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ORIGINAL ARTICLE

Winged insects associated with the poorly studied forest fire ant *Solenopsis virulens* (Hymenoptera: Formicidae)

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Abstract. The present study documents new records of winged insects collected from fragments of nests of the tropical fire ant *Solenopsis virulens* (Smith), using a trap, which is also described in detail. The emergence chamber consisted of a 5L opaque plastic container, a 50 ml transparent vial and a 1.5 ml microcentrifuge tube. This trap captured 70 insects from 12 nests of *Solenopsis virulens*. They were classified into two orders, 13 families, 18 genera and 39 morphospecies. The most abundant order was Diptera, with nine families. The richest family was Cecidomyiidae, with 12 morphospecies, followed by Sciaridae with seven. Ceratopogonidae, Chironomidae, Chloropidae, Drosophilidae, Hybotidae, Phoridae and Psychodidae were also represented. Hymenoptera included a total of three families: Figitidae (Eucoilinae), Platygastridae (Scelioninae and Platygastrinae) and Diapriidae. Although the association of several genera (Diptera: 11/ Hymenoptera: 1) and families (Diptera: 1/ Hymenoptera: 1) with ant nests was recorded for the first time, it is likely that many of these insects are only fortuitous or temporary inquilines and not obligatory myrmecophiles. The trap described is also useful for catching insects that emerge from other substrates such as leaves, logs, galls, termite nests, etc. Furthermore, it could enable researchers to sample and further study important material in the laboratory and decrease the costs of sampling during field trips.

INTRODUCTION

In myrmecological studies, investigating the diversity of arthropods associated with ant nests is especially challenging (Delabie et al., 2021). Over the years, the diversity of arthropods associated with ant nests has been studied for a range of species (Hölldobler & Wilson, 1990; Hölldobler & Kwapich, 2022), e.g. the fauna of commensals in nests of Paraponerinae and Ponerinae ants (Araújo et al., 2019; Castaño-Meneses et al., 2019; Moreira et al., 2020). Ant nests provide a safe, stable, food-rich and temperature-controlled environment, which attracts many organisms [commensals, opportunists, mutualists and parasites (generally invertebrates)] (Kronauer & Pierce, 2011; Zarka et. al., 2022). Furthermore, it is also known that pheromones and specific chemicals also cause some accidental symbionts to infiltrate nests (Adams et al., 2020).

When it comes to fire ants or other ants of economic interest, concerns about studying and discovering their potential natural enemies may greatly increase interest due to their potential effects on the biological control of pests (Fowler et al., 1989). In the last decade, there have been numerous studies on ant parasitoids (Bragança et al., 2021) and other insects associated with ants (Parker, 2016; Rocha et al., 2020), however, most of these focus on aspects of ecology and control (Chen & Fadamiro, 2018 – review).

The Neotropical Forest fire ant *Solenopsis virulens* was described by Frederick Smith (1858), but there are very few studies on its biology. This species belongs to the fire ant *Solenopsis virulens* species-group (Pitts et al., 2018) and is more poorly studied than other species of fire ant (Fox, 2014). Only in the last decade have aspects of its biology and nests been published (Pereira et al., 2015, 2021). This ant has been collected almost exclusively in three different forest biomes in Brazil: Atlantic Forest (Resende et al., 2011), Caatinga Dry Forest (Arnan et al., 2018) and Amazonian Forest (Prado et al., 2019). Papers on this species are limited to worker ants captured by traps (Oliveira et al., 2009) or taxonomic studies (Pitts et al., 2018). This is probably related to the fact that colonies of these ants are rarely found compared to other species of fire ant. Their larval stages, adult males and queens remain undescribed (Pitts et al., 2018).

In the field, there are many difficulties in collecting commensals present in ant nests at the right development stage for identification; furthermore, the costs of collecting this kind of biological material are generally high. Estimating biodiversity has become a challenging task, mainly in undeveloped countries, due to limited (or scarce) investment, time, difficulty of collecting data in the field and the high extinction rate associated with human activities (Otto, 2018). There is also a growing interest in cheap entomology traps e.g., those using low-cost materials, such as, PET bottles [e.g., used to monitor the dispersal of necrophagous Diptera (Oliveira et al., 2019)] or even entomological umbrellas (Bczuska et al., 2018).

