The second area was a *Eucalyptus* ssp. Forest, approximately 22 years old, with little shading. And the third is a fragment of Atlantic Forest with high shading. The choice of anthills in each area was defined by assessing the activity rhythm, by counting the ants on the trail, thus selecting the anthills that had their most intense activities. After the choice, the anthills were marked, isolated and each anthill was identified as a repetition, totaling three anthills per treatment.

Data collection

The parasitoids were collected from April to July 2017 and the number of collections each month ranged from two to three, with a duration of six hours of observation divided into eight 45-minute intervals (Gomes, 2011). In each interval, observations were made in the scouts and trails, starting at 8:00 am and ending at 5:00 pm. In the observation periods where there was no activity of ants, disturbances were caused in the colonies to instigate the workers to leave and thus attract the phorids. After the departure of the first workers, the time required for the appearance of the phorids was counted, totaling 216 hours of field observation.

Foraging activity was quantified on the scout and on the trail 50 cm from the scout by means of a manual counter, counting the number of foragers and collectors in the first 15 minutes of each interval. The remaining 30 minutes were dedicated to the observation of the phorids.

The phorids that attacked the ants around the colony (trails and scouts) were collected with the aid of plastic containers (2.5 x 8.5 cm). At the end of each observation interval, the number of phorids collected was recorded, labeled, conditioned in 70% GL alcohol and were taken to the Entomology Laboratory of the Federal University of Reconceive the Bahia (UFRB) to carry out the identification of biological material.

In the field, in addition to the foraging rhythm of the ants and the activity of the phorids, the behavior of the ants in the face of attacks by the parasitoid (defense mechanism) was also observed. In each observation, the time of collection of the phorids, temperature and the relative humidity of the air were checked. Meteorological data were obtained from the National Institute of Meteorology (Inmet, 2017), based on local stations.

Abundance of parasitoids

To determine the abundance of parasitoids, a 10 x 8.5 cm adhesive trap was impregnated with entomological glue on both sides (Fig 1). The traps remained in the field for 24 hours. Four traps were placed in each colony, two cards on the foraging trail and two on the scout's entrance. The phorids captured by the traps were collected and taken to the Entomology Laboratory of the Federal University of Reconceive the Bahia (UFRB), counted and conditioned in 70% GL alcohol.

Ant identification

Specimens of ants from each of the anthills observed were taken to the Entomology Laboratory at the Federal University of Reconceive the Bahia and identified using the key from Della Lucia (1993).

Phorid identification

The identification of the genders was made based on the work key of Uribe et al. (2014). To confirm the genera, each specimen collected was taken to the Biology Laboratory of the Federal University of Reconceive the Bahia (UFRB), placed in a Petri dish and photographed with the aid of a stereomicroscope (Leica EZD4) using the Application Suite application, the photos of each of the phorids were sent to a specialist at the National University of Colombia.

