



With the dead under the mat: the zombie ant extended phenotype under a new perspective

Fernando Sarti Andriolli¹ · José Aragão Cardoso Neto² · José Wellington de Moraes¹ · Fabricio Beggiato Baccaro³

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Abstract

Some parasitic fungi can increase fitness by modifying the behavior of their hosts. These behaviors are known as extended phenotypes because they favor parasitic gene propagation. Here, we studied three lineages of *Ophiocordyceps*, a fungus that infects ants, altering their conduct before death. According to fungal strategy, ants may die in leaf litter, with entwined legs in branches, under the moss mat, or biting plant tissue. It is critical for parasites that the corpses stay at these places because *Ophiocordyceps* exhibit iteroparity, possibly releasing spores in multiple life cycles. Thus, we assumed substrate cadaver permanence as a fungi reproductive proxy and corpse height as a proxy of cadaver removal. We hypothesize that biting vegetation and dying in higher places may increase the permanence of ant corpses while avoiding possible corpse predation on the forest floor. We monitored over a year more than 4000 zombie ants in approximately 15 km² of undisturbed tropical forest in central Amazonia. Our results show a longer permanence of corpses with increasing ground height, suggesting that the parasites may have better chances of releasing spores and infecting new hosts at these places. We found that the zombie ants that last longer on the substrate die under the moss mat in tree trunks, not necessarily biting vegetation. The biting behavior appears to be the most derived and complex mechanism among *Ophiocordyceps* syndromes. Our results put these findings under a new perspective, proposing that seemingly less complex behavioral changes are ecologically equivalent and adaptative for other parasite lineages.

Keywords Natural history · Coevolution · Selective pressure · Parasitism · Formicidae

Introduction

Parasitism is one of the earth's most successful ways of life (Poulin and Morand 2000; Okamura et al. 2018). Half of the known species are parasites if each free-living metazoan can host at least one parasite species in its organism (Schmid-Hempel 2011; Price 1980; Windsor 1998). Consequently, addressing the intimate interactions between hosts and parasites is necessary to deeply understand the behavior, specialization, and ecological diversification of the tree of life (de Meeûs et al. 1998).

Typically, an individual's behavior heads to propagating its genes, expressed through the generations of its descendants (Hirshfield and Tinkle 1975). However, some hosts can contribute to the survival of parasitic genes at the expense of life itself (Dawkins 1982; Dawkins and Krebs 1979). In these cases, the parasites go beyond causing damage by absorbing resource hosts. For instance, *Ophiocordyceps* (order: Hypocreales, family: Ophiocordycipitaceae) is one of the largest genera of entomopathogenic fungi, with ants

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✉ Fernando Sarti Andriolli
andriollifernando@gmail.com

José Aragão Cardoso Neto
jneto_bio89@hotmail.com

José Wellington de Moraes
moraesjw@gmail.com

Fabricio Beggiato Baccaro
baccaro@ufam.edu.br

¹ Instituto Nacional de Pesquisas da Amazônia - INPA, Coordenação de Biodiversidade - COBIO, Av. André Araújo 2936, Petrópolis Manaus, AM 69083-000, Brazil

² Instituto Federal de Educação, Ciência e Tecnologia do Amazonas – IFAM/CMA, Estradas Dos Moraes, S/N, Senador José Esteves, Maués, AM 69190-000, Brazil

³ Departamento de Biologia, Universidade Federal Do Amazonas (UFAM), Av. General Rodrigo Octávio, Manaus, AM 620069.080-900, Brazil

(Formicidae) being their hosts (Araújo and Hughes 2017). The ants infected by fungal spores belonging to the genus *Ophiocordyceps* show a marked change in behavior, dying in specific locations with better microclimatic conditions that benefit parasite development and production of spores (Andersen et al. 2009; Andriolli et al. 2019; Hughes et al. 2011, 2016; Mongkolsamrit et al. 2012).

Infection caused by *Ophiocordyceps* leads to a progressive loss of motor function, convulsions, and a tremulous, erratic gait in hosts (Andersen et al. 2009; Evans and Samson 1982). These symptoms culminate in the ants dying after attaching their jaws to leaves and other plant tissue, burying themselves in leaf litter and twisting their way through the moss of a tree trunk, or with their legs entwined in shrub branches, depending on the parasite's *modus operandi* (Andersen et al. 2009; Araújo and Hughes 2017; Evans and Samson 1982). The death sites are on the outskirts of foraging trails and colonies, sufficiently distant for the corpses not to be removed from the site by your conspecifics (Gracia et al. 2018). The fungus grows and completes its development with the ant attached to the substrate, releasing spores that can infect new ants (Evans and Samson 1984; Evans et al. 2011). These altered behaviors by fungi, turning the hosts into vehicles to parasitic genes, are considered examples of extended phenotypes and why these parasitized insects became known as zombie ants (Araújo and Hughes 2017; Dawkins 1982; Evans and Samson 1984; Evans et al. 2011).