All the issues mentioned above stimulated us to document the fauna of flying insects associated with the poorly studied forest fire ant *Solenopsis virulens* and describe an inexpensive and simple emergence chamber. This is the first emergence chamber trap used to collect the winged insects associated with ant nests, which can be studied in the laboratory and by so doing decrease the cost of collecting data during field trips.

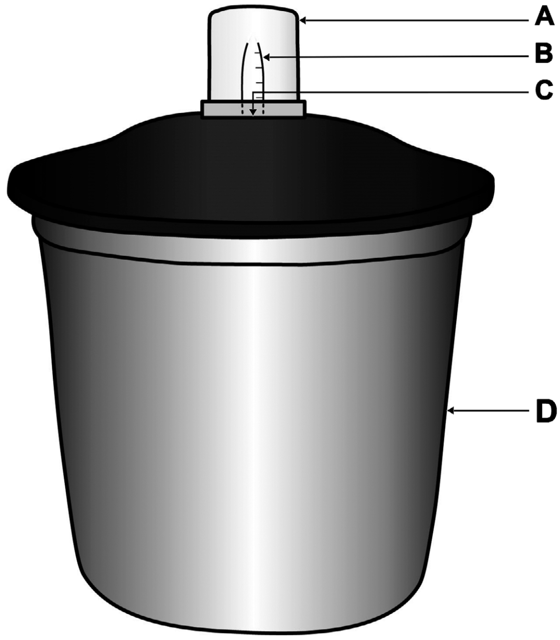

Fig. 1. Emergence chamber. Longitudinal section of the trap: A – transparent container, B – 1.5 ml microcentrifuge tube cut open at the base, C – opening for passage of light and insects, D – opaque plastic container and lid.

MATERIAL AND METHODS

Study area

This study was carried out at the Michelin Ecological Reserve (REM) – Ouro Verde, The Private Natural Heritage Reserve (RPPN) (13°47′05.1″S, 39°10′32.1″W), located in territories of both the Ituberá and Igrapiúna municipalities, Bahia State, Brazil. The reserve lies within the Atlantic Forest biome characterized as dense lowland ombrophilous rainforest. The climate is wet tropical (Köppen-Geiger climatic classification: Af). The Ouro Verde RPPN has an average annual rainfall of 2,000 mm and temperatures between 18°C and 30°C, with rainfall occurring throughout the year (Flesher, 2021).

Emergence chamber

The trap used is categorized as an "emergence chamber" according to Ferro & Summerlin (2019) as the material is removed from the original site, as opposed to an emergence trap where there is a full or partial enclosure of material in the field. It consists of a 5L opaque plastic container that is not hermetically sealed, enabling air to circulate, but tight enough to prevent insects from escaping; and a lid with a 50 ml transparent vial and a 1.5 ml microcentrifuge tube (Fig. 1) on the top. A small aperture in the centre of the container's lid, and the microcentrifuge tube was then fixed in an upside-down position with the bottom cut open. This aperture allowed newly emerged insects to pass through and be trapped inside the transparent vial. This chamber has a single source of light located in the upper part, which attracts emerging insects that are positively phototropic. A dusting of talcum powder (no ethanol added) on the inner wall of the opaque plastic container prevents ants (as well as other organisms) from escaping, thereby only collecting flying insects. It is worth mentioning that the ant nests were collected in the field and put directly into the emergence chamber.

A step-by-step video illustrating the material used (Fig. 2) and how to assemble the trap is provided (Supplementary file S1).

Sampling

Nests of *Solenopsis virulens* were collected from April to May 2013. This species of ant nests in the soil and builds conspicuous earthen mounds, as do other species of the *Solenopsis geminata* group, e.g., *Solenopsis saevissima* (Smith) and *Solenopsis geminata* (Fabricius) (Taber, 2000). Twelve nests were collected from different strata, including trunks of fallen trees (n = 1), large stones near the river (n = 1) and at the base of trees (n = 8) and shrub plants (n = 2) (e.g., *Piper* spp., Piperaceae).

