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Parasitism of *Atta sexdens* Forel, 1908 (Hymenoptera: Formicidae) by phorids (Diptera: Phoridae) in Londrina-PR

Parasitismo de *Atta sexdens* Forel, 1908 (Hymenoptera: Formicidae) por forídeos (Diptera: Phoridae) em Londrina-PR

Julio Cesar de Almeida[1]; Adriano Thibes Hoshino[2]; Thalles Platiny Lavinscky Pereira[3]; Amarildo Pasini[2]*

Highlights

Natural biological control of *Atta sexdens.*
Behavioral patterns of parasitoid attacks on their hosts.
The action of parasitoid phorids interferes with foraging by leaf-cutting ants.

Abstract

Leaf-cutting ants belonging to the genus *Atta* are considered fundamental ecosystem engineers while also posing a threat to production systems due to their extensive defoliation behavior on several plant species. Several flies of the family Phoridae are parasitoids of social insects and engage in the biological control of leaf-cutting ants. This study aimed to assess the prevalence and diversity of parasitoids affecting *Atta sexdens*, a leaf-cutting ant species, in Londrina, state of Paraná, Brazil. Additionally, the study sought to record parasitism rates and the behavioral and biological traits of the species. Between June 2019 and May 2020, we conducted collections of worker ants and their associated phorid flies at previously selected nests located on the campus of the State University of Londrina and within the native forest of the Botanical Garden of Londrina. The collected phorid flies were preserved in 70% alcohol for subsequent species identification, while the leaf-cutting ants were brought to the laboratory to assess parasitism, following established protocols in the literature. Collected ants were frequently inspected and dead individuals were placed individually in plastic tubes for further analysis. A total of 216 adult phorid flies were captured in both environments, with the following parasitoid species identified: *Apocephalus attophilus, Eibesfeldtphora declinata, Eibesfeldtphora elongata, Eibesfeldtphora tonhascai,* and *Myrmosicarius grandicornis.* Among

[1] Masters Student in Graduate Program in Agronomy, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: julio.bras7@gmail.com
[2] Profs. Drs., Department of Agronomy, UEL, Londrina, PR, Brazil. E-mail: hoshinoagro@gmail.com; pasini@uel.br
[3] Dr. Researcher, Harvard University, Museum of Comparative Zoology, Cambridge, Massachusetts, USA. E-mail: thallesplp@gmail.com
* Author for correspondence

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these, *Myrmosicarius grandicornis* predominated when employing the 'APC' adult parasitoid survey method. Only 1.28% of the collected leaf-cutting ants were parasitized, with *A. attophilus* emerging as the most abundant species with a relatively short developmental cycle. Parasitism rates remained below 2% throughout the year, with the highest rate observed in September (1.81%). The expansion of the geographic distribution of these flies to Paraná contributes relevant information about the biological and behavioral characteristics of some parasitoid species and their influence on nest activities. This underscores their potential as agents of biological control for managing *A. sexdens* in integrated management programs.
Key words: *Apocephalus.* Biological Control. *Eibesfeldtphora.* Leaf-cutting ants. *Myrmosicarius.*

Resumo

As formigas-cortadeiras do gênero *Atta* são consideradas engenheiras de ecossistemas e fundamentais para o ambiente, por outro lado, são também prejudiciais aos sistemas produtivos pela severa desfolha que ocasionam em diversas espécies vegetais. Diversas moscas da família Phoridae são parasitóides de insetos sociais, e atuam no controle biológico das cortadeiras. O estudo objetivou verificar a ocorrência e riqueza de parasitóides que incidem sobre a saúva-limão, *A. sexdens*, em Londrina-PR, além de registrar a taxa de parasitismo e algumas características comportamentais e biológicas das espécies. Entre junho/2019 e maio/2020 coletas de formigas-operárias e forídeos associados às mesmas foram realizadas, em ninhos previamente selecionados, no Campus da Universidade Estadual de Londrina e na mata nativa do Jardim Botânico, em Londrina, PR. Os forídeos coletados foram preservados em álcool 70%, para posterior identificação das espécies e as saúvas foram mantidas em laboratório, para a verificação do parasitismo, conforme descrições na literatura. Formigas coletadas foram frequentemente inspecionadas, e uma vez constatada a sua morte, foram individualizadas em tubos plásticos. Foram capturados, nos dois ambientes, 216 forídeos adultos, sendo identificadas as seguintes espécies parasitóides: *Apocephalus attophilus, Eibesfeldtphora declinata, Eibesfeldtphora elongata, Eibesfeldtphora tonhascai* e *Myrmosicarius grandicornis*, sendo este último, predominante pelo método de coleta de parasitóides adultos 'CPA'. Das saúvas coletadas, apenas 1,28% estavam parasitadas, sendo *A. attophilus* a espécie emergida mais abundante e ciclo reduzido de desenvolvimento. As taxas de parasitismo mantiveram-se abaixo dos 2% ao longo do ano, com maior taxa em setembro (1,81%). A ampliação da distribuição geográfica destas moscas para o Paraná contribui com relevantes informações sobre as características biológicas/comportamentais de algumas espécies parasitóides e a interferência que provocam nas atividades dos ninhos, ressaltando-se a importância destas como potenciais agentes de controle biológico da saúva-limão, em programas de manejo integrado.
Palavras-chave: *Apocephalus.* Controle Biológico. *Eibesfeldtphora.* Formigas-cortadeiras. *Myrmosicarius.*

Introduction

Leaf-cutting ants belonging to the genus *Atta fabricius,* 1804 are social hymenopterans known for their complex nest structures, organized into castes. To ensure survival and population growth, these ants forage in various plant areas, cutting and transporting fresh material to the symbiotic fungus *Leucoagaricus gongylophorus* (Singer) Möller (Agaricaceae: Leucocoprineae), primarily to nourish their larvae (Hölldobler & Wilson, 2009; Wartchow, 2018).