The *Ophiocordyceps* complex comprises five primary lineages or species complexes: *O. myrmecophila* and *O. irangiensis*, *O. lloydii*, *O. australis*, *O. kniphofioides*, and *O. unilateralis*. These complexes are not commonly found sympatrically (Araújo and Hughes 2017). However, in the Central Brazilian Amazon, species belonging to three complexes were recorded: *O. unilateralis* sensu lato, *O. kniphofioides* sensu lato, and *O. australis* sensu lato (Cardoso Neto et al. 2019). Each group, per se, has specific extended phenotypes to achieve reproductive success and continue infecting healthy ants (Andersen et al. 2009; Araújo et al. 2018; Evans and Samson 1982, 1984).

Strategically, ants infected by fungi of the species complex *O. unilateralis* s.l., such as *Camponotus*, *Colobopsis*, *Dinomyrmex*, and *Polyrhachis*, climb bushes (summit behavior) and lock their jaws on plant tissue (biting behavior) just before dying from the fungus infection (Hughes et al. 2011). With the corpse fixed, the reproductive phase of the parasite begins by growing the fruiting body and culminating in the release of spores to enable new infections (Andersen et al. 2009; Loreto et al. 2014). In contrast, ants infected by *O. kniphofioides* s.l., such as *Cephalotes*, *Paraponera*, *Dolichoderus*, and *Daceton*, mostly die in tangles under the moss mat at the base of tree trunks (Araújo and Hughes 2017; Evans 1982; Evans and Samson 1982; Imirzian et al. 2020).

On the other hand, *O. australis* s.l. that infects *Pachycondyla*, *Neoponera*, *Paltothyreus*, *Crematogaster*, and *Odonotomachus* genera can induce a variety of extended phenotypes. Despite the hosts found in the mosses of tree trunks, they also can die with their legs entwined in branches or bury themselves a few centimeters in the leaf litter (Araújo and Hughes 2017; Evans and Samson 1982). Though in *O. kniphofioides* s.l. and *O. australis* s.l., scaling bushes and biting plant tissue are not evident behaviors as in *O. unilateralis* s.l., the ant corpses remain attached to the substrate not necessarily by the mandibles, allowing the development of the parasite reproductive phase (Araújo et al. 2018).

Opportunistic and generalist soil and litter invertebrates dominate tropical forests, consuming everything from fermented fruits to droppings and carcasses of all types of animals (Carroll and Janzen 1973; Griffiths et al. 2018). During an experiment in Thailand, dead *Camponotus leonardi* infected by *O. unilateralis* s.l. were removed from the locations originally found (jaws stuck in bush leaves) and allocated to higher bushes and the forest floor. The ants relocated to the ground disappeared within a few days, while the others in the uppermost forest bushes had the reproductive stage developed abnormally, not producing fruiting bodies (Andersen et al. 2009). Thereby, the place where the infected ants die (hereafter called “tomb type”) and the fitness of the parasite groups seem to depend on the extended phenotype (Loreto et al. 2018). Generally, hosts with modified behavior by parasites die in different niches from uninfected hosts, making it difficult to encounter opportunistic host predators (Moore 2002).

Most studies with zombie ants investigated the complex *O. unilateralis* s.l. and infection strategies of other *Ophiocordyceps* groups are still poorly understood (Barbosa et al. 2015; Loreto et al. 2018; Somavilla et al. 2019). We assumed that the permanence of the corpses in the tomb type allows parasites to produce fruiting bodies. For example, after the ant death, the fungi need an amount of time varying from a few weeks in tropical forests, just over a month in subtropical forests, to more than a year in temperate forests to fructify (Andersen et al. 2009; Loreto et al. 2018; Will et al. 2023). Moreover, the parasite can produce a new batch of spores several times, providing greater chances of infecting new hosts if the ant remains attached to the substrate, and biting the vegetation was previously posited as an additional way to secure the corpses in place (Andersen et al. 2012; Evans and Samson 1982; Mongkolsamrit et al. 2012).