Fragments of each nest (~2,000 cm³) were collected using a garden trowel and transferred to an enclosed opaque 5L plastic container. The samples were transported to the Myrmecology Laboratory of Centro de Pesquisa do Cacau, Ilhéus, Bahia and kept in emergence chambers for 15 days. This was the average time a colony survived in vitro; after 15 days ants were no longer alive (workers and the queen). We chose to collect only the insects that were caught while the ants were still alive. Traps were inspected daily by carefully unscrewing the 50 ml transparent vial and quickly using an aspirator or forceps to collect insects that were then preserved in 70% ethanol, as described in Pereira et al. (2015).

Flies were identified to genus level using the Manual of Central American Diptera (Brown et al., 2009, 2010). The species of Psychodidae collected were the same as those recorded by Pereira et al. (2015). Hymenoptera were identified using Fernández (2000) (Platigastridae classification updated by Aguiar et al., 2013). The identified specimens were deposited in the Prof. Johann Becker Entomology Collection of the Zoological Museum of the Universidade Estadual de Feira de Santana, Bahia, Brazil (MZFS).

RESULTS

A total of 70 insects emerged from 12 nests of *S. virulens* and were classified into two orders, 13 families, 18 genera and 39 morphospecies (Table 1). Eleven genera of Diptera and a single genus of Hymenoptera were recorded for the