The foraging behavior of these insects can have positive effects on increasing organic matter in the soil (Swanson et al., 2019). However, in monocultures, their defoliation activities result in production losses and increased expenses.

A species of particular concern is *Atta sexdens* Forel, 1908, commonly known as "saúva limão" in its native region (Antwiki, 2021). This economically significant species inflicts substantial damage to plant development through persistent and intensive defoliation. Their coexistence and sharing of resources with humans even leads to landscape degradation in urban areas (Bueno et al., 2017).

As a control strategy, the application of insecticides with diverse active ingredients is widely adopted. When used correctly, chemicals can achieve satisfactory results in managing these ants. However, relying solely on chemical methods may not yield the desired control effect due to the excellent strategies employed by leaf-cutting ants, such as trophallaxis and hormone release. Furthermore, this method may not have the desired control effect, besides causing environmental contamination and intoxication to humans, as exemplified by fipronil (Medina et al., 2007).

In recent years, there has been a growing number of studies on biological control methods, involving the utilization of beneficial insects to combat leaf-cutting ants either through mortality (Guillade & Folgarait, 2014) or disruption of their activities. A notable example are flies from the family Phoridae (Diptera), which exhibits various life habits, including parasitism during the larval stage (De Araújo Galvão et al., 2019).

Tropical regions feature a high diversity of these parasitoids, with genera such as *Apocephalus* Coquillett, 1901, Myrmosicarius Borgmeier, 1928, and *Eibesfeldtphora* Disney, 2009 (Disney et al., 2009) standing out. Natural parasitism rates are known to range from 2 to 5% (Araújo Galvão et al., 2019; Souza, 2013), and the mere presence of phorid flies flying near ant nests can disrupt ant activities, leading to the abandonment of loads (transported plant material), early return to the nest, or a reduction in worker recruitment for foraging (Elizalde & Folgarait, 2011).

Given the expanding areas infested by leaf cutters in various regions, it is reasonable to assume that the mentioned parasitoid genera are present in the Londrina-PR region. If present, these parasitoids could serve as potential biological control agents due to their specificity.

Therefore, this study aims to investigate the occurrence and diversity of parasitoid flies that affect *A. sexdens*, a leaf-cutting ant species, in Londrina-PR, Brazil. Additionally, we aim to document parasitism rates and gather information on behavioral and biological traits of the species.

Material and Methods

Study sites

The study was conducted from June 2019 to May 2020 at two distinct sites in Londrina-PR, Brazil: the State University of Londrina (UEL) Campus (23º19' S; 51º12' W) and the native forest of Botanical Garden of Londrina (JBL) (23º21' S; 51º10' W). These locations fall within the humid subtropical climate zone (Cfa) according to the Köppen-Geiger climate classification. The climate is characterized by an average air temperature during the coldest month of less than 18 °C and an average air temperature during the hottest month exceeding 22 °C. This region experiences hot summers with infrequent frosts and a tendency for rainfall to concentrate in the summer months, without a distinct dry season (Alvares et al., 2013).

The UEL campus covers an area of 235 ha and is situated to the southwest of the municipality of Londrina, within a more urbanized environment. In contrast, JBL spans 57 ha and represents a more preserved area with a diverse flora consisting of different strata, including herbaceous/shrub (Pilocarpus microphyllus Stapf), understory (Plinia cauliflora (DC.) Kausel), canopies (Caesalpinia pluviosa DC.), and arboreal stratum (Aspidosperma polyneuron Müll. Arg.). The soil in both locations is classified as Eutroferric Red Nitisol (NVef) (Bhering et al., 2007).

Ant and parasitoid sampling

In each study site, four ant nests were selected, ensuring a minimum distance of 100 m between each nest. The areas were inspected every two weeks to collect ants and parasitoid phorids, resulting in a total of eight nests being surveyed.

During each inspection, 100 leaf-cutter ants were collected per nest, from foraging trails and active feeding holes. We recorded the total number of flies captured during a 30-min observation period per nest. Flies were captured either in flight or while attacking the ants. These collection methods are known as larval parasitoid survey ("larval parasitoid collection", LPC)) and adult parasitoid survey ("adult parasitoid collection", APC) (Elizalde & Folgarait, 2011). Visits to the ant nests were standardized, occurring either in the morning from 09h00am to 11h00 and/or in the afternoon from 17h00 to 18h00.

In the collection process, cylindrical tubes (50 mL) were used along with a suction device to capture the phorids. Tweezers were used to collect the grass cutters (Figure 1).