Using a large dataset of more than 4000 infected ants, sampled over a year in ~ 15 km² of undisturbed tropical forest, we investigate the relationships between tomb types, the height of the ant cadaver, and parasite extended phenotypes. We hypothesized that the corpse permanence at the substrate is affected differently by the height of the ant corpse and the extended phenotype mode in three *Ophiocordyceps*

species complexes in Central Amazonia (*O. australis* s.l., *O. kniphofioides* s.l., and *O. unilateralis* s.l.). We predict that the permanence of the dead ants will be related to the extended phenotypes mode, with a longer permanence of ants that bite plant tissue. We expect that corpses near the ground will be removed by invertebrate predators-scavengers more frequently, leaving more dead ants at higher distances from the soil.

Material and method

We carried out this study at Ducke Forest Reserve (02° 55' 12" S, 59° 57' 36" W), located north of the city of Manaus, Amazonas state, Brazil (Fig. 1). The Reserve has low annual temperature variation, intense rainfall from December to May and predominant “terra-firme” Tropical Humid Forest vegetation (Falesi et al. 1969; Ribeiro 1976; Guillaumet 1987). We used the perpendicular north–south and east–west trails of the Ducke Reserve that give access to the set of permanent plots maintained by the Biodiversity Research Program-PPBio and the Long-term Ecological Research Program-PELD (Costa et al. 2015, 2020).

We searched for zombie ants on 15 plots of 250 m in the Ducke Reserve (Fig. 1C). We search leaves and branches

of shrubs and palm trees, the moss on tree trunks, and leaf litter in a ~3 m buffer around the main trail of each plot up to ~2 m high. Dead ants found in leaf litter and at the base of tree trunks were detected primarily by fungal fruiting bodies (Evans 1982; Evans and Samson 1982). We individually numbered the ant bodies with adhesive plastic tapes in the substrate closest attached to the dead ant and measured the height from the ground and the tomb type. We did the fieldwork in seven events every ~60 days from November 2014 to December 2015, covering the annual rainfall regime (see Cardoso Neto et al. 2019 for more details). In each field collection event, we recorded the new corpses and the absence of the numbered ants. We considered a removed ant when the individual disappeared from the tomb type but had the adhesive tape intact, indicating its initial location. We removed from our analysis the cases when the ant and the numbered plastic tag were lost to avoid mixing a natural leave fall with a predation or remotion event. Therefore, we could record the ant corpse permanence or loss over 14 months.

We identified the ants using taxonomic keys (Mackay and Mackay 2010; Ortiz and Fernández 2011; Baccaro et al. 2015) comparing with specimens from the INPA (Instituto Nacional de Pesquisas da Amazônia) Entomological Collection with confirmation by ant Taxonomist Itanna Fernandes, Ph.D. We identified the fungi using the morphology of the fruiting bodies and the parasite-host specificity criterion (Evans et al. 2011; Kobmoo et al. 2012 and 2015). To date, evidence points to a species-specific relationship between the ants and the parasite fungus species (Araújo et al. 2018; Sakolrak et al. 2018). We grouped and distinguished the ants into three complexes—*O. unilateralis* s.l., *O. kniphofioides* s.l., and *O. australis* s.l., according to the literature description of infections recorded and fruiting body fungal morphology (Evans and Samson 1982, 1984; Araújo et al. 2015, 2018; Araújo and Hughes 2017). The *unilateralis* complex infects exclusively Camponotini ants that bite substrate, producing brown fruiting bodies attached laterally to emerged stroma. In contrast, in the *kniphofioides* complex, the ascoma (or fruiting body) is orange, covering totally the emerged stroma (or stalk) from the hosts that often die in the moss of tree trunks (Araújo et al. 2018). On the other hand, the *australis* complex generally produces long stalks with a terminal ovoid and red fruiting body (Araújo and Hughes 2017). These features associated with species-specific relationships made distinguishing the three parasite groups possible. We also classified the places where we find the dead infected ants into three different tomb types: leaves and branches, moss trunks, and palm trees. We separated palm tree leaves from other plant leaves because palms are conspicuous elements of the forest understory, where we find many dead ants.

