

1 **Title:**

2 3D mapping of disease in ant societies reveals a strategy of a specialized parasite

3 **Authors:**

4 Raquel G. Loreto\*<sup>a,b</sup>, Simon L. Elliot<sup>b</sup>, Mayara L. R. Freitas<sup>b</sup>, Thairine M.  
5 Pereira<sup>b</sup>, & David P. Hughes\*<sup>a,c</sup>

6 **Affiliation:**

7 <sup>a</sup>Department of Entomology and Center for Infectious Disease Dynamics,  
8 Pennsylvania State University, University Park, PA, USA.

9 <sup>b</sup>Department of Entomology, Universidade Federal de Viçosa, Viçosa, MG, BR.

10 <sup>c</sup>Department of Biology, Pennsylvania State University, University Park, PA,  
11 USA.

12 **\*Corresponding authors:** Raquel G Loreto. W241A Millennium Science  
13 Complex, Pennsylvania State University, Pennsylvania State University, University Park,  
14 PA 16802, USA. Email: rzg148@psu.edu

15 David Hughes. W129 Millennium Science Complex, Penn State University,  
16 Pennsylvania State University, University Park, PA 16802, USA. Telephone: 814-863-  
17 6073. Email: dph14@psu.edu

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22 **Abstract:**

23           Despite the widely held position that the social insects have evolved effective  
24 ways to limit infectious disease spread, many pathogens and parasites do attack insect  
25 societies. Maintaining a disease-free nest environment is an important evolutionary  
26 feature, but since workers have to leave the nest to forage they are routinely exposed to  
27 disease. Here we show that despite effective social immunity, in which workers act  
28 collectively to reduce disease inside the nest, 100% of studied ant colonies of  
29 *Camponotus rufipes* in a Brazilian Rainforest were infected by the specialized fungal  
30 parasite *Ophiocordyceps unilateralis s.l.* Not only is disease present for all colonies but  
31 long-term dynamics over 20 months revealed disease is a permanent feature. Using 3D  
32 maps, we showed the parasite optimizes its transmission by controlling workers' behavior  
33 to die on the doorstep of the colony, where susceptible foragers are predictable in time  
34 and space. Therefore, despite social immunity, specialized diseases of ants have evolved  
35 effective strategies to exploit insect societies.

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## 44 **Introduction**

45 High density living in human settlements, or among the animals and plants we  
46 raise for food can result in both major epidemics and the emergence of novel pathogens  
47 [1]. Dense societies also occur in natural systems and the prime example are the social  
48 insects. Their colonies can contain thousands and sometimes millions of highly related  
49 individuals [2] which might suggest constant epidemics. However, such societies have in  
50 fact achieved both ecological dominance and long evolutionary stability. For instance,  
51 ants have become dominant in terrestrial biomes accounting for over 50% of the biomass  
52 despite making up less than 2% of all insect species [3]. This success implies that their  
53 societies have evolved ways to reduce disease pressure, a phenomenon known as social  
54 immunity [4]. Therefore, important lessons for limiting disease spread might be gained  
55 from examining societies that have evolved by the process of natural selection over long  
56 periods of time.

57 Many studies have demonstrated that ants, termites, bees and wasps successfully  
58 defend their colonies from a range of parasites, through an integration of different levels  
59 of immunity, from cellular to behavioral [4-7]. However, this raises a paradox since we  
60 know that many different groups of parasites do infect social insects and, based upon  
61 their life history, the majority appear to be specialized parasites requiring infection of  
62 colony members for lifecycle completion [6-8]. If colonies are so adept at defending the  
63 nest, an important question is: How do specialized parasites transmit despite such  
64 effective group defense?

65 The majority of studies on disease dynamics in ants have focused their attention  
66 on colony response to generalist parasites introduced inside the nest, under laboratory

67 conditions [5,9-11]. These studies can control numerous factors and capture quantitative  
68 details of collective defensive strategies. However, such studies minimize environmental  
69 complexity, missing important components of the host parasite interaction. For example,  
70 they do not focus on the ants that are more exposed to infection (foragers) and do not  
71 consider specialized parasites, which have adaptive traits that drive transmission. Without  
72 incorporation these factors, they do not explain if and how co-evolved parasites infect  
73 their host, somehow circumventing the social immunity. Thus, we set out to study a  
74 disease of ants in a rainforest ecosystem, incorporating environmental complexity.  
75 Further we addressed specialization by focusing on a parasite that has evolved the ability  
76 to control host behavior to affect transmission [12-16] and is specific to its host species  
77 [14].