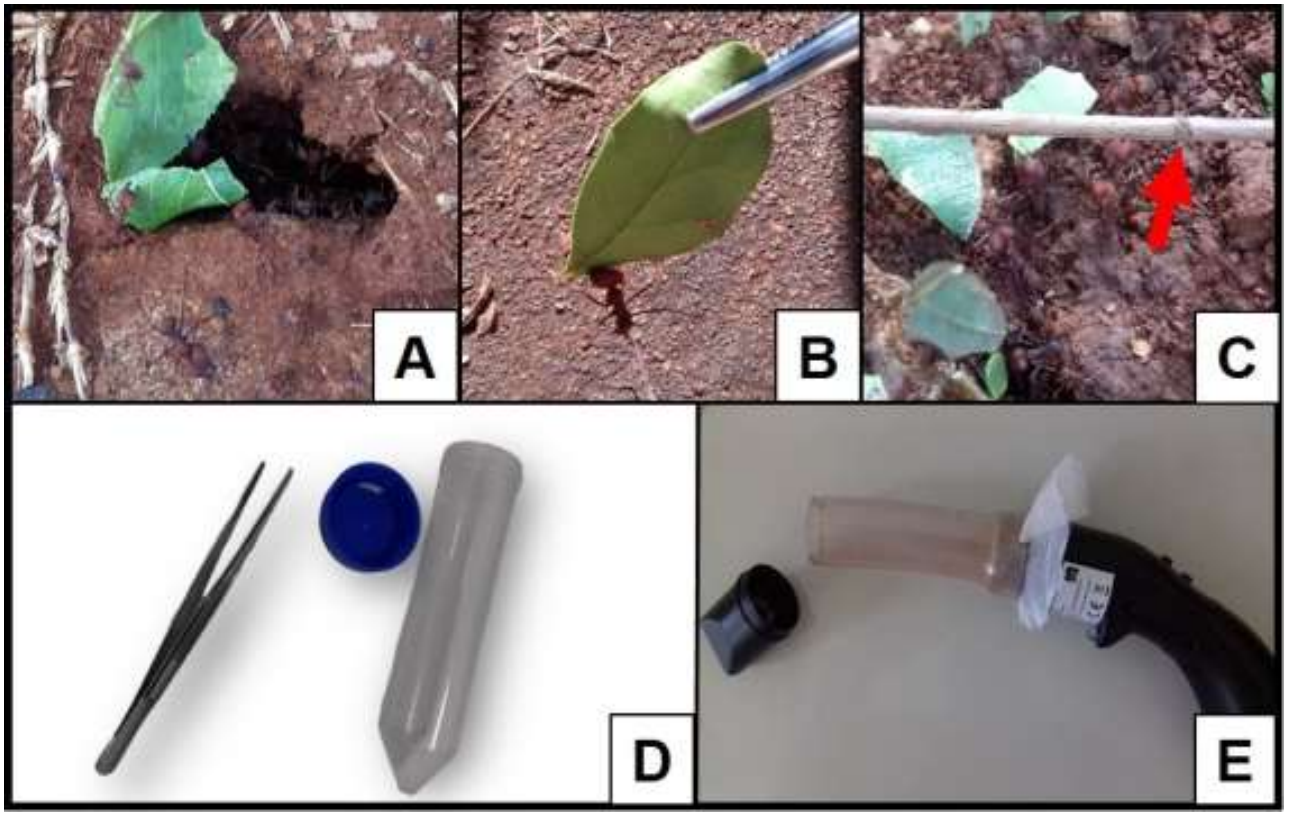

Figure 1. Procedures for collecting ants and flies in the field. Feeding hole of a nest (A), worker ant carrying leaf (B), and parasitoid fly (C) (red arrow). Materials used: tweezers, cylindrical tubes with screw cap (D), and insect suction device (E).

The collected ants were placed in plastic pots (400 mL), while flies were placed in cylindrical tubes (50 mL), and both were transported to the Multiagro-UEL laboratory. The grass cutters were maintained at room temperature, grouped in pots containing 20 individuals each, and fed twice a week with a honey solution diluted in water (Bragança & Medeiros, 2006), administered via a 1-mL syringe, applied to the container's walls. Phorids were euthanized by freezing for 30 min and then preserved in 70% alcohol in small glass bottles, separated by the collection location for subsequent identification (Figure 2).

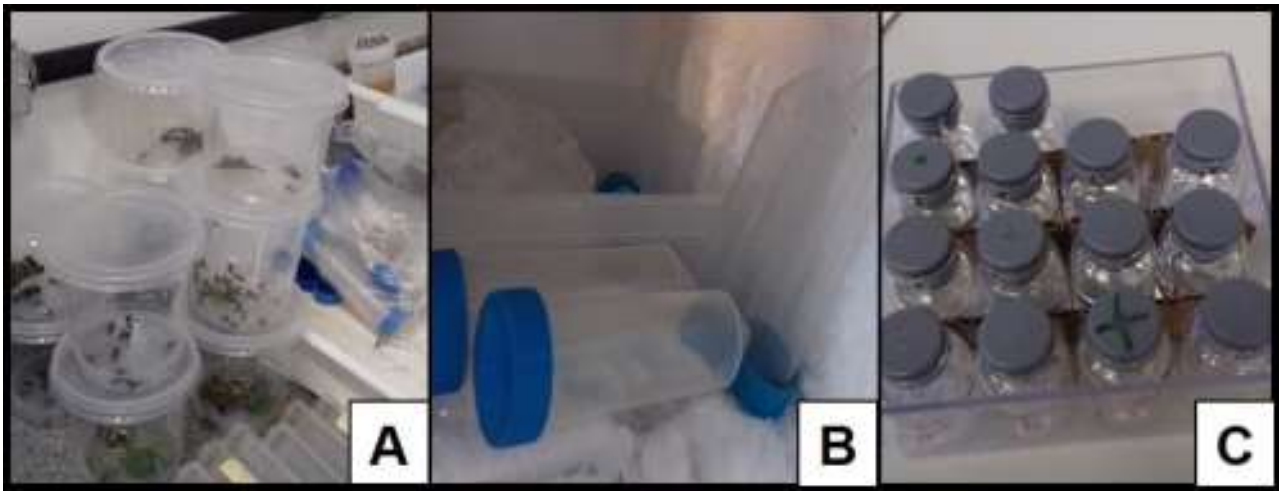

Figure 2. Laboratory steps. Maintenance of ants to examine parasitism (A), death of parasitoids by freezing (B), and temporary storing of parasitoids for later identification (C).