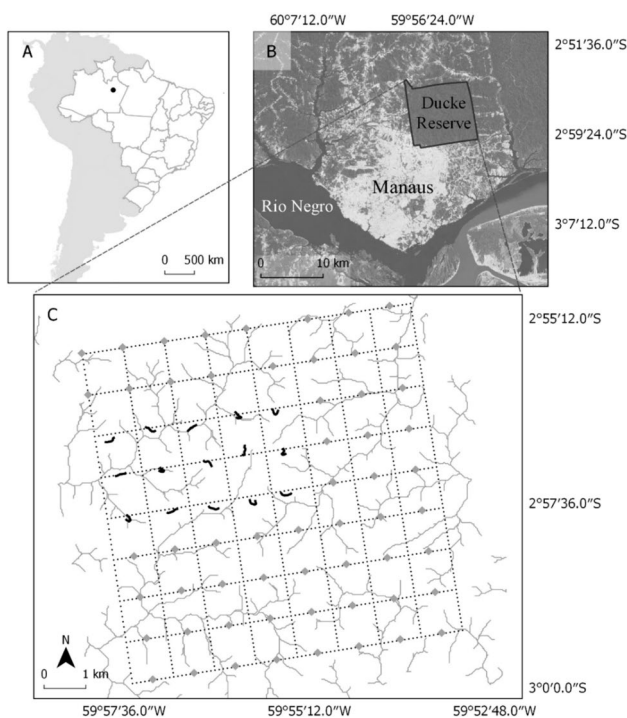


Fig. 1 **A** Brazilian territory state limits, the dot shows the location of the Ducke Reserve **B** Manaus region and highlighted delimitation of the Ducke Reserve. **C** Permanent plots sampled (250 m) in bold (the gray diamonds represent the other permanent plots of the Reserve). The dotted lines represent the access along trails (PPBio-PELD)

Data analysis

In this study, the event of interest and response variable was the time required to lose or remove corpses from where we found the dead ants (i.e., tomb types). We also call it “survival of the corpses” due to analysis terminology. We considered as censored events the ants found on the first fieldwork day, as we do not have information on the period of arrival and death of ants, and fresh corpses (ants that appeared and died after the first fieldwork day) as uncensored events (Muenchow 1986). Therefore, we considered the survival data from the second event fieldwork, with the new corpses found starting that period. We used Cox regression models to test whether the predictor covariate height affects cadaver survival (Cox 1972). This analysis allows modeling the effect of covariables concomitant with survival, in our case, the permanence of ant cadavers at the substrate. To assess whether there were patterns between species within their respective groups, we ran models considering cadavers from different parasitic complexes (*Ophiocordyceps: australis s.l.*, *kniphofioides s.l.*, and *unilateralis s.l.*) for all dead ant species that have at least ten individuals. We also used chi-square tests to investigate correlations between the tomb types and parasite complexes. We performed all the analyses and built the graphics in the software R (R Core Team 2021) with the package survival, ggplot2, survminer, and tidyverse (Kassambara et al. 2021; Therneau 2021; Wickham 2016, Wickham et al. 2019).

Results

Ground height of the dead ants

We found 4118 infected dead ants during the fieldwork period. The most abundant species complex was *O. unilateralis s.l.* (3674 individuals) followed by *O. kniphofioides s.l.* (402 individuals) and *O. australis s.l.* (42 individuals). The

most abundant cadavers were *Camponotus atriceps* (2505 ants, *O. unilateralis s.l.*) and *Camponotus bispinosus* (746 ants, *O. unilateralis s.l.*), followed by *Cephalotes atratus* 383 ants, *O. kniphofioides s.l.*), respectively (Fig. 2).

The hosts of *O. australis s.l.* (42 individuals) presented an average height of 80.2 cm (sd = 49.3), of which 19 ants (45.23%) were tangled in the moss mat of tree trunks, 17 (40.47%) in leaves or branches of shrubs, and 6 (14.28%) in leaves or branches of palm trees with most ants embracing or biting plant tissue. The average height of the corpses of the *O. kniphofioides s.l.* (402 individuals) was 48.14 cm (sd = 53.9), with 388 ants (96.52%) found dead buried in the moss mat of tree trunks, 9 (2.24%) palm tree leaves, and 5 (1.24%) in shrubs, biting or embracing vegetation. The average height of ant corpses of the *O. unilateralis s.l.* (3674 individuals) found was 101.8 cm (sd = 48.6). The *Camponotus* individuals dead by *O. unilateralis s.l.* were mainly found in palm leaves (75.25%), followed by shrub leaves or branches (23.21%), and (1.54%) in the moss on tree trunks, always with lock jaws in plant tissue (Fig. 3).

The survival of the dead ants

The chi-square tests reveal a correlation between the parasite complexes and all tomb types. However, the *unilateralis* complex presented more dead ants than expected by chance in palm trees and leaves and branches ($p < 0.001$ and $p = 0.039$, respectively). In contrast, the *kniphofioides* complex shows more corpses than predicted by chance in the moss trunk ($p < 0.001$). Furthermore, the *australis* complex shows more dead ants than expected by chance in leaves and branches and moss trunks ($p = 0.004$ and $p < 0.001$, respectively).