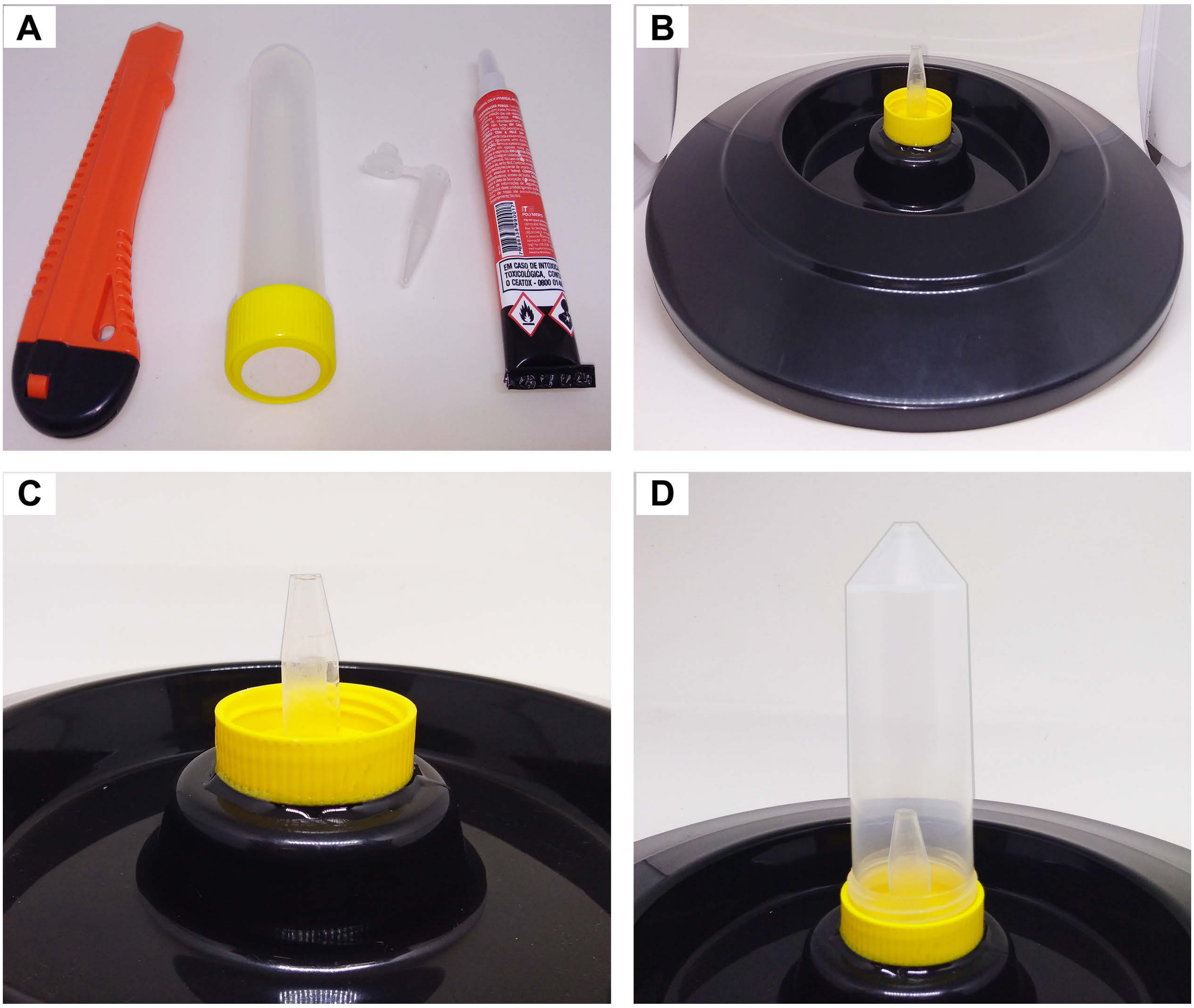

Fig. 2. The material used to construct the trap. A – knife, transparent container, 1.5 ml microcentrifuge tube and super glue, B – detail of the lid of a container, C – detail of the opening with the microtube cut open at the base, D – detail of the lid of a container ready to use.

first time associated with ant nests (* Table 1). The most family-rich order was Diptera, with nine families. Sciaridae was the most abundant family with 22 specimens, followed by Cecidomyiidae with 18. On the other hand, the richest family was Cecidomyiidae, with 12 morphospecies, followed by Sciaridae with seven. Chironomidae, Chloropidae, Drosophilidae and Phoridae were also represented.

A total of three families of Hymenoptera were caught: Figitidae (Eucoilinae), Platygastridae (Scelioninae and Platygastrinae) and Diapriidae. Platygastridae were the most abundant with five specimens.

DISCUSSION

Diptera

Chironomidae

An unidentified species of *Lipurometriocnemus* Sæther, belonging to the subfamily Orthocladiinae, was recorded in ant nests. A terrestrial habit is unusual for Chironomidae (Hudson, 1987) as the larvae of most species are aquatic, however, species of Orthocladiinae live in soil (Delettre, 2005). Larvae move into soil or humus during droughts (Delettre, 1984). The immature stages and habitat of *Lipurometriocnemus* are unknown (Spies et al., 2009). It is the first time that this genus is recorded from an ant nest.

Ceratopogonidae

We found four morphospecies of Ceratopogonidae: two species of *Forcipomyia* Meigen, one of *Culicoides* Latreille and a single specimen of another unidentified genus (id. Borkent et al., 2009). Wasmann (1893) described *Forcipomyia* (*Forcipomyia*) *braueri* (Wasmann) from nests of *Formica fusca* Linnaeus and *Forcipomyia* (*Forcipomyia*) *myrmecophila* Egger is associated with many species of the genus *Formica* Linnaeus (Formicinae) (Bernard, 1968). Three other midges are reported as myrmecophilous: *Forcipomyia* (*Forcipomyia*) *squamipes* (Long) associated with *Labidus coecus* (Latreille) (Dorylinae), *Forcipomyia stenammatis* Long. with *Aphaenogaster fulva* Roger (Myrmicinae) and *Forcipomyia* (*Forcipomyia*) *wheeleri* (Long) found in an unidentified abandoned ant nest (Long, 1902).

Table 1. List of the insects collected by emergence chambers containing material from *Solenopsis virulens* (Smith) nests collected in the Michelin Ecological Reserve (REM), Bahia – Brazil, between July 2012 and April 2013. Trophic guild is based on the literature for each family/genus. *Recorded for the first time in Formicidae nests. **Recorded for the first time in fire ant nests. ***Recorded for the first time in *Solenopsis virulens* nests.

