Sociobiology 72(3): e11408 (September, 2025)
DOI: 10.13102/sociobiology.v72i3.11408

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

First Record of Parasitoidism by Phoridae Flies in *Atta opaciceps* Borgmeier (Hymenoptera: Formicidae), a Leafcutter Ant from the Northeast Region of Brazil

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Article History

Edited by
Elmo Borges Koch, UEFS, Brazil
Received 31 October 2024
Initial acceptance 15 December 2024
Final acceptance 17 February 2025
Publication date 14 July 2025

Keywords
Natural enemies, biological control, parasitoidism rate, Neotropical Region.

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Abstract

Leafcutter ants of the genus *Atta* Fabricius, 1804 (Hymenoptera: Formicidae) are important actors in natural environments, as they are part of crucial ecological processes; however, they are also considered important pests in agroecosystems. Numerous species of flies from the Phoridae family have been recorded as parasitoids of several species of leafcutter ants, with details of their biology, life cycle, and forms of attack behavior. For the leafcutter *Atta opaciceps* Borgmeier, 1939, there is no record of associated phorids or even records made in the state of Sergipe on parasitoid phorids. This study is the first to report the occurrence of phorids associated with *A. opaciceps* and the observed parasitoidism rates. Workers collected in the field were kept in the laboratory for parasitoid emergence. The parasitoidism rate was 8.4%, with most workers being parasitized by *Myrmosicarius grandicornis* Borgmeier, 1928 (n = 267; 99%). There were two other records: one parasitized by *Eibesfeldtphora declinata* (Borgmeier, 1925) and another worker hosting *Apocephalus setitarsus* Brown, 1997. This study expands the knowledge about phorids associated with *Atta* in Brazil, including *A. opaciceps* on the list of natural hosts, the state of Sergipe in its distribution area, and information on parasitoidism rates and biological characteristics of this interaction.

Introduction

Leafcutter ants of the genus *Atta* Fabricius, 1804 are important actors in the Neotropical region, playing diverse and antagonistic roles, sometimes contributing to fundamental ecological processes in ecosystems, such as nutrient cycling (Farji-Brener & Werenkraut, 2015, Nascimento et al., 2024), and sometimes causing economic loss in agroecosystems, due to the severe defoliation they promote in vegetation (Fowler et al., 1989; Cherrett, 1986; Della Lucia, 2011; Leal et al., 2014).

In the Northeast region of Brazil, *Atta sexdens* (Linnaeus, 1758) stands out for its wide distribution (Delabie et al., 1997; Barrera et al., 2022), while other species occur more locally, according to the predominant vegetation cover, such as *Atta cephalotes* (Linnaeus, 1758) in fragments of the Atlantic Forest between the states of Pernambuco and Bahia (Corrêa et al., 2005), as well as *Atta opaciceps* Borgmeier, 1939, a species endemic to the northeast, but which has high plasticity, occurring in many other environments, such as the semiarid Caatinga, forest fragments and pastures (Delabie et al., 1997, Santos & Sousa-Souto, 2023). *A. opaciceps* has proliferated in anthropogenic landscapes, inhabiting from forests to agricultural fields (Siqueira et al., 2017; Sousa-Souto et al, 2022), reducing the availability of biomass for livestock or even damaging urban afforestation in parks and forest fragments in cities, due to the consumption of seedlings of tree species.

Open access journal: http://periodicos.uefs.br/index.php/sociobiology
ISSN: 0361-6525

Although the use of chemical baits is still one of the primary methods for controlling leafcutter ants (Mota Filho et al., 2021a), more sustainable methods have been researched, especially those that promote natural biological control (Araujo et al., 2022; Mota Filho et al., 2021b). Parasitoid flies of the family Phoridae are among the most common natural enemies of leafcutter ants (Feener & Moss, 1990; Feener & Brown, 1997; Brown, 2001; Pereira et al., 2022a). These flies are often observed near leafcutter ant nests, attacking workers on their trails, nest entrance, or foraging areas throughout the year (Erthal & Tonhasca, 2000; Tonhasca et al., 2001; Bragança, 2011; Arruda et al., 2019; Souza et al., 2021). The attacks are carried out by females when they lay their eggs on the host ant, usually in the head or abdomen (Bragança & Medeiros, 2006; Bragança et al., 2016). It has also been shown that host size is an important factor in female decision-making in different species (Souza et al., 2021; Pimentel et al., 2022; Bragança et al., 2023). Once parasitized, workers have a reduced life expectancy, and attacks affect the foraging behavior of leaf-cutting ants, reducing the transport of plant material owing to the reduction in the size and activity time of workers, making them potential agents for biological control programs (Bragança et al., 1998; Tonhasca & Bragança, 2000; Tonhasca et al., 2001; Almeida et al., 2021).