Dr. Thalles P. L. Pereira, from the Museum of Comparative Zoology at Harvard University, conducted the identification of fly species. This process involved morphological examination with the aid of a Leica MZ16 stereomicroscope and identification keys proposed by Brown (1997), Brown et al. (2010), Disney et al. (2006), and Uribe et al. (2014).

After a 12-day period, the ants that remained alive were discarded, as previous studies have indicated that this duration is sufficient for the host ants to die (Erthal & Tonhasca, 2000; Tonhasca et al., 2001). The dead ants were individually isolated in cylindrical tubes (50 mL) for parasitism observation (Table 1), while the Phoridae flies were deposited in the collection of the Zoology Museum of the University of São Paulo (MZUSP).

Table 1
Evidence of parasitism in *Atta spp*. leaf-cutter ants of each phorid genus

Parasitoid genus	Indicator
Eibesfeldtphora	Presence of puparia between the mandibles (Bragança, 2011)
Apocephalus	Presence of larvae inside the host's head or outside the body; opening between the pronotum region and the propleura through which the larva or pupa of the parasitoid was observed (Bragança & Medeiros, 2006)
Myrmosicarius	Head without mandibles, with the right side dark and the left side translucent (Tonhasca et al., 2001)

Source: adapted from Pimentel (2017).

Analyzed variables

Parasitism rates were calculated for each month based on the ratio of parasitized ants to the total number of ants collected. To confirm parasitism, we considered the indicators outlined in Table 1.

The emergence rate of phorids in the laboratory was determined after pupae maintenance by calculating the ratio of emerged parasitoids to the total number of pupae.

Subsequently, the emerged phorids were euthanized and preserved in 70% alcohol for the identification phase.

Results and Discussion

A total of 216 adult phorids were meticulously collected and subsequently classified into two subfamilies, six genera, and five distinct species (Table 2). The phorids recognized as parasitoids, i.e., females with sclerotized ovipositors, encompassed the following species: *A. attophilus* Borgmeier, 1928, *E. declinata* Borgmeier, 1925, *E. elongata* Brown, 2001, *E. tonhascai* Brown, 2001, and *M. grandicornis* Borgmeier, 1928. These findings align with prior studies conducted by Bragança et al. (2016), Araújo Galvão et al. (2019), and Silva et al. (2007). These parasitoids were predominantly captured in flight or while attacking leaf-cutter ants at feeding holes, along trails, and in foraging areas. While other captured flies were thought to be associated with leafcutter nests, there was no concrete evidence of parasitism.

The genus *Apocephalus* (Metopininae), described by Brown (1997) and further revised by Brown (2000), encompasses all species featuring a distinct posterior apical sclerite separate from the ovipositor. Currently, it stands as the second-largest genus of phorids after *Megaselia Rondani,* 1856. Within the American context, the genus comprises two common subgenera: *Apocephalus* and *Mesophora Borgmeier,* with the former known as an ant parasitoid.

Table 2
Species of flies captured in interaction with grass cutters, Londrina-PR, Brazil. 2019/2020

Phorid/ant relationship	Phorid	Subfamily
Parasitoid	*Apocephalus attophilus* *Eibesfeldtphora declinata* *Eibesfeldtphora tonhascai* *Eibesfeldtphora elongata* *Myrmosicarius grandicornis*	Metopininae
Associated	*Allochaeta sp.* *Megaselia sp.*	
	Dohrniphora sp.	Phorinae

The species *A. attophilus* (Figure 3A) has also been noted by Farder-Gomes et al. (2018) as a natural predator of *Atta bisphaerica* (Forel, 1908), *Atta laevigata* (Smith, 1858), and *Atta cephalotes* (Linnaeus, 1758), with Bragança et al. (2003) adding *Atta colombica* Guérin as an additional host. It is a comparatively smaller fly than the aforementioned genus, demonstrating high potential for control and physiological plasticity, allowing it to thrive in adverse environmental conditions (Araújo Galvão et al., 2019). Its presence is documented along the Brazilian coast (São Paulo and Rio de Janeiro), as well as in the states of Minas Gerais, Goiás, Tocantins, and Santa Catarina (Brown, 1997).

The genus *Eibesfeldtphora* (Metopininae), initially classified as *Neodohrniphora,* was later recognized as a distinct genus by Disney et al. (2009). In this study, we observed the presence of *E. declinata* (Figure 3B), one of three identified species, foraging on *A. sexdens* workers, corroborating findings by Brown (2001) and Silva et al. (2008), who reported this parasitoid's activity on the same host as well as in nests of *A. laevigata* and *A. cephalotes.*

Eibesfeldtphora tonhascai (Figure 3C), similar to the previous parasitoid, is known to infest nests of *A. sexdens* and *A. laevigata* ants (Pesquero et al., 2010). It is believed that this parasitoid, similar to other *Eibesfeldtphora* spp., is relatively sensitive to reduced environmental diversity, with higher parasitism rates being recorded in forest fragments (Araújo Galvão et al., 2019).

Eibesfeldtphora elongata (Figure 3D) exhibits a significant presence in nests of *A. sexdens* and *A. laevigata* (Gazal et al., 2009; Silva et al., 2008). A preference for attacks in the morning was observed, in line with the observations recorded by Bragança et al. (2008). These three species belonging to the genus *Eibesfeldtphora* have been identified in Rio de Janeiro, Minas Gerais, São Paulo (Brazil), Leticia (Colombia), and Berbice (Guyana) (Brown, 2001).