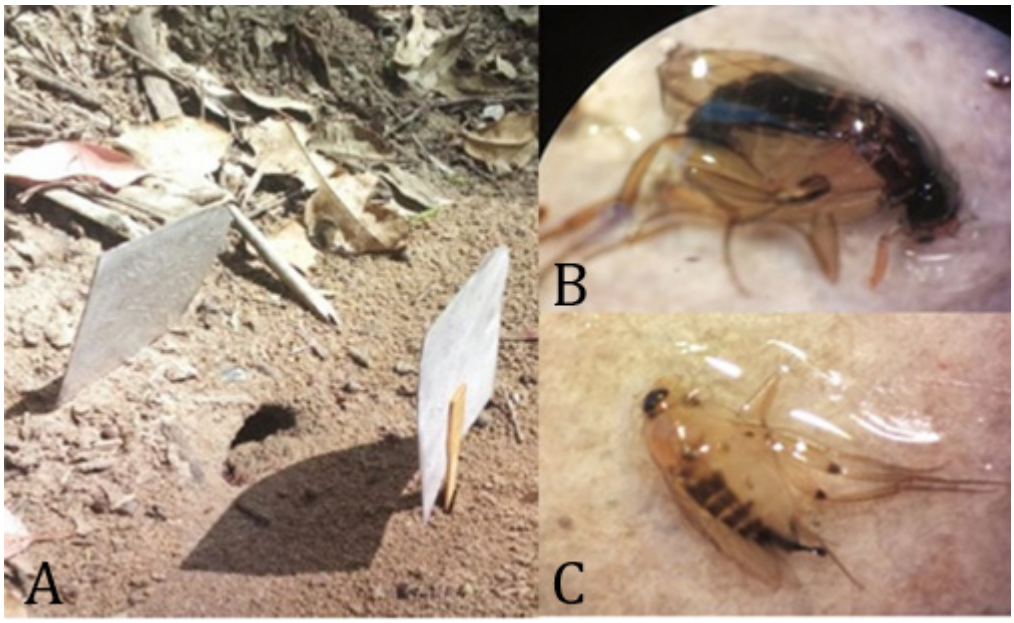

Fig 1. (A) Details and distribution of the traps, (B) Phorids captured from the genus *Myrmosicarius*, (C) Phorids captured from the genus *Eibesfeldtphora.*

Statistical analysis

The total abundance of phorids in the different areas and months of collections was compared using ANOVA with a randomized block design and the means compared by the Tukey test at 5% significance. Climatic data were compared with Pearson's correlation coefficient, comparing the two variables (temperature and abundance of phorids) (SAS University, 2002).

Results and Discussion

A total of 112 specimens of phorids were collected, 71% of the genus *Eibesfeldtphora* and 29% of the genus *Myrmosicarius*, associated with *Atta sexdens sexdens* and *Atta laevigata*. Only one specimen of the genus *Myrmosicarius* was found associated with *Atta sexdens sedens* (Table 1).

The abundance of phorids between the collection areas differs statistically only in the natural area (fragment of Atlantic Forest) when compared to the other areas (pasture area and *Eucalyptus*). The two agricultural areas will not differ statistically ($F_{(2,318)} = 4.09$; $p = 0.46$). The total number of phorids captured per area is 10 in the natural area, in the eucalyptus planting area 39 and in the pasture area 44 (Fig 2).

Table 1. Number of individuals of *Eibesfeldtphora* and *Myrmosicarius* (Diptera: Phoridae) in different species of *Atta*, in Cruz das Almas-BA, Brazil.

Species of Ants	Phoridae genera	
	Eibesfeldtphora	***Myrmosicarius***
Atta sexdens sexdens	41	1
Atta laevigata	23	47
Total	64	48

The sampling made with the use of adhesive traps in the period of 24 hours, results in a total of 53 individuals collected, being 19 of the genus *Myrmosicarius* (Fig 3) and 34 of the genus *Eibesfeldtphora* (Fig 4). There is no significant difference in the abundance of phorids when comparing the two sampling methods performed in this work (observation and trap). In the method using traps it is possible to notice the preference of the attack for the activity areas of the ants, being more abundant in the trails (68.7%) and scouts (31.3%) (Fig 2). The number of phorids is influenced by the traffic of ants, in all study areas.