Overall, the survival or permanence probability of the corpses from one event to the next dropped from 70–80 to 5–25% after 1 year of monitoring (Fig. 4). The Cox regression models with height as a covariate showed significant values for most host species analyzed (Table 1, species

Fig. 2 Boxplot shows the cadaver species' height by parasite complex with five or more individuals found at Ducke Reserve. The ant species were ordered from left to right according to the mean height (cm)

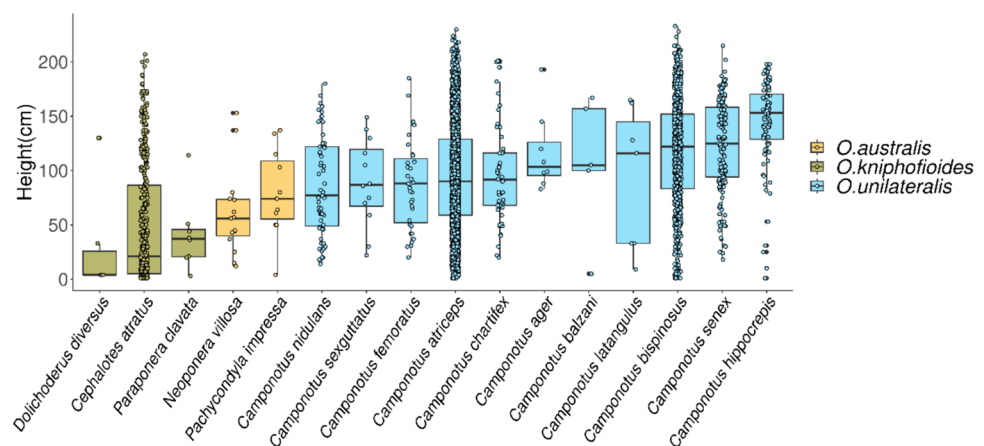


Fig. 3 Violin plot representing the height (cm) of the most infected ant species found as a function of tomb types (*C. atratus* and *P. clavata*: *O. kniphofioides* s.l.; *N. villosa* and *P. impressa*: *O. australis* s.l.; *C. atriceps* and *C. bispinosus*: *O. unilateralis* s.l.)

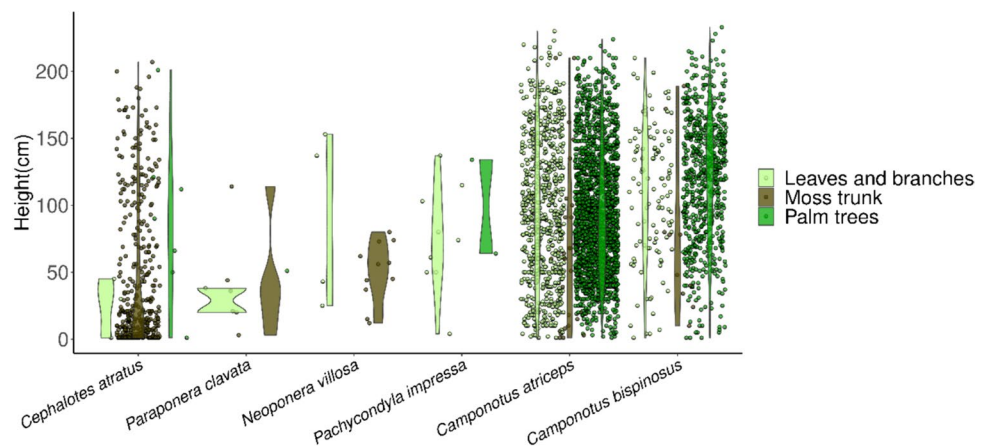


Fig. 4 Survival plots controlled by the height of the corpses for the most ants (at least ten individuals) of each parasite complex. *C. atriceps*, *C. bispinosus*, *C. senex*, *C. hippocrepis*, *C. chartifex*, *C. femoratus*, *C. nidulans*, and *C. sexguttatus* (*O. unilateralis* s.l.); *C. atratus* and *P. clavata* (*O. kniphofioides* s.l.); *N. villosa* and *P. impressa* (*O. australis* s.l.)