Myrmosicarius (Metopininae) was described by Disney et al. (2006), and one of the species identified in this study is *M. grandicornis* (Figure 3E), known to parasitize not only *A. sexdens* but also *A. laevigata* and *A. bisphaerica* (Arruda et al., 2019; Bragança et al., 2003). These parasitoids were captured at feeding holes, with their attacks at feeding holes also reported by Tonhasca et al. (2001). These species predominantly targeted medium-sized workers, a behavior consistent with observations made by Elizalde and Folgarait (2011), which suggested a preference for ants with higher reserves, thereby enhancing the parasitoid's likelihood of survival and reproduction. *Myrmosicarius grandicornis* is distributed across Brazil (Rio de Janeiro, Minas Gerais, and Tocantins) and Paraguay (Disney et al., 2006).

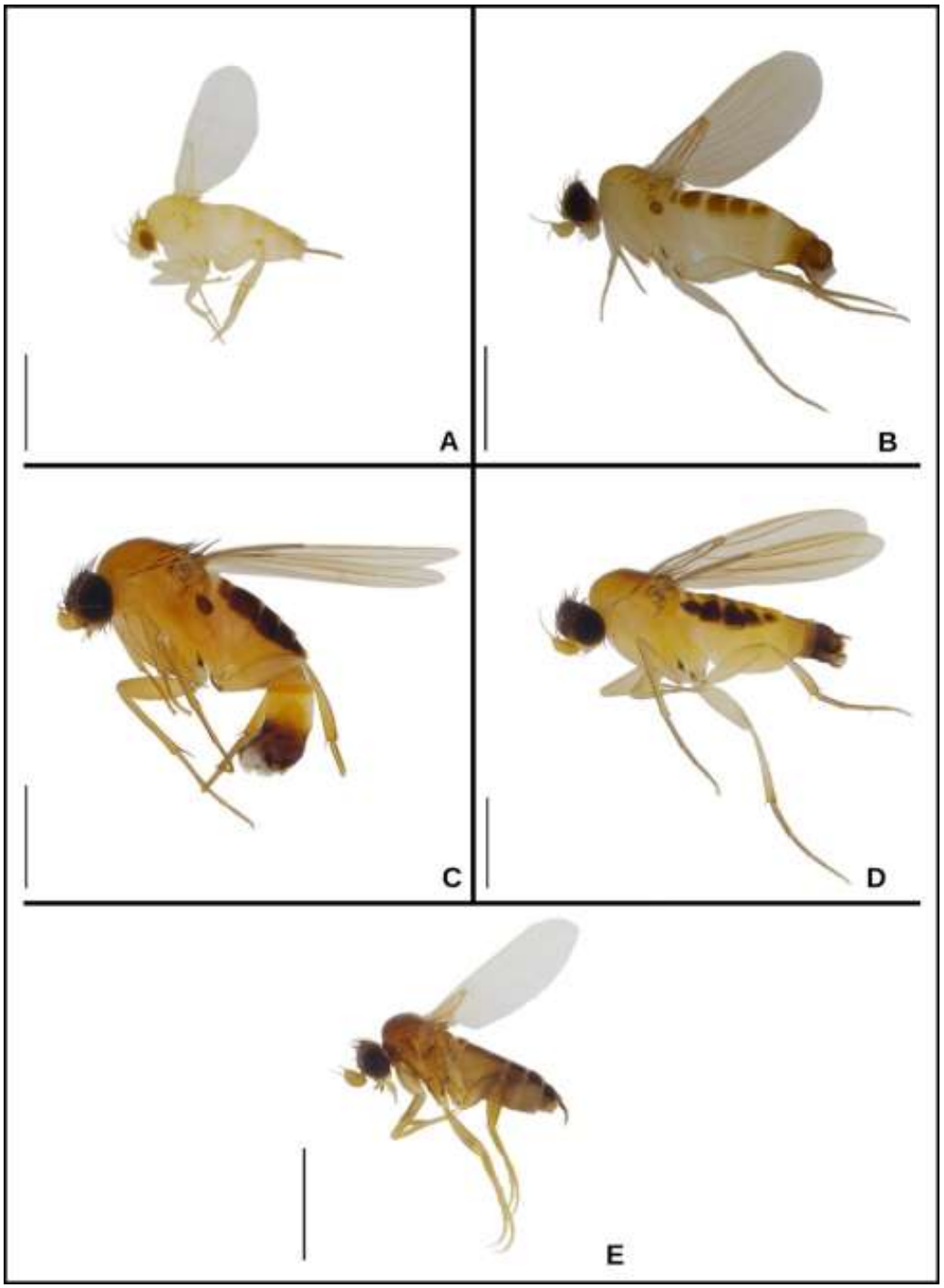

Figure 3. *Apocephalus attophilus,* lateral view of the adult female (A). *Eibesfeldtphora declinata,* lateral view of the adult female (B). *Eibesfeldtphora tonhascai,* lateral view of the adult female (C). *Eibesfeldtphora elongata,* lateral view of the adult female (D). *Myrmosicarius grandicornis,* lateral view of the adult female (E). Scale: 1 m.