78

## 79 **Material and Methods**

### 80 *Study area and host and parasite species.*

81 Fieldwork was carried out at the Research Station of Mata do Paraíso, Universidade  
82 Federal de Viçosa, Minas Gerais, Southeast Brazil (20°48'08 S 42°51'31 W). The  
83 carpenter ant *Camponotus rufipes* is very abundant in this habitat. The ants forage on  
84 trails, being active at night with activity peaking in the early evening [17]. The trails are  
85 built mainly on twigs and branches lying on the forest floor so the ants use the 3D space  
86 of the forest, not walking only on the floor. Ant trails are permanent with the same trail  
87 being used for weeks [17]. The entomopathogenic fungi *Ophiocordyceps unilateralis*  
88 *sensu lato* is a specialized parasite of ants that must kill the host to complete its life cycle.  
89 Before killing the infected ant, the fungal parasite manipulates the behavior of host,

90 leading the hosts to climb the vegetation, bite the veins and margins of leaves in  
91 rainforests [12-16], that then serve as a platform for fungal growth and spore release from  
92 the dead ant [15]. *Ophiocordyceps unilateralis s.l.* transmission requires the growth of a  
93 long stalk from the head of a dead ant from which spores are released onto the forest  
94 floor to infect other workers. The newly described parasitic fungus *Ophiocordyceps*  
95 *camponoti-rufipedis*, previously known as *O. unilateralis s.l.* [14], is a parasite that has  
96 *C. rufipes* as its host, and it is also abundant in the study area [17].

### 97 ***Disease within the nest.***

98 Experimentally it has been shown that the fungus cannot grow either on the forest floor  
99 or in the dry upper canopy [12], thus the manipulation is adaptive for the parasite. While  
100 informative, these earlier experiments left open the question of if the parasite could  
101 develop inside the nest. To determine whether the fungus is able to grow normally inside  
102 an ant mound, we collected a whole nest of *C. rufipes* (including nest material, ants and  
103 brood) and a recently abandoned nest (only the nest material, no ants or brood). Both  
104 were directly placed in buckets (volume = 8L), maintaining the original characteristics of  
105 the nest, and kept under natural day/night light and temperature regime. We collected 28  
106 ants freshly killed by *Ophiocordyceps camponoti-rufipedis*, took pictures of their initial  
107 conditions and attached them to flags (so as not to lose them inside the nest). Each  
108 cadaver was at the fungal pre-emergence stage, meaning the ant's body had been  
109 colonized by fungal blastospores and hyphae but the stalk required for transmission had  
110 not yet grown. Since the stalk is crucial for transmission we identified this stage as being  
111 crucial for fungal lifecycle completion. The 28 ants were placed in two different  
112 treatments: (1) nest with ants, n=14; (2) nest without ants, n=14. In the treatment with

113 ants, sugar/water 50% and canned tuna were used to feed the ants. In both treatments the  
114 fungal killed ants were placed on the top of 10 cm of nest material and covered with 20  
115 cm of the same nest material. The ant cadavers were removed 10 days later, and pictures  
116 were taken to evaluate the development of the fungus.

117 ***Disease surrounding the nest.*** Because social immunity is well known from  
118 experimental studies in the laboratory to be effective and rapidly deployed [5,9-11] we  
119 might expect colonies in nature to be disease-free. We therefore set out to ask how  
120 common infection by *O. camponoti-rufipedis* was at the population level. In order to  
121 identify nests of *C. rufipes*, we made 22 transects of 2,000m<sup>2</sup> each (100x20m), covering  
122 44,000m<sup>2</sup>. The first 15 transects were initiated on the main path of the research station,  
123 and were taken 100m into the forest, using string as a guide reference. From the string,  
124 10m for both sides were covered. In order to obtain more complete coverage of the site,  
125 we delineated a new path from which we traced the other six transects, covering the  
126 2,000m<sup>2</sup> area for each one. The distance between the start points of each transect was  
127 100m and the transect direction alternated between the left and right sides of the path.  
128 Using this methodology we found 9 nests. Another 8 previously identified nests were  
129 used in this study. We examined the vegetation within a 1m radius around each nest  
130 looking for ants killed by the *O. camponoti-rufipedis*, attached to the underside of leaves.  
131 The nests that had dead ants on the adjacent vicinity were recorded.