Therefore, the objectives of this study were to report, for the first time, the occurrence of phorid flies parasitizing *A. opaciceps*, to assess the rates of parasitoidism, and to evaluate the biological characteristics of this interaction, including the duration of the pupal period, the emergence rate of the parasitoids, and the size and lifespan of parasitized and non-parasitized workers.

Materials and Methods

Study area

The study was performed at three sites (locations) in state of Sergipe, Brazil (Fig 1) between September 2022 and August 2023. Site 1 (10°94’68.7″ S, 37°05’44.5″ W) is an urban forest area of approximately 10,000 m^2. Site 2 was established on the campus of the Federal University of Sergipe (UFS) (10º92’42.5” S 37º10’01.2” W), which also presents an urban forest-type physiognomy with a total area of approximately 40,000 m^2. Site 3 (10º95’88.7” S 37º26’32.3” W) is a pasture area of approximately 30,000 m^2. The distance between the sites was approximately 6–30 km. These places fall within the tropical climate, which presents winter rains (As) according to the Köppen-Geiger climate classification. The climate is characterized by an average air temperature of 29 ^{o}C with a strong rainfall gradient (east-west) of 1,500 to 700 mm, with a rainy period concentrated between April and August, and an intense dry period (September to March) (Álvares et al., 2013). The average temperature in the study areas varies between 26.8 ^{o}C and 28.8 ^{o}C throughout the year (INMET, 2024). During the experiment, the rainiest month was June 2023 (200.2 mm) and the driest month was December, with 24 mm (INMET, 2024).

The soil type in the study region is Spodozol, mainly deep sandy clay with low fertility, high porosity (draining rainfall), high acidity, and salinity (Sousa-Souto et al., 2012). In urban forest areas, the dominant vegetation consists of grasses and herbs, mainly *Paspalum notatum* Flügge (Poaceae), *Cynodon dactylon* L. (Poaceae), and *Richardia brasiliensis* Gomes (Rubiaceae) (Poderoso et al., 2009), in addition to tree vegetation commonly used in urban afforestation in the region, composed of native and introduced species, such as *Terminalia catappa* L. (Combretaceae), *Moquilea tomentosa* (Benth.) (Chrysobalanaceae), *Cenostigma pyramidale* (Tul.) E. Gagnon & G. P. Lewise (Fabaceae), *Anadenanthera colubrina* (Vell.) Brenan (Fabaceae) and *Cocos nucifera* (Arecaceae). The dominant species in the pasture area was *Brachiaria decumbens* (Poaceae).

The map (Fig 1) was created using SimpleMappr (Shorthouse 2010). Specimens were photographed using a 3MP camera attached to a Leica EZ4-HD stereomicroscope. Editing of the drawings and final plates was performed using Inkscape 0.91.

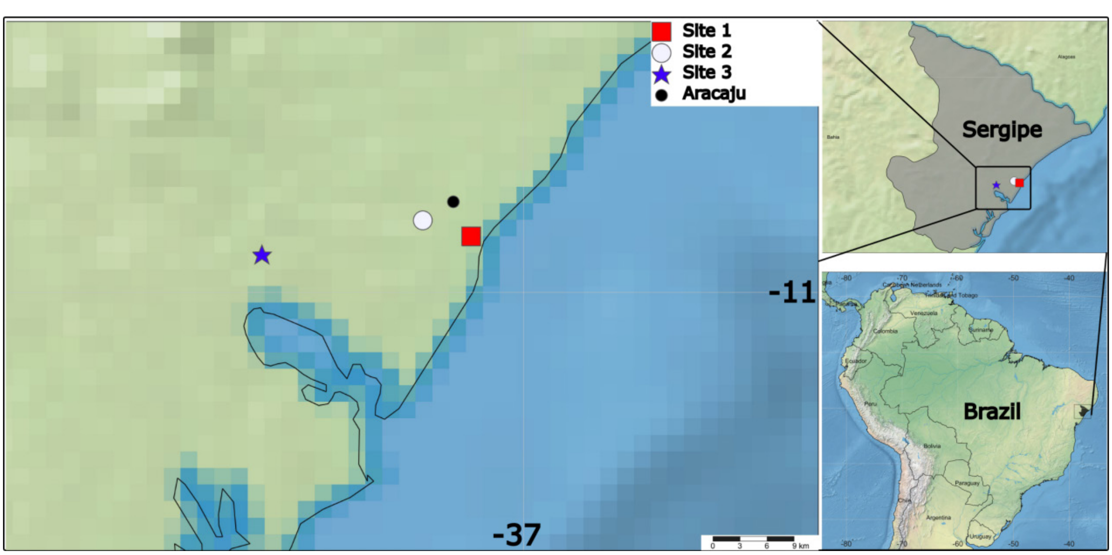

Fig 1. Study area locations: site 1, urban forest area; site 2, campus of the Federal University of Sergipe (UFS), which also presents an urban forest-type physiognomy; site 3, pasture area.