All five species of adult parasitoids collected in this study were found in both environments (UEL and JBL campuses). These locations are characterized by numerous plant species, likely providing essential resources that support increased fly diversity, thus favoring parasitism. Additionally, the sites offered alternative food sources (nectar/pollen) and shelter for adult flies (Landis et al., 2000). Notably, the JBL nests were located approximately 550 m from a forest fragment, as well as in proximity to the agricultural area of IDR-Paraná (Paraná Rural Development Institute - IAPAR-EMATER) at around 500 m. Meanwhile, the UEL nests were situated at a distance of 300 to 400 m from a forest fragment on the campus.

In studies conducted with *A. sexdens* in Campos dos Goytacazes - RJ, it was observed that the abundance of phorids did not exhibit significant variations across different habitat types, namely native forest, eucalyptus plantation, and agricultural areas. This lack of distinction can be attributed to the relatively short duration of the study and the remarkable adaptability of certain parasitoid genera, such as *Apocephalus* sp. and *Myrmosicarius* sp., to unfavorable environmental conditions (Araújo Galvão et al., 2019).

The 'APC' method revealed that *M. grandicornis* was the predominant species, and out of the 19,200 grass cutters collected by the 'LPC' method, 246 (1.28%) were parasitized by phorids. These parasitism rates displayed seasonal fluctuations, with a peak of 1.81% observed in September 2019, followed by subsequent months ranging from 0.63% to 1.69% (Figure 4).

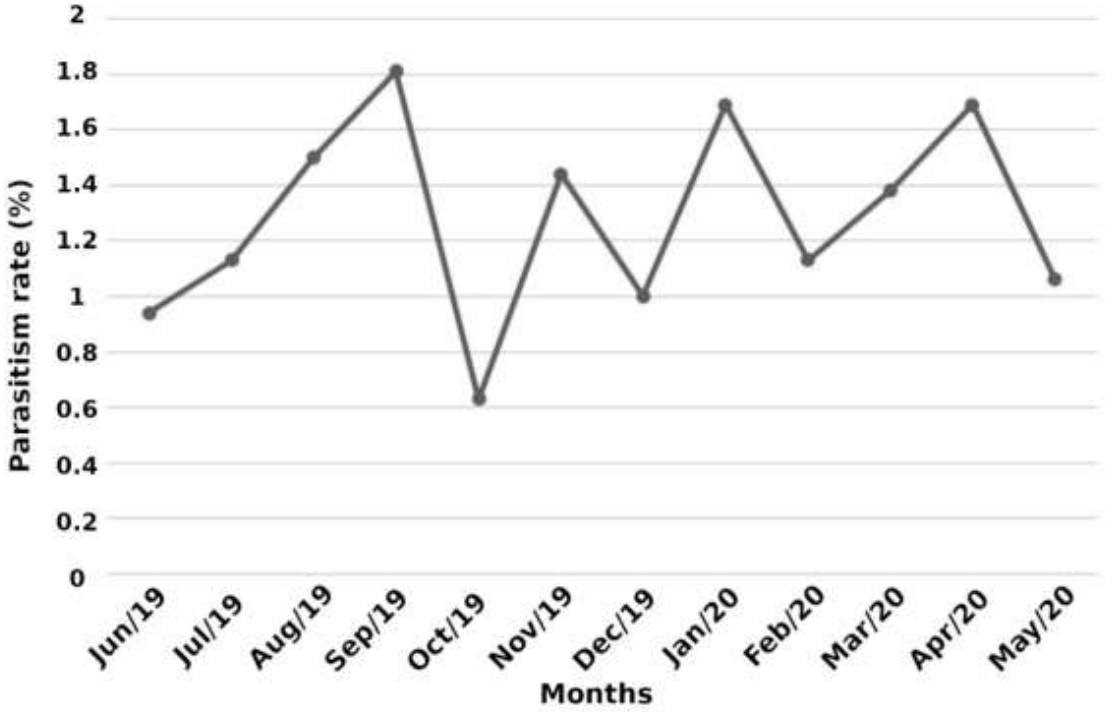

Figure 4. Total parasitism rate (n=8 per month) on *A. sexdens* obtained from workers collected at UEL and JBL, Londrina-PR, Brazil, 2019/2020.

These findings are consistent with the studies by Araújo Galvão et al. (2019) and Bragança et al. (2016), which reported parasitism rates below 2% for *A. sexdens* in Rio de Janeiro and Tocantins, respectively.

During maintenance at the Insect Ecology-UEL laboratory, on average, there were 6.95±1.02 parasitoids per worker ant, with a range of four to eight flies per parasitized ant. This observation aligns with the gregarious behavior characteristic of these parasitoids (Pesquero et al., 2010). Araújo Galvão et al. (2019) recorded a range of 1 to 17 parasitoid individuals per host, while Farder-Gomes et al. (2017, 2018) reported 15 and 7 larvae per cutter ant, respectively.

Of the 1,696 pupae obtained during the study period in *A. sexdens,* 1,613 (95%) successfully developed into adults, which is consistent with findings reported by Bragança et al. (2016), who observed similar percentages of flies emerging from *A. sexdens* and *A. laevigata* (96.8% and 90%, respectively).

Regarding the behaviors exhibited by *A. attophilus,* as reported in the literature, we observed its rapid approach to worker ants involved in fragmenting flowers and leaves. Once the host was located, the fly carried out oviposition at the oral opening. Successful oviposition often resulted in the interruption of the cutting activity and the abandonment of the selected plant fragment by the targeted ant, which attacked the phorid with its mandibles and antennae. The ant also assumed a defensive position with its head raised, mandibles open, and gaster positioned between its legs to form a "C" shape. In unsuccessful cases, the fly circled around the ant and faced it (Bragança et al., 2003; Bragança & Medeiros, 2006; Bragança et al., 2016; Erthal & Tonhasca, 2000). Bedoya Cochet et al. (2017) also observed similar behavior in attacks by *Apocephalus colombicus* Brown, 1997, on *Atta colombica* Guerin-Meneville, 1844, specifically in the mandible region.