Order	Family	Tribe/Genus/Species	N ind	Trophic guild
Diptera	Sciaridae	*Chaetosciara* sp. 1*	1	Mycetophagous
		Pseudosciara sp.1*	12	Mycetophagous
		Scatopsciara sp.1*	1	Mycetophagous
		Scatopsciara sp.2	4	Mycetophagous
		Schwenckfeldina sp. 1*	1	Mycetophagous
		Sciaridae sp.1	2	Mycetophagous
		Sciaridae sp.2	1	Mycetophagous
			22	
	Cecidomyiidae	*Brachineura* sp.1*	3	Woody midge
		Clinodiplosis sp.1*	1	Gall inducer
		Neolasioptera sp.1***	1	Gall inducer
		Cecidomyiidi sp.1	1	Gall inducer
		Cecidomyiidi sp.2	1	Gall inducer
		Cecidomyiidi sp.3	3	Gall inducer
		Cecidomyiidae sp.1	1	Gall inducer
		Cecidomyiidae sp.2	1	Gall inducer
		Cecidomyiidae sp.3	1	Gall inducer
		Cecidomyiidae sp.4	2	Gall inducer
		Cecidomyiidae sp.5	2	Gall inducer
		Cecidomyiidae sp.6	1	Gall inducer
			18	
	Psychodidae	*Trichomyia annae* Bravo	1	Saprophagous
		Trichomyia myrmecophila Araújo & Bravo	1	Saprophagous
		Quatiella truncata Chagas & Cordeiro	1	Saprophagous
			3	
	Ceratopogonidae	Ceratopogonidae sp. 1	1	Saprophagous
		Forcipomyia sp. 1**	3	Saprophagous
		Forcipomyia sp. 2	3	Saprophagous
		Culicoides sp. 1*	2	Predator
			9	
	Chironomidae	*Lipurometriocnemus* sp. 1*	**2**	Unknow
	Hybotidae	Tachydromiinae sp.1**	**5**	Predator
	Phoridae	*Syneura* sp.1*	1	Parasitoid
		Puliciphora sp. 1***	1	Saprophagous
			2	
	Chloropidae	*Apallates* sp. 1*	1	Saprophagous
		Apallates sp. 2*	1	Saprophagous
			2	
	Drosophilidae*	*Drosophila* sp. 1*	**2**	Saprophagous
Hymenoptera	Platygastridae	Scelioninae sp.1	2	Parasitoid
		Scelioninae sp.2	1	Parasitoid
		Inostemma sp.1*	1	Parasitoid
		Platygastridae sp.2	1	Parasitoid
			5	
	Diapriidae	Diapriidae sp.1	**1**	Parasitoid
	Figitidae*	Eucoilinae sp.1	**1**	Parasitoid
Total			70	

Sciaridae

The most common species of Sciaridae associated with ant nests are *Lycoriella subterranea* (Markel), *Bradysia chandleri* Menzel and *Bradysia placida* (Winnertz). *Lycoriella subterranea* is recorded in red wood ant nests (*Formica rufa* Linnaeus) and is considered to be a true myrmecophile (Robinson & Robinson, 2013). Four genera were recorded for the first time in this study: *Chaetosciara* Frey, *Pseudosciara* Schiner, *Scatopsciara* Edwards and *Schwenckfeldina* Frey.

Cecidomyiidae

Larvae of two genera of Cecidomyiidae, *Neolasioptera* Felt and *Cecidomyia* Meigen, are recorded in nests of *Solenopsis richteri* in the United States (Collins & Markin, 1971). In this study, we report Cecidomyiidae in *S. virulens* nests for the first time. This family is widely known for galling plants, however, some species live in decaying matter or soil fungi (Gagné & Jaschhof, 2009). Gall midges are also associated with termites (Gagné & Jaschhof, 2009). All the cecidomyiids recorded in this study are gall inducers, except *Brachineura* Rondani, which is associated with decaying wood. *Solenopsis virulens* nests in the soil and builds conspicuous earthen mounds, collecting organic matter from around its nest and incorporate it into their nests. It is probable that galled leaves or even seedlings with galls were incorporated into these nests. The recovered parts of the plants remain alive and are protected from herbivory, allowing gall insects that could already be in an advanced stage of development to emerge.

Psychodidae

There are reports of 3 species of the family Psychodidae in ant nests: larvae and adults of *Alepia longinoi* Quate & Brown associated with *Azteca* sp. (Dolichoderinae) (Quate & Brown, 2004), *Nemapalpus mopani* De León (Bruchomyiinae) in an *Eciton hamatum* (Fabricius) (Dorylinae) bivouac in Guatemala and Ecuador (Kistner et al., 2001) and adults of *Lutzomyia texana* Dampf, 1938 (Phlebotominae) in a nest of *Atta texana* Buckley (Myrmicinae) in southern U.S.A. (Dampf, 1938; Young & Perkins, 1984). Herein, we identified three other species: *Trichomyia annae* Bravo, *Trichomyia myrmecophila* Araújo & Bravo and *Quatiella truncata* Chagas & Cordeiro (Pereira et al., 2015). The last two were described by Pereira et al. (2015) based on specimens collected from the emergence chamber described in this paper. It is worth mentioning that other papers report the genus *Trichomyia* in the Michelin Ecological Reserve (REM) (Araújo & Bravo, 2016; Araújo et al., 2018), which were collected using different methods (e.g. Light and Malaise traps), during the same field trip, but up until now, *Trichomyia myrmecophila* is the only species collected from an ant nest using the emergence chamber described in this paper. During their larval stage, flies of this family decompose organic matter (Wagner & Ibáñez-Bernal, 2009; Ježek et al., 2010) and may have been feeding on decaying material and took advantage of the protection provided by the ants.