Ants sampling

Between September 2022 and August 2023, 17 colonies were mapped to three sites for sampling workers. Due to the availability of the incubator, between two and four colonies could be sampled each month, with a minimum of 100 workers per colony. The collection time was standardized and always occurred in the morning from 7:00 am to 10:00 am. The sampling time was determined for logistical reasons, and although this time did not correspond to the peak activity of the colonies sampled in the dry season, there was sufficient ant flow to collect 100–150 workers in less than 20 min in all sampling events. Due to the higher density of colonies, collection at site 2 occurred in all months of the experiment, totaling 2,080 workers collected. In the urban forest area (site 1), three collections were carried out in November, December 2022, and July 2023, totaling 354 workers. At site 3, four samples were collected in September and October 2022 and February and March 2023, totaling 763 workers (Supplementary Table).

Forceps were used to collect workers along foraging trails and active nest entrance holes. The collected ants were placed in plastic pots (400 mL) and transported to the Insect Ecology Laboratory (UFS). In the laboratory, the workers collected from the same nest were placed in groups of 20 individuals in a 250 mL plastic container (78 mm long, 78 mm wide, and 70 mm high), with small holes in the lid to allow oxygen renewal. The containers were maintained in a growth chamber (26 ± 1 ºC, 85 ± 5% RH). Workers were fed every 48h using a cotton pad soaked in a solution of water and 10% honey, placed in the center of the container (Bragança et al., 2016). Daily, dead ants were placed in Petri dishes (60 × 15 mm) and inspected under a stereomicroscope (Leica EZ4HD) for 7 days after death. During this period, all dead workers which showed signs of parasitoidism, as described by Almeida et al. (2023), were individually placed in a 1.5 ml microcentrifuge tube sealed with cotton until the parasitoids emerged. After 7 days, dead workers without signs of parasitoidism were considered in calculating the parasitoidism rate and discarded. After 15 days of observation, the number of remaining live workers was considered in the parasitoidism rate and subsequently discarded (Tonhasca et al., 2001). Between 24 and 36 hours after emergence, adult phorids were killed by freezing for 30 min, and each fly was stored in a vial containing 70% alcohol. This period was necessary to allow for complete cuticle sclerotization for subsequent identification.

Adult parasitoid collection

Along with monthly collections of workers in the field, in November 2022 – January 2023 (dry period) and April – June 2023 (rainy period), an active search was carried out for phorids observed in active feeding holes or attacking workers on foraging trails. Specimens were collected twice per month, always in the first and last week of each month, with sampling for 30 minutes of two colonies each week, totaling 4 colonies and 2 hours of sampling effort per month. The collection of phorids began immediately after the collection of workers, in the same colonies and active trails. Flies were collected using an entomological aspirator. The collected flies were taken to the laboratory, killed by freezing for 30 min, and placed in vials containing 70% alcohol for identification. This collection method aimed to complement the information on the diversity of parasitoids.

The fly species were identified through morphological examination using a Leica MZ16 stereomicroscope, and identification keys proposed by Brown (1997), Brown et al. (2010), Disney et al. (2006), and Uribe et al. (2014). Phoridae flies were deposited in the Insect Ecology Laboratory (UFS) and Zoology Museum of the University of São Paulo (MZUSP) collection. Major workers were identified using the keys available in Della Lucia (1993) and compared with the specimens deposited in the UFS entomological collection.

Data analysis

Parasitoidism rates were calculated monthly, based on the ratio of parasitized workers to the total number of workers collected from the set of colonies. The emergence rate of phorids in the laboratory was determined by calculating the ratio of emerging adult parasitoids to the total number of pupae. Parasitoidism and emergence rate data were pooled for all colonies. The lifespan of the collected workers (parasitized or non-parasitized) and the duration of the pupal period (i.e., the difference in days between the date of pupal formation and adult emergence) were recorded. The head capsule width (the widest point of the head in mm at the lower margin of the compound eyes) was measured for comparison, using a stereomicroscope equipped with a millimeter ocular lens to test for possible differences between the size of parasitized and non-parasitized workers (Farder-Gomes et al., 2018). The data were subjected to the Shapiro-Wilk normality test (α = 5%). The width of the head capsules of non-parasitized and parasitized workers was compared using a t-test (α = 5%). For this t-test comparison, 267 non-parasitized ants were selected randomly from the pool of collected ants to obtain samples of the same size.