The larvae of *A. attophilus* developed within the ant's head, consuming its entire brain contents. The immatures exited the host through the oral cavity and pupated outside the host (tube walls), remaining until the adult emerged (Figure 5). This pattern of development highlights the continued parasitoid life cycle in the soil while also exposing the larvae to environmental risks such as predation and pathogens (Farder-Gomes et al., 2017; Bragança & Medeiros, 2006).

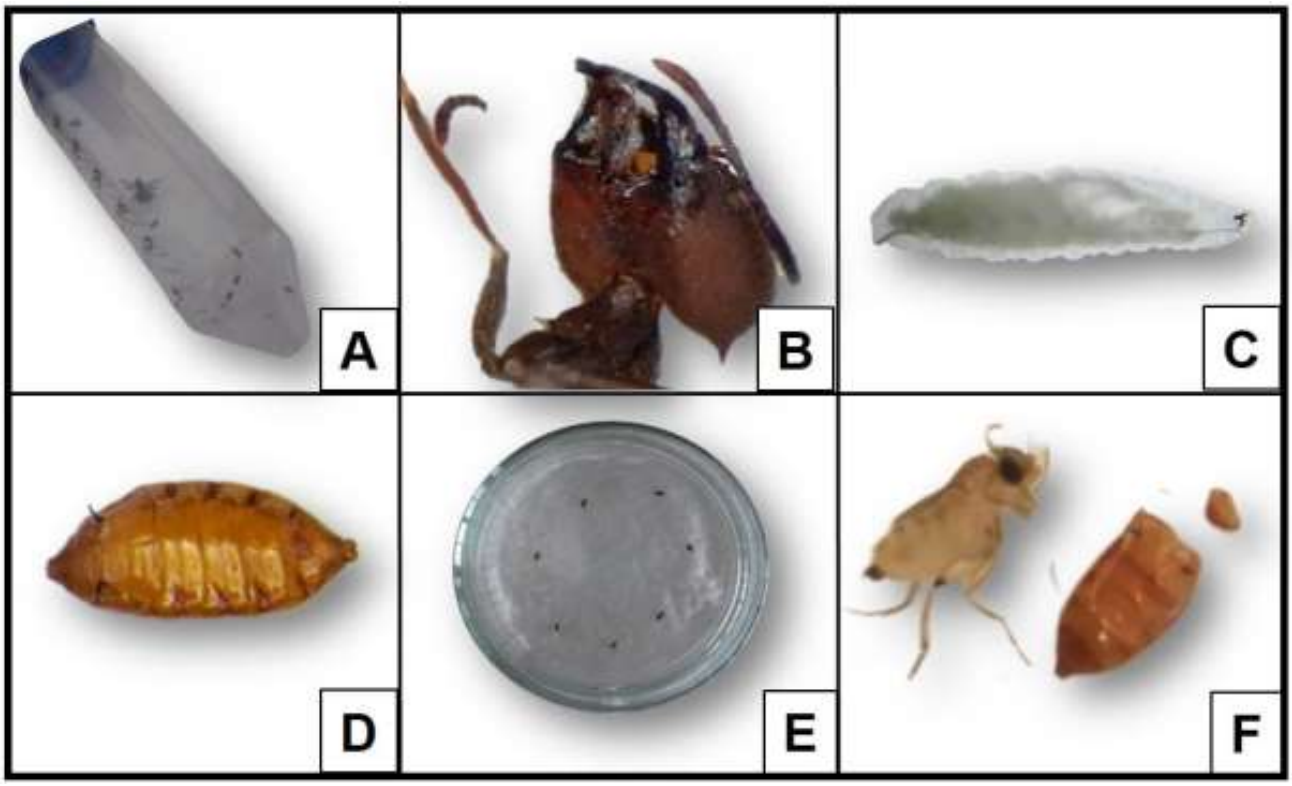

Figure 5. Parasitism of *A. sexdens* by *A. attophilus.* Sequence of events observed in the laboratory. Falcon tube with host and pupae (A). Exit opening of the parasitoid on the ant (B). Lateral view of the parasitoid larva (C). Pupa with respiratory siphons (D). Conservation of pupae (E). Newly emerged adult (male) (F).

In the case of *M. grandicornis,* prior investigations by Martins (2015) involving *Atta bisphaerica* documented a singular pupa per host. Similarly, Guillade and Folgarait (2011) observed a one-to-one association between *Atta vollenweideri* Forel, 1893 and the parasitoid species *Myrmosicarius brandaoi* Disney, 2006 and *Myrmosicarius gonzalezae* Disney, 2006. However, our study diverged from these findings, as we observed the development of three larvae within an *A. sexdens* worker, with only one adult fly emerging (Figure 6). This discrepancy underscores the existing gaps in our understanding of the larval development of this parasitoid, a subject that merits further in-depth biological investigations, as previously noted by Brown (1992).