Hybotidae

Larvae of Hybotidae are predators (Cumming & Sinclair, 2009; Sinclair & Cumming, 2017). In the literature, there is a single record of this family associated with ants. Marshall et al. (2007) report the genus *Drapetis* Meigen on leaves being carried by a column of *Atta colombica* (Guérin-Méneville) (Myrmicinae). He suggests the larvae are predators of fly larvae or other immature insects living in ant nests, although they could also be predators of ant brood. The specimens that we found belong to the same subfamily as the genus *Drapetis*. We suggest that the larvae of the specimens collected may be predators of ant larvae and pupae, as Marshall et al. (2007) suggest.

Phoridae

The Phoridae is a very diverse group consisting of decomposers, parasitoids, predators, etc. (Brown, 2010). The genera recorded were *Syneura* Brues and *Puliciphora* Dahl. According to Brown (2010), *Syneura cocciphila* (Brues) is a well-known parasitoid of scale insects. Coincidentally, in some *S. virulens* nests there are scale insects that are farmed by the ants (Pereira et al., 2021). Species of the genus *Puliciphora* Dahl have apterous females (Brown, 2010) and are commonly found in decomposing organic material, especially dead or injured insects. For that reason, ant refuse would be a likely source of food for this genus.

Although the genus *Pseudacteon* is the most well-known fire ant parasitoid (Chen & Fadamiro, 2018 – review) and the genus *Megaselia* Rondani is also reported in fire ant (Collins & Markin, 1971) and leaf cutter ant nests (Walter et al., 1938), we did not record these genera.

Drosophilidae

The lifestyle of this family is very diverse and species are known to reproduce in decaying fruit and vegetables, live flowers, fresh fungi, etc. Despite the fact that they have been found in many situations, Drosophilidae has never been reported in association with ant nests (Grimaldi, 2010). We present the first report of the genus *Drosophila* Fallén in *Solenopsis* nests. However, we hypothesize that their occurrence could be incidental, since we only found two specimens in one colony.

Chloropidae

We collected individuals of two morphospecies of the genus *Apallates* Sabrosky. This New World genus is diverse and abundant in disturbed areas such as roadsides and margins of agricultural fields (Wheeler, 2010). This is the first report of this genus associated with an ant. Other cases of myrmecophily for this family are reported. *Pseudogaurax paratolmos* Wheeler is reported as having an association with the fungus-growing ant *Apterostigma dentigerum* Wheeler (Myrmicinae) in Panama (González et al., 2016). Imagoes of *Dasyopa pori* (Harkness & Ismay) are also found in association with the Mediterranean ant *Cataglyphis bicolor* (Fabricius) (Formicinae) (Nartshuk, 2010). Clark & Blom (1992) document an unknown species of fly of the genus *Incertella* Sabrosky, approaching and touching the heads of workers of *Pogonomyrmex salinus* Olsen (Myrmicinae).

Chloropidae is also known for its wide range of lifestyles. The immature stages of some species are saprophytic, many others are phytophagous and a minority are inquilines, predators and there is even a gall-inducer in this family (Wheeler, 2010).

Hymenoptera

Three families of Hymenoptera were recorded in this study (Table 1): Figitidae, Platygastridae and Diapriidae. Figitidae (Eucoilinae) are reported here for the first time in association with ant colonies. Consequently, these results increased the number of families of Hymenoptera associated with Formicidae colonies to 17: Bethylidae, Braconidae, Ceraphronidae, Chalcididae, Diapriidae, Encyrtidae, Eucharitidae, Eulophidae, Eurytomidae, Figitidae*, Ichneumonidae, Mutilidae, Perilampidae, Platygastridae, Pompilidae, Pteromalidae and Sphecidae. These families are reported as parasitoids, specialist predators or inquilines (Kieffer, 1904, 1905, 1909, 1913; Collins & Markin, 1971; Hölldobler & Wilson, 1990; Lachaud & Pérez-Lachaud, 2012; *new report).