Finally, the survival probability of parasitized and non-parasitized workers was compared using survival analysis (Kaplan-Meier), followed by pairwise comparisons using log-rank tests at a significance level of 5% (Crawley, 2013). The Kaplan-Meier method is widely used to estimate the survival function when dealing with censored data, that is, when the event of interest (ant death) is not observed for all replicates in the group or both treatments during the observation period (Barber & Jennison, 1999). Additionally, a Student's t-test was performed to compare the average survival (in days) of parasitized and non-parasitized *A. opaciceps* workers. Data were analyzed using the R software (R Core Team, 2023).

Results

A total of 3,197 workers were collected, of which 269 (8.4%) were parasitized by phorids. The majority of workers (n = 267; 99%) were parasitized by *Myrmosicarius grandicornis* Borgmeier, 1928, followed by one record of *Apocephalus setitarsus* Brown, 1997, and another by *Eibesfeldtphora declinata* (Borgmeier, 1925) (Fig 2).

The main signs of parasitoidism observed in the workers were the same as those described by Almeida et al. (2023). For example, the host of *E. declinata* showed a dark spot on the inner central part of the head when viewed against a light source approximately 36h after the ant's death. The sides were translucent, and approximately 72 hours later, the puparium was observed positioned between the jaws (Fig 3A), remaining approximately half of its length inside the head until parasitoid emergence, which occurred 16 days after the pupa was exposed. Thus, the period from host death to adult emergence was 22 days. In the case of *A. setitarsus*, three larvae were observed freely on the ant's body around 72h after its death, and three pupae were observed near the host body between four and six days later (Fig 3B). Adult emergence occurred between 12 and 13 days after puparium formation.

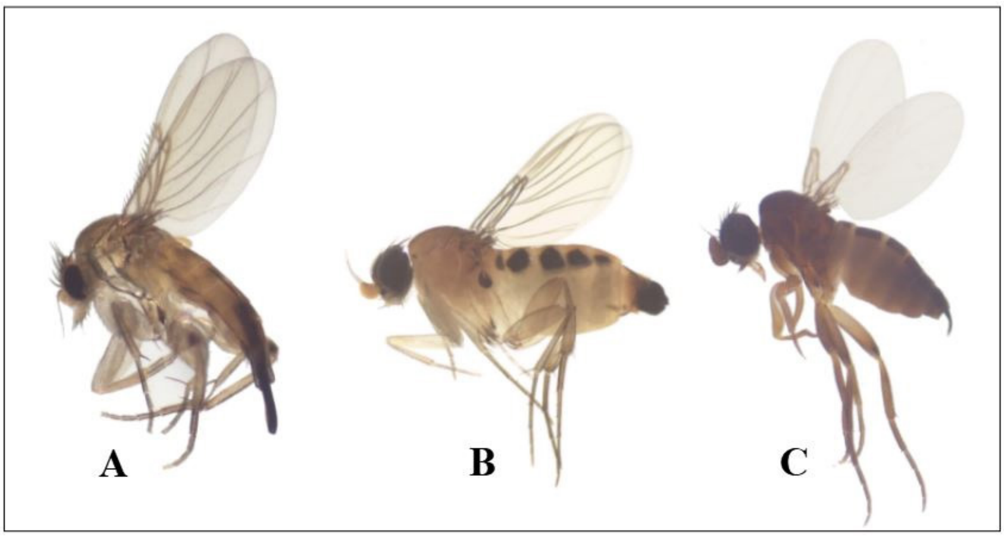

Fig 2. Females of the three species of parasitoid phorids collected in the present study: *Apocephalus setitarsus* (A), *Eibesfeldtphora declinata* (B), and *Myrmosicarius grandicornis* (C). Flies are not in their original scale.