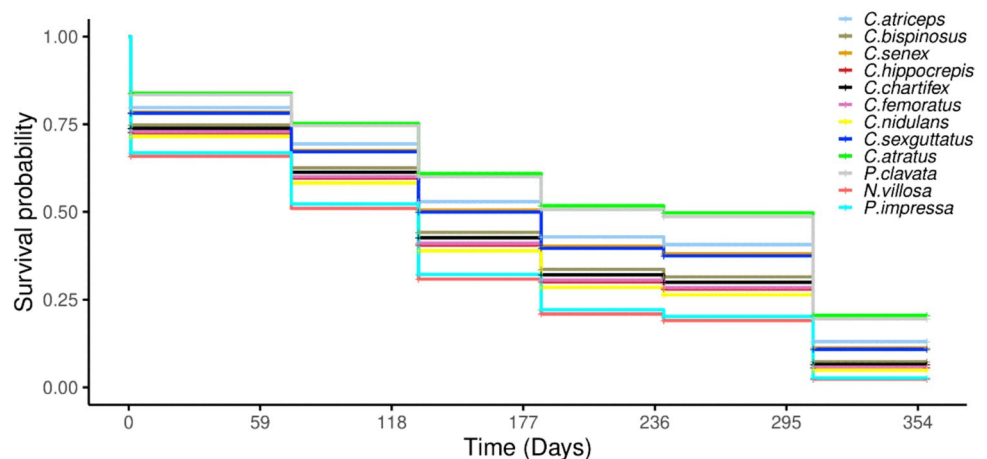


Table 1 Cox regression model results of the host species (at least ten individuals) concerning the height covariate in cadaver permanence. The model uses *C. atriceps* species as a comparison factor

Parasite hosts	Parasite complex	Pr(> z)
<i>Neoponera villosa</i>	<i>O. australis</i>	0.009*
<i>Pachycondyla impressa</i>	<i>O. australis</i>	0.046*
<i>Cephalotes atratus</i>	<i>O. kniphofioides</i>	0.016*
<i>Paraponera clavata</i>	<i>O. kniphofioides</i>	0.763
<i>Camponotus bispinosus</i>	<i>O. unilateralis</i>	<0.001*
<i>Camponotus chartifex</i>	<i>O. unilateralis</i>	0.046*
<i>Camponotus hippocrepis</i>	<i>O. unilateralis</i>	0.018*
<i>Camponotus nidulans</i>	<i>O. unilateralis</i>	0.001*
<i>Camponotus senex</i>	<i>O. unilateralis</i>	0.824
<i>Camponotus sexguttatus</i>	<i>O. unilateralis</i>	0.783
<i>Camponotus femoratus</i>	<i>O. unilateralis</i>	0.054

marked with asterisk), except for *P. clavata* (*O. kniphofioides* s.l.), *C. atriceps*, *C. sexguttatus*, *C. senex*, and *C. femoratus* (*O. unilateralis* complex). For the remaining species, the covariate height influences the permanence of ant corpses in their tomb types. The hosts *C. atratus* (Fig. 5b–e) and *P.*

clavata were the dead ants with higher chances (~25%) of survival or permanence at the tomb types after a year.

Discussion

Our results reveal that the permanence of the dead ant species differs among parasite complexes, partially supporting our initial hypotheses. We predicted that the survival of the dead ants would be related to the extended phenotype type, with a longer permanence of *O. unilateralis* infected ants that express biting behavior (Andersen et al. 2009). However, we found that hosts of the *O. kniphofioides* complex have longer permanence in tomb types. Cox models show the tendency of most corpses fixed at some distance from the ground to remain longer in tomb types. The *O. australis* complex hosts have the shortest permanence among tomb types, and infected ants may display several extended phenotypes before dying. Overall, our results suggest that height and tomb type may affect one facet of parasite fitness differently among *Ophiocordyceps* lineages.