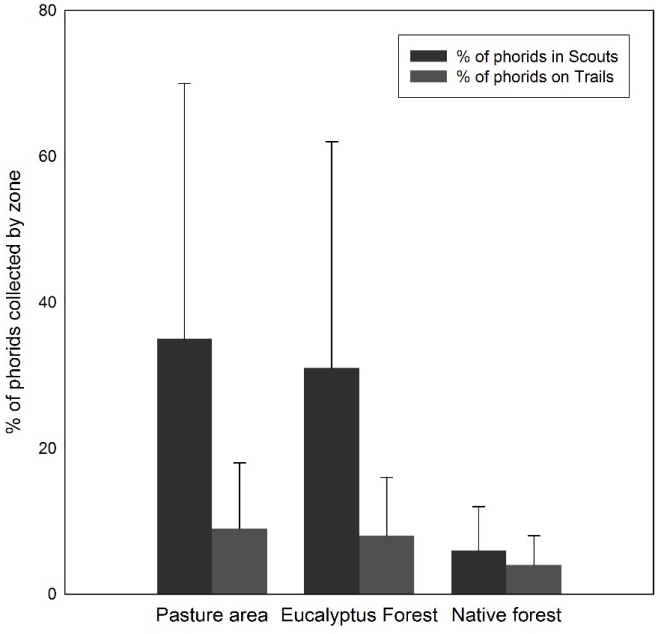

Fig 2. Percentage of phorids collected in each activity zone (scout and trails).

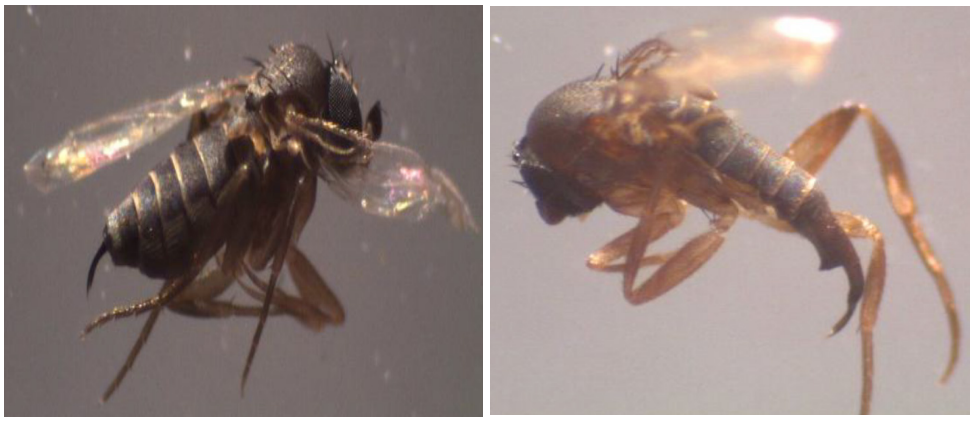

Fig 3. Species collected from the genus *Myrmosicarius*.

Considering the three areas, phorids of only two genera are identified, being *Eibesfeldtphora* and *Myrmosicarius*, with a greater occurrence of the genus *Eibesfeldtphora*. Regarding the climatic factors evaluated, the correlation is significant only for temperature, and the abundance of phorids has a strong to very strong correlation (Pearson r = 0.97; n = 8; p = 0.02). The relative humidity of the air does not show a correlation and is therefore not significant (Pearson r = -0.57; n = 8; p = 0.08).

Only two genera of parasitoids were collected when attacking the workers of *A. laevigata* and *A. sexdens sexdens*. The results will indicate parasitoid preferences among ant species, having a direct relationship with the place of occurrence, since *Eibesfeldtphora tonhascai* (Brown, 2001) and *Myrmosicarius grandicornis* (Borgmeier, 1928), have already been reported as parasitoids of *A. sexdens sexdens* in the states from Minas Gerais, Goiás and Rio de Janeiro (Tonhasca et al., 2001; Silva et al., 2008; Souza, 2013). However, there are no reports in the literature of the occurrence of these parasitoids in the state of Bahia, which allows us to affirm that the attacks of this Phorid on *Atta* in different latitudes suggest a process of adaptation to parasitism in a new host (Pereira, 2001).

Fig 4. Species collected from the genus *Eibesfeldtphora*.

Important variations in the occurrence of these phorids can happen throughout the year and in certain environments (Elizalde, 2011; Silva et al., 2008; Guillade et al., 2011), showing these clear dynamics between the species of ants. It is observed that the experiment shows the preference of some genera of phorids for a given environment, with the largest number of phorids found in areas with less dense vegetation and with a higher incidence of sunlight. Elizalde and Folgarait (2010), carried out studies where they associated species of phorids with specific habitats, and the results showed the preference of *Myrmosicarius* for closed environments and *Eibesfeldtphora* in open environments or, for areas with some type of culture.