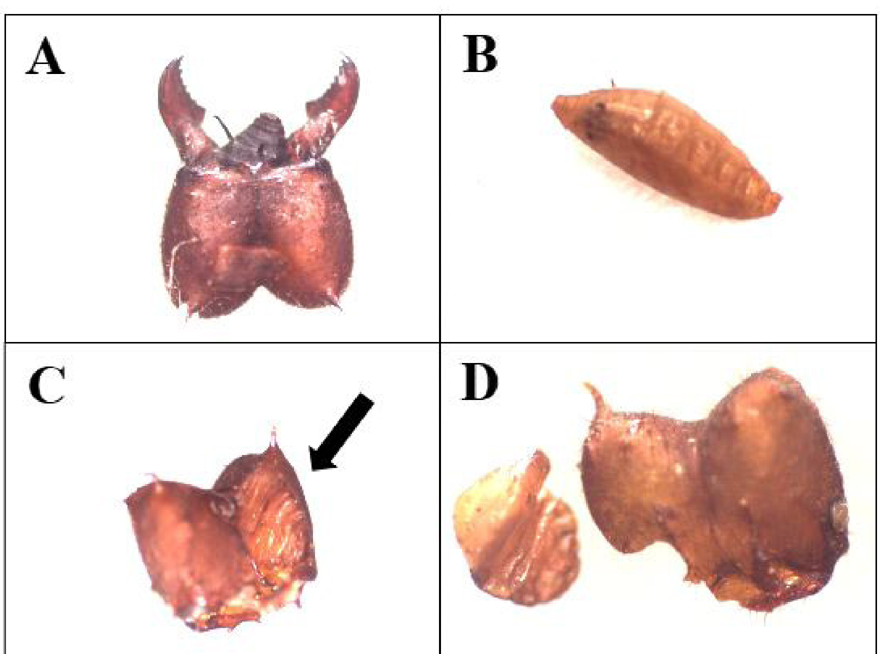

Fig 3. Signs of parasitoidism in *Atta opaciceps*. The puparium of *Eibesfeldtphora declinata* was observed between the worker's jaws (A). Pupa of *Apocephalus setitarsus* developed outside the ant's body (B). Head of *A. opaciceps* with translucent lobe, allowing visualization of the pupa of *Myrmosicarius grandicornis* inside (arrow) (C). The same head and pupa, after being dissected under a stereomicroscope (D).

Finally, workers parasitized by *M. grandicornis* showed head detachment from the body and loss of the mandibles, approximately 24h to 72h after the ant's death. During this period, a single larva was easily seen inside the head, often positioned in the right lobe, with the left side of the head completely translucent (Fig 3C-D). All puparia remained inside the ant head capsules until adulthood, with an emergence success rate of 58.4%.

A single worker (head capsule width of 3.38 mm) observed to host *E. declinata* was collected on September 22, 2022 (the beginning of the dry period in the region), with adult emergence (female) on October 14. A single worker parasitized by *A. setitarsus* was collected on August 10, 2023. Both *E. declinata* and *A. setitarsus* individuals were obtained from hosts collected from the Federal University of Sergipe (UFS) Campus. Worker ants parasitized by *M. grandicornis* occurred in all collection months and at all three sampling sites. Due to its dominance, all information on parasitoidism rates, monthly fluctuations in these rates, and average measurements of the cephalic capsule mentioned below was calculated only for *M. grandicornis.*

Parasitoidism rates displayed seasonal patterns. The highest rates were observed in January, June, and August 2023 (above 14%), whereas the months with the lowest rates (below 5%) were September, October 2022, March, and May 2023 (Fig 4). The values of the head capsule measurements did not differ from those of the normal distribution ($P = 0.15$). The average width of the head capsules of parasitized workers (2.18 ± 0.29 mm; n = 267) and non-parasitized (2.10 ± 0.31 mm; n = 267) did not differ significantly, at a significance level of 5% (t = 1.89; $P = 0.076$).

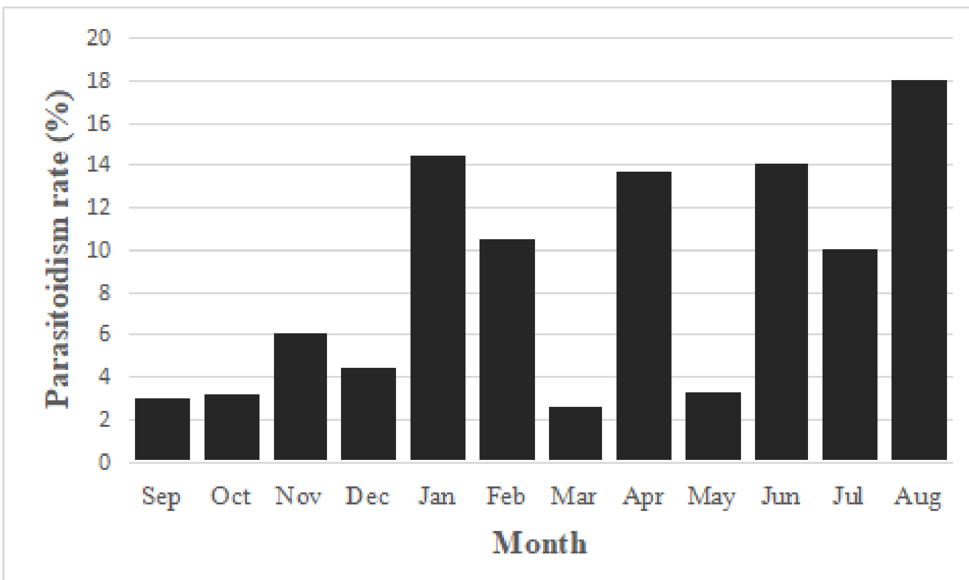

Fig 4. Parasitoidism rate (%) caused by *Myrmosicarius grandicornis* on *Atta opaciceps* workers in Sergipe, Brazil, from September 2022 to August 2023.