Diapriidae includes more than 17 genera associated with ant nests, and some studies have shown that their larvae are fed by the ants (Hölldobler & Wilson, 1990). Other studies report some parasitic diapriid wasps (e.g., *Acanthopria* spp. and *Mimopriella* spp.) are brood parasitoids in Attini nests (Fernández-Marín et al., 2006). Within this family, *Bruchopria hexatoma* Kieffer is known as a myrmecophile and is associated with the fire ant *Solenopsis richteri* (Kieffer, 1921). Loiácono et al. (2002) suggests that species of the genus *Bruchopria* could be parasitoids of ant larvae.

For the Platygastridae, one morphospecies of the genus *Inostemma* Haliday was recorded, which is known to parasitize flies of the family Cecidomyiidae (Diptera) (Maia & de Azevedo, 2001). It is likely it was parasitizing the cecidomyids recorded in the colonies, but further studies are necessary to confirm this possible tritrophic relationship between ants, cecidomyids and parasitoids.

Species of Figitidae are known as hyperparasitoids of braconids and chalcidoids, parasitoids of syrphids (Diptera), chamaemyiid larvae (Diptera: Shizophora) and Neuroptera, or even gall inducers (Buffington et al., 2007). However, despite this great diversity of lifestyles, the subfamily Ecoilinae is mainly composed of Schizophora parasitoids.

Ant nests

As previously stated, *S. virulens* build conspicuous earthen mounds 10–20 cm high often at the base of tree trunks, which could account for the occurrence of dozens of Diptera and non-ant Hymenoptera in these nests. Under these nests, there could be living plants (leaves, roots, etc.) and leaf-litter. We presume that some of the insects recorded in nests in this study were accidental or temporary inquilines. Some of them may have morphological, behavioural or chemical mechanisms that allow them to remain unnoticed by ants, as previously reported (Fiedler & Maschwitz, 1989; Hölldobler & Wilson, 1990; Akino et al., 1999). Hölldobler & Wilson (1990) argue that an ant colony acts as an ecological island, a partially isolated mini-ecosystem. Ant nest environments consist of a wide variety of richly structured microhabitats such as refuse areas, galleries and storage chambers (Hölldobler & Kwapich, 2022). These microhabitats are occupied by a range of commensals adapted to each of these niches.

Emergence chamber

Emergence chambers are used to collect insects (Perich et al., 1989), especially beetles from rotting wood (Ferro & Carlton, 2011) and evaluating the nesting habits of ground-nesting bees (Sardinas & Kremen, 2014). For collecting insects associated with ant nests, emergence chambers are used to fully or partially enclose material in the field, with the aim of collecting specific insects (e.g. Lepidopteran parasites in ant nests, Thomas & Elmes, 1998) and for determining ant–plant interactions (Scharmann et al., 2013). This is the first time an emergence chamber has been used to collect ant nest-associated fauna.

CONCLUSIONS AND RECOMMENDATIONS

It is likely that many of the insects recorded are temporary inquilines and not obligatory myrmecophiles, but the exact role of all these organisms in ant nests remains unknown. The trap used in this study should be useful for further research, such as: (a) collecting plant litter near a nest and comparing the emerging fauna with that coming from an ant colony, and (b) observations of galls included by the ants inside a nest, attempting to understand if the gall-inducing insect are more likely to survive in than outside an ant's nest. Therefore, this type of trap could be used to clarify the role of these organisms in ant nests or whether their presence is just a matter of chance, eg. through the sampling of the substrate around in which the nest was built or where they forage.

Furthermore, emergence chambers could also be used to collect insects that emerge from other substrates, e.g., leaves, logs, galls, etc. In addition, many RNA studies need living or fresh insects for research, which could also be obtained by using such traps.

The discussion offers hypotheses that can be experimentally tested. In addition to describing the emergence chamber and providing a list of the insects found in ant colonies, we hope that this article provides researchers with an easy and inexpensive tool that inspires further studies on insect/ant relationships.

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