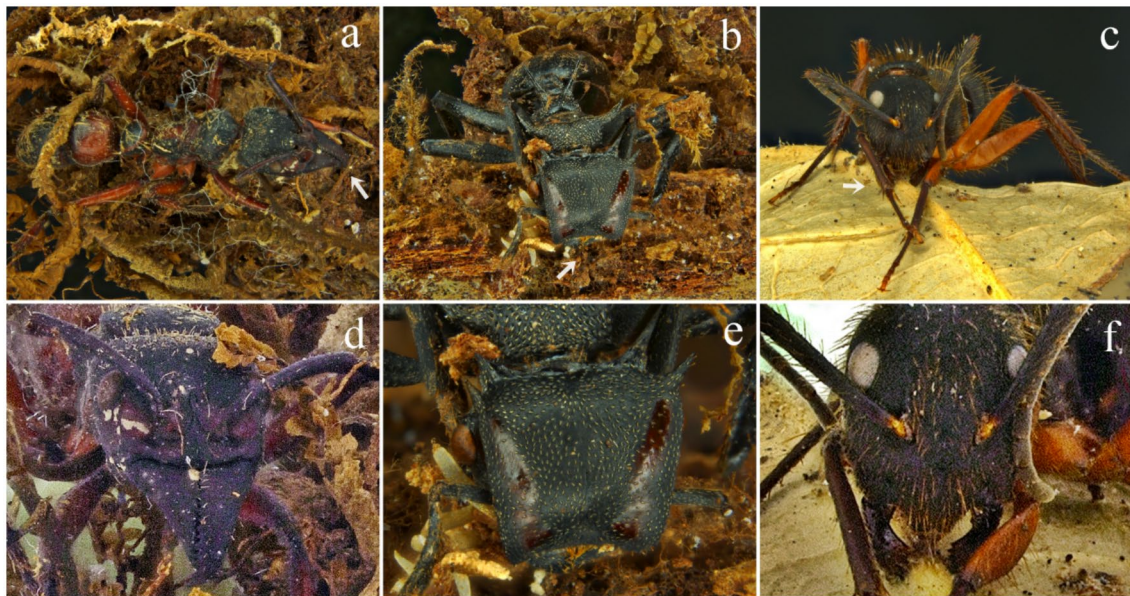


Fig. 5 The zombie ant extended phenotype under a new perspective. **a, d** *Pachycondyla crassinoda* (infected by *O. australis* s.l.) showing no biting behavior at the moss mat in a tree trunk found next to leaf litter level. **b, e** *Cephalotes atratus* (infected by *O. kniphofioides*

s.l.) found on moss at the base of tree trunks, not biting the substrate. **c, f** *Camponotus atriceps* (infected by *O. unilateralis* s.l.) biting the central vein's leaf. The white arrows indicate the ants' mandibles. Photographs by S. P. de Lima

Our findings indicate a niche selection process among parasite species and tomb types. *Ophiocordyceps* lineages generally follow a consistent pattern, yet they do not limit infected dead ants to a single tomb type. For instance, some extended phenotypes, such as the biting behavior, are unanimous within some species (all *O. unilateralis* s.l. complex), yet not by others (Andersen et al. 2009 Araújo et al. 2018). Even considering dying under the moss mat (*O. kniphofioides* s.l. besides *O. australis* s.l.) as a typical feature within parasitic species, the hosts may manifest other extended phenotypes like die biting other substrates or in different ways, albeit less frequently. Though there is variation in the place of death and height within each parasite lineage, our findings indicate more corpses in palm leaves for *O. unilateralis*. Simultaneously, mosses were the site with the highest number of ants killed by *O. kniphofioides* s.l. and *O. australis* s.l. Further, the number of ant corpses we found hugging sticks or biting leaves was similar within the *O. australis* lineage. In other words, defining more precisely if it exists and what the typical tomb type of the *O. australis* lineage would be remains a challenge, given its low frequency in the environment.

Climbing and biting before death, the remarkable and final acts in the life of a zombie ant infected by *O. unilateralis* s.l. were well investigated in several studies (Andersen et al. 2009, Andriolli et al. 2019, de Bekker et al. 2015, de Bekker et al. 2017, de Bekker and Das 2022, Chung et al. 2017, Hughes et al. 2011). Our findings suggest that most of

the dead ants infected by *O. unilateralis* s.l. were in leaves on understory bushes, mostly in palm trees. At these locations, the biting behavior seems primary for the parasites to achieve reproductive fitness. We expected that the biting behavior was the most effective mechanism that kept the ant attached to plant tissue. However, our findings indicate that other extended phenotypes and tomb types may be just as or even more efficient to parasites. The dead ants with higher permanence in the tomb types (*C. atratus* and *P. clavata*) belong to the *O. kniphofioides* complex. Although *C. atratus* may bite plant tissue before dying by infection (Evans and Samson 1982), this behavior is not always expressed. Moreover, *Paraponera clavata*, *Dolichoderus bispinosus*, and *Daceton armigerum* (all hosts of *O. kniphofioides* complex) also typically die without biting (Araújo et al. 2018). In this regard, ants in the moss mat may not need the biting behavior to fix at the substrate and enable parasite reproductive fitness.