The probability of survival was lower in parasitized workers ($P = 0.007$; estimates obtained from 267 parasitized and 2,928 non-parasitized individuals), with an average lifespan of four days less than that of non-parasitized ants (Fig 5). The same results were verified by t-test comparison in which the average survival of parasitized workers (5.0 ± 3.28 days; n = 243) was significantly lower (t = 2.61; $P < 0.014$) than that of non-parasitized workers (8.6 ± 4.3 days; n = 228) kept in the growth chamber. The pupal period was 22 ± 6.3 days, with a minimum of 6 days and a maximum of 37 days.

Finally, 28 phorids were collected during active sampling (adult parasitoid collection), with 17 individuals collected in November and January and 11 collected in April and June (Supplementary Table). All individuals were female and identified as *M. grandicornis*.

Discussion

In the present study, it was possible to observe the occurrence of three species from three different genera of parasitoid phorids in *A. opaciceps*, with *M. grandicornis* accounting for 99% of the records. The present study also expanded the geographic distribution of these phorid species along the northeastern region of Brazil, adding the State of Sergipe.

The two occasional species in the present study have a distinct distribution pattern in Brazil. On one hand, the species *E. declinata* has records of parasitoidism in *A. sexdens* (Tonhasca, 1996; Almeida et al., 2023), *A. cephalotes* (Brown, 2001), *Atta colombica* Guérin-Méneville, 1844 (Disney et al., 2016), and *Atta laevigata* (Smith, 1858) (Silva et al., 2008),