The greatest abundance of phorids is obtained in the pasture area. Gomes (2011) reports that there is a greater number of parasitoid flies in areas with a higher incidence of light, as they are insects with daytime activity. This fact justifies the number of captured phorids being greater in areas with little vegetation. Bragança et al. (2008) performing laboratory tests on ants of the genus Atta where they were subjected to three different proportions of light to verify its influence on the action of parasitoids, the luminosities were high (simulating daytime light), medium (simulating dusk and dawn) and absence of light (simulating night). They note that there were only attacks by parasitic phorids at the high light level, suggesting that some species of parasitic phorids

are only active during the day and not active during dawn, dusk or at night, thus concluding that the visual stimulus may be an essential factor for the location of the host.

The collection techniques used prove to be efficient to assess the abundance of phorids in the three study areas. Despite the lower number of phorids being collected in the natural area, in contrast to the results obtained by Galvão (2016), the detection of the presence of these flies in specific environments, already shows their activities in any environment. The rhythm of activity and the behavior of the attack against the species of ants in each environment may have made it difficult to visualize the phorids in the natural landscape area (Bragança et al., 2008). Eucalyptus plantations and pasture areas create an environment that facilitates the visualization of parasitoids since, it is believed that this is the main means of interaction between host and parasitoid (Bailez, 2016).

At first, the number of captured phorids seems to have been low, especially of the genus *Myrmosicarius*. Gomes (2011) states that the duration of sampling, in addition to the time and time of year in which these surveys are carried out, can influence the abundance of phorids, which in fact would justify the low number of captured parasitoids, and the study in question it was driven most of the time in rainy season. Phorids of the genus *Myrmosicarius* do not seem to be affected by environmental changes, as well as their hosts (Wirth et al., 2007), whereas those of the genus *Eibesfeldtphora*, are more susceptible to climate changes, due to their greater sensitivity to light, having preference for brighter environments (Bragança, 2011). The abundance of parasitoids being negatively influenced by temperature, it is assumed that these phorids are literally linked to light and are more efficient in dry periods (Martins, 2015).

All species of ants studied have a defense mechanism against the action of parasitoids, such as changing the cutting time and protection of the body where the parasitoids can oviposit. during foraging. This behavior has been mentioned in other studies with other species of ants of the same genus, in order to hinder the action of parasitoids, since they prefer the size of the ants, always attacking larger ants (soldiers) (Feener & Brown 1993; Tonhasca, 1996; Bragança et al., 2009).

Linksvayer et al. (2002), studied the way that the smaller workers defend the bigger workers against oviposition during the daytime, and called this hitchhiking mechanism, which means "*hitchhiking*". It is not yet known for certain whether it is really a defense mechanism against the parasitoid or if it has some other function, however during field observations it was possible to notice this behavior when it had the presence of phorids. The number of phorids is influenced by the traffic of ants in all areas of study, due to this, the hypothesis arises that the abundance of phorids may be associated with the release of allelochemicals, because when a disturbance was made in the colonies, instigating the output of ants also increased the number of captured phorids.

Communication between workers (smaller ants) and soldiers, as if it were a request for help, involves chemicals called alarm pheromone, which triggers the defense behavior in the colony. The constituent is composed of volatile substances which can be one of the main factors that attract phorids. As it is a mechanism that has not been studied yet, further studies are necessary between the pheromone of leaf-cutting ants and phorids. However, according to the observation of this work, everything indicates that it may be among the main interaction routes, in addition to visualization.

Conclusion

This work was the first report of parasitoid phorids associated with ants of the genus *Atta* in the Recôncavo da Bahia. The abundance of these parasitoids may prove to be a control method for leaf-cutting ants, however, the results found suggest the need for future studies, which can understand the role of these natural enemies of leaf-cutting ants in different habitats.

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