The most recent phylogenetic studies suggested that *Ophiocordyceps* may have initially infected Coleoptera larvae (Araújo and Hughes 2019) or social cockroaches (Araújo et al. 2021). The Coleoptera larvae and termites inhabit the soil and fallen logs, and the parasite, through niche overlap and selective pressures, diversifying (host-jumping) and reaching the ants. In both predicted cases, the infection originates from soil insects (Coleoptera larvae and termites), where the parasite kills the host in the same place or close to the original infection location

(Araújo and Hughes 2019, Araújo et al. 2021). Although the ants became infected in foraging trails in the colony vicinity, they did not die inside colonies or in foraging trails (Andersen et al. 2009). In these places, they would be removed by conspecific ants attempting to avoid newly infected nestmates (Evans and Samson 1982; Hughes et al. 2011; Gracia et al. 2018). Similar pressure occurs by predators during foraging, as predators search for food where the prey live. Therefore, manipulating hosts to climb shrubs, biting leaves (*O. unilateralis*), or twisting under the moss mat (*O. kniphofioides* and *O. australis*) are refined and derived ways to mislead a predator or a conspecific ant (Araújo et al. 2018; Evans and Samson 1982; Imirzian et al. 2020; Moore 2002). On this point, our results suggest that the predation pressure exerted at ground level seems to have influenced the death places of the ants independently of the *Ophiocordyceps* lineages. Thus, allocating ants at some distance from soil appears adaptative to most parasite species' fitness.

In the most abundant species infected by *O. kniphofioides* s.l., *Cephalotes atratus*, the infection success is low and credited to their foraging habits. Although they mostly die at the base of tree trunks under the moss mat, contact between healthy ants and infected conspecific cadavers is infrequent (Imirzian et al. 2020). Moreover, the structures of the sexual (teleomorphs) or asexual phases (*O. kniphofioides* anamorphic forms) need physical contact with the hosts during infection (Evans and Samson 1982). When these forms come into contact with the conspecific healthy ant, they can promote new contamination (Araújo et al. 2018; Evans and Samson 1982). Our results show a longer resilience of *C. atratus* (along with *P. clavata*) corpses in the environment. In this regard, the moss carpet may be a protective barrier to the ants' corpses, minimizing the chances of finding and removal by conspecific ants and opportunistic scavenger insects (Evans and Samson 1982; Imirzian et al. 2020). Furthermore, with the dead ants under the moss mat, the parasite may access better and more stable microclimatic conditions to reproduce at fluctuating heights above ground since the moss can grow over large parts of the trees (Eldridge et al. 2023).

Ants infected by the *O. australis* complex are less abundant in tropical forests, building colonies consistently smaller in individual number (Holldober and Wilson 1990). These ants die in different places and in multiple ways, suggesting these parasites do not have such a fine adjustment of the extended phenotypes. Although the lack of enough data might preclude a possible trend, the higher-variable extended phenotype reported here could be in response to the low abundance of host ants. Diverse extended phenotypes may increase the range of possibilities of infection. Consequently, it can bring a

lower efficiency in the adjustment of each one of them. We hypothesize that the variation of extended phenotypes was initially intrapopulation and may co-evolve with ant death sites while becoming increasingly adaptive to *Ophiocordyceps* lineages. Attempting to look for the present temporal scale of these complex and diversified parasites, the niches or tomb types seem to have selected common characteristics within the species complexes, such as biting palm leaves and burying themselves in moss mats. Despite this, many factors may also influence the location of the ant's death and corpses, such as places with better microclimatic conditions, environmental and seasonal traits, and other selective pressures like possible predation at the ground forest level, as we suggest here (Andersen et al. 2009; Andriolli et al. 2019; Loreto et al. 2018, Will et al. 2023).

The present study places the zombie ant extended phenotype under a new perspective by including less-studied *Ophiocordyceps* syndromes. Although we have not monitored the formation of the parasites' fruiting bodies, fungal iteroparity supports our hypothesis. We have shown that the dead ants' permanence changes between parasitic lineages in most species according to fungi manipulation and height from the ground. We propose that niche selection, as a possible response to predation (or consumption) pressure with the microclimatic conditions, composes a selective force for the extended phenotypes of parasite syndromes. Zombie ants that die minimally above ground seem more likely to survive, favoring the parasites' increased fitness.

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Author contribution FSA and FBB contributed to the study's conception and design. JACN conducted the data collection. FSA and FBB performed the analysis. JWM and FBB supervised the project and reviewed versions of the manuscript. All authors contributed, commented, and approved the submitted manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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