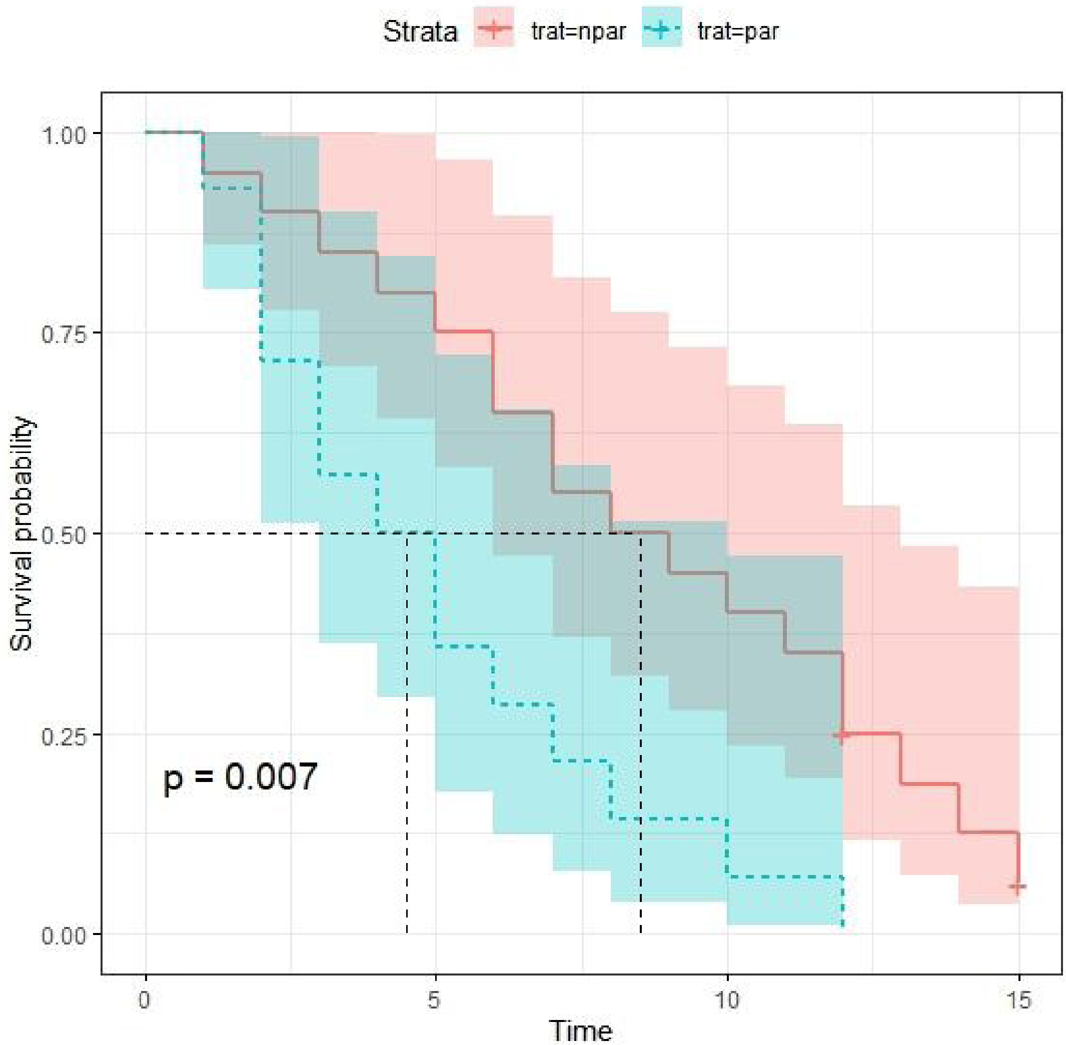

Fig 5. Survival curve of parasitized (par) and non-parasitized (npar) *Atta opaciceps* workers by *Myrmosicarius grandicornis*. The bold dotted line indicates the period in days when the probability of survival reaches 50% between treatments.

and its distribution is restricted to the North, South, Southeast, and Central-West regions of Brazil. On the other hand, the phorid *A. setitarsus* had just one recorded occurrence in Brazil, more specifically a male collected using a light trap, near the city of Manaus (2.67°S, 60.02°W) in the northern region of Brazil (Brown, 1997). The low abundance of *A. setitarsus* was also reported by Elizalde and Folgarait (2012) for *Atta saltensis* Forel, 1913 and *Atta vollenweideri* Forel, 1893 in Argentina. These authors point out that the attacks were made while the workers cut leaves and that these phorids were rarely observed on foraging trails. The emergence of multiple specimens of *A. setitarsus* (n = 3) from a single worker agreed with previous reports on *Apocephalus* spp. For example, Bragança et al. (2021) observed a variation of one to seven pupae of *A. attophilus* in *Atta bisphaerica* Forel, 1908, but there are records of up to 14 parasitoids in a single host of *A. laevigata* (Bragança et al., 2016).

Although we only considered *M. grandicornis*, the parasitoidism rate of 8.4% observed here can be considered exceptionally high compared to other studies citing the same parasitoid. For example, Farder-Gomes et al. (2018) found only two *A. bisphaerica* workers parasitized by more than 23,700 workers collected (0.008% rate), and Bragança et al. (2021) observed 1,238 workers parasitized by *M. grandicornis* among 91,544 workers sampled (1.35%), while for *A. sexdens,* other studies reported natural parasitoidism rates of 0.2% (Pereira et al., 2022a) and 0.89% (Bragança et al., 2016).

The species *M. grandicornis* has a high host plasticity, parasitizing several species of *Atta* in a wide range of environments in Brazil, Paraguay, and Argentina (Arruda et al., 2019; Galvão et al., 2019; Bragança et al., 2003; Disney et al., 2006; Elizalde & Folgarait, 2010). Despite this fact, the favorability of the occurrence of a given parasitoid species depends on the host species and location of the sampling sites (Bragança et al., 2016; Farder-Gomes et al., 2018; Galvão et al., 2019). For example, Bragança et al. (2016) found that the proportion of workers parasitized by *M. grandicornis* ranged from 57% for *A. sexdens* to 1.43% for *A. laevigata* in the same area of the Cerrado in the state of Tocantins. For our study area, however, this hypothesis should be tested in future studies that compare the richness of phorids and the frequency of parasitoidism between *A. opaciceps* and other conspecific leafcutter ants.

As previously mentioned, the worker collection time in the present study did not coincide with the peak of colony activity during the dry season, and this factor may have influenced the frequency of larger workers on the foraging trails and, consequently, our results. Host selection based on size is an important factor that explains the preference of phorid flies to attack workers (Farder-Gomes et al., 2018; Bragança et al., 2021, 2023; Pereira et al., 2022b), and this effect of host size may explain the predominance of *M. grandicornis* over other species in the present study. For example, Bragança et al. (2023) demonstrated that *M. grandicornis* presented the highest rates of parasitoidism in colonies whose workers had the smallest size (Bragança et al., 2021, 2023). The average size of the workers parasitized observed here (2.17 ± 0.35 mm) is quite smaller than the average size reported by Bragança and Medeiros (2006) for *A. laevigata* workers parasitized by *Neodohrniphora erthali* (Brown, 2001) (4,3 ± 0.9 mm). Thus, the few records by *A. setitarsus* and *E. declinata* found here suggest that, at least in part, the greater occurrence of *M. grandicornis* was due to the smaller size of *A. opaciceps* workers sampled in our study areas, favoring the parasitoid species that has a preference for workers with a narrow head width.