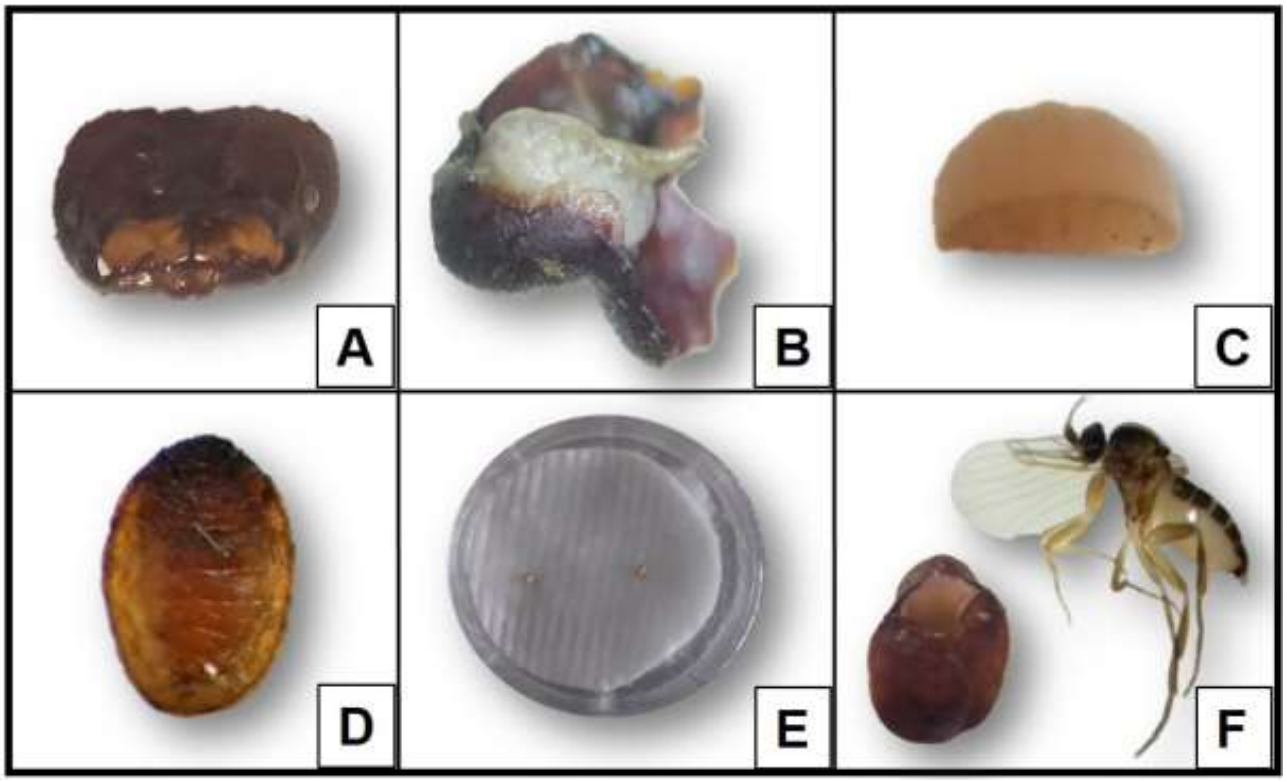

Figure 6. Parasitism of *A. sexdens* by *M. grandicornis.* Sequence of events observed in the laboratory. Leaf cutter without mouthparts (A). Larva inside the host's head (B). Lateral view of the parasitoid larva (C). Pupa (D). Conservation of pupae (E). Newly emerged adult (male) (F).

Tonhasca et al. (2001) also emphasize that the infrequent attacks on workers carrying plant fragments suggest that *M. grandicornis* can rapidly discern between potential hosts. Upon an attack, the affected workers typically displayed a temporary immobility, during which their nestmates engaged in a series of tactile examinations using antennae and mouthparts before eventually dispersing. The larval stage of this phorid species, as reported by Bragança et al. (2016), proceeded to consume the entire contents of the ant's head and pupated beneath the tentorium, at the base of the head capsule. Consequently, the decapitated heads, lacking mandibles and antennae, were commonly discovered outside the nest (Tonhasca et al., 2001). The retention of the phorid larva within the host's head may serve as a strategy to reduce its exposure to the external environment, potentially shielding it from other potential predators.

Among the parasitoid specimens observed by the 'LPC' method, *A. attophilus* promptly killed its host within an average of 2.36±0.54 days (field collection - death of *A. sexdens*), followed by a relatively short development period of 19.16±1.29 days (pupa - emergence of the fly). These findings align with previous studies by Bragança et al. (2016). Erthal and Tonhasca (2000)

noted a pupal stage lasting around 15 days, consistent with reports by Bragança and Medeiros (2006), Farder-Gomes et al. (2018), and Guillade and Folgarait (2014). This suggests that *A. attophilus* may have potential for future use as a control agent in management programs.

The complex social structure of *A. sexdens* enables it to adapt to environmental adversities, aiming to maximize energy input into the nest (Viana-Bailez & Endringer, 2016) and establish themselves in the area. Nevertheless, this study highlights the ecological significance of their natural enemies, which can disrupt their normal activities. Further research on parasitism, the expansion of the geographic distribution of these ants, and a comprehensive understanding of the biology of phorid parasitoids is warranted.

Conclusions

Atta sexdens is parasitized by five distinct species of phorid flies, which are categorized into three separate genera: *Apocephalus attophilus, Eibesfeldtphora declinata, Eibesfeldtphora tonhascai, Eibesfeldtphora elongata,* and *Myrmosicarius grandicornis.*

The occurrence and prevalence of the collected parasitoids fluctuated over a year, resulting in a generally low parasitism rate of *Atta sexdens* by these flies (< 2%).

Species within the genera *Eibesfeldtphora* and *Myrmosicarius grandicornis* attack *Atta sexdens* individuals both at feeding holes and along foraging trails. In contrast, *Apocephalus attophilus* only occurs in plant-cutting areas.

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