In the present study, the longevity of parasitized workers was approximately 50% lower than that of non-parasitized workers, which is in line with other studies that have already demonstrated the same effect, regardless of the parasitoid or host species (Bragança & Medeiros, 2006; Elizalde & Superina, 2019; Farder-Gomes et al., 2018). For *M. grandicornis*, the survival results observed here were similar to the survival time reported by Bragança et al. (2021) of approximately four days, indicating slight variation between the different regions where the parasitoid occurs. However, differences in longevity occur when comparing different species of parasitoids, with workers parasitized by *A. attophilus* having a shorter lifespan than those parasitized by other species (Farder-Gomes et al., 2016; Bragança et al., 2021). This difference may be due to the shorter larval and pupal development times (Farder-Gomes et al., 2016), as well as the greater number of larvae that develop in the same host of *A. attophilus*, thus accelerating the consumption of internal tissues and consequently host mortality (Bragança et al., 2021).

This study reports, for the first time, the parasitoidism of the leafcutter ant *A. opaciceps* by Phoridae flies. The three species of phorids also parasitize other *Atta* species in Brazil and Argentina, expanding the geographic distribution of these flies along the northeastern region of Brazil, now including the State of Sergipe. The high rate of parasitoidism observed, as well as the significant reduction in the longevity of workers parasitized by *M. grandicornis*, reinforces the potential of this phorid as a biological control agent, offering a new perspective for managing *A. opaciceps* colonies in anthropized areas. Future studies focusing on the biology and behavior of phorids in both field and laboratory settings could help elucidate the factors contributing to the occurrence of these species in the study areas, as well as provide insights into strategies that could favor their presence and increase their parasitoidism rates.

Acknowledgments

We want to thank the team at the Insect Ecology Laboratory (UFS), especially Gilvan S. Silva, Lucas Pajeu, Carlos Lemos, Geovanna Amaral, Catharine Menezes, Elvira Carvalho, and Alex Pinto, for helping with field collections and maintaining the insects in the laboratory. Nascimento LCF was supported by a COPES/PNAES/UFS scientific initiation grant. TPLP thanks the Biodiversity Post-doctoral Initiative (MCZ - Harvard) for the support of the Post-Doctoral Fellowship. Our thanks to two anonymous referees, who contributed significant improvements to the final version of the text.

Authors' Contributions

LCFN: Conceptualization, methodology, investigation, writing original draft.
TPLP and MALB: Phorid identification, writing original draft, writing review & editing.
LSS: Conceptualization, project administration, supervision, resources, investigation, formal analysis, writing original draft, writing review & editing.

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Supplementary Table

Table S1. Nest identification, year, month, sampling site, and the number of workers and phorids sampled at each sampling event. S1 = Site 1: Urban forest area in Aracaju - SE (10°94’68.7″ S, 37°05’44.5″ W); S2 = Site 2: Campus of the Federal University of Sergipe (UFS) (10º92’42.5” S 37º10’01.2” W). S3 = Site 3: Pasture area in São Cristóvão - SE (10º95’88.7” S 37º26’32.3” W). (*) Phorids collected during active searches.

Nest ID	Year	Month	Site	nº Workers	nº Phorids*
1	2022	Sep	S2	122	
2	2022	Sep	S3	182	
3	2022	Oct	S2	130	
4	2022	Oct	S3	191	
3	2022	Nov	S2	150	5
5	2022	Nov	S1	100	3
6	2022	Dec	S2	134	
7	2022	Dec	S1	100	
6	2023	Jan	S2	118	4
8	2023	Jan	S2	127	5
4	2023	Feb	S3	178	
9	2023	Feb	S2	111	
10	2023	Mar	S2	135	
11	2023	Mar	S3	102	
12	2023	Mar	S3	110	
13	2023	Apr	S2	104	2
14	2023	Apr	S2	120	3
1	2023	May	S2	125	
9	2023	May	S2	115	
8	2023	Jun	S2	100	2
15	2023	Jun	S2	105	4
5	2023	Jul	S1	154	
16	2023	Jul	S2	140	
15	2023	Aug	S2	108	
17	2023	Aug	S2	136	
Total				3,197	28