132 To investigate the disease surrounding the nest with more details and over longer  
133 periods of time, we mapped the dead ants surrounding 4 nests over 20 months (Dec 2010-  
134 Jul 2012). Because the ants are known to travel long distances from their nests [18], we  
135 limited the mapping to the area surrounding the nest. This area is critical because the ants

136 must walk throughout there when leaving or returning to the nest. We also refer to this  
137 area as “doorstep” of the colony. For 4 of the 17 previously discovered nests, we  
138 demarcated a study area of 200m<sup>3</sup> (10m x 10m x 2m) (from now called plot) that were  
139 centered on the nest. Thus, we observed the long-term dynamic of the fungal infection in  
140 four distinct colonies.

141 In order to determine the 3D position of ants killed inside the four studied plots,  
142 we used the coordinate system relative to the nest, determining the x, y and z position of  
143 each dead ant, having the left bottom corner as beginning. For example, all the four nests  
144 had the coordinates (500, 500, 0) because they were in the center of the plots (x=500cm,  
145 y=500cm) and on the forest floor (z=0cm). We measured the disease in 3D (x,y,z  
146 coordinates) because the ants are manipulated to die attached to the underside of leaves  
147 on plants in the understory vegetation of tropical forests [12-16].

148 Before beginning the 3D measures, in November 2010 we tagged all dead ants in  
149 those plots checking every single leaf inside the plots, up to 2m from the forest floor.  
150 Across the first six consecutive months (December 2010- May 2011) we identified,  
151 tagged and mapped (x,y,z coordinates) every single newly killed ant attached to leaves  
152 within each of the plots. None of the dead ants that we counted were removed from the  
153 plots so we did not reduce the naturally occurring disease pressure. To capture long-term  
154 dynamics, we left the area for seven months following the May 2011 census and mapped  
155 the new cadavers in January 2012. Finally, we returned in July 2012 to check if each of  
156 the four nests had new dead ants on the immediate vicinity of the colony.

157 Since the potential hosts are encountered on the foraging trails, we also measured  
158 and mapped in 3D the trails formed by the ant. The foraging trails were marked with

159 small flags placed every 30cm, starting at the nest and continuing until they left the plot.  
160 The coordinates of each flag inside the area were determined the same way as we did for  
161 the dead ants. The z positions, measured from the forest floor, were included because the  
162 trails pass along on branches, lianas and roots above the forest floor [17]. Combining  
163 those coordinates we were able to access the exact location of each trail in space, related  
164 to the nest. We also did not disturb the ant trails. The trails and dead ants were mapped  
165 once a month, being necessary a day for each plot (trails and dead ants for each plot were  
166 mapped at the same day). The 3D data were plotted using the Grasshopper® plugin for  
167 the 3D modeling platform Rhino®. Statistical analyses were conducted using R (version  
168 2.15.2). We used generalized mixed models to avoid temporal pseudo-replication, using  
169 the variable “Month” as repeated factor.

170

## 171 **Results:**

### 172 *Disease within the nest.*

173 Of the total of 28 samples placed inside the nest, none developed normally (Fig. S1).  
174 Eight (53%) of the cadavers placed within the nest material without ants did not grow at  
175 all, and the remaining six (47%) grew abnormally in a way that ensured spore  
176 transmission would not occur, since it occurs from a specialized structure (ascoma) that  
177 grows near the top of that stalk that grows from the ant’s head) (Fig. S1 B, D). Of the  
178 cadavers placed into nests containing live ants, nine were removed from the leaf they  
179 were attached to (64%) and it was not possible to find them. This suggests that the  
180 healthy ants removed the cadavers, possibly destroying them since we could not recover  
181 them. The remaining five (36%) failed to grow normally meaning, as occurred in the nest



182 without ants, that sporulation did not happen and hence no transmission occurred. In  
183 summary, the fungal parasite was incapable of reaching the infective stage inside ant  
184 nests, whether ants were present or not.

185 ***Disease surrounding the nest.*** We discovered 17 nests that were patchily distributed in  
186 the study area. All 17 nests had ant cadavers attached to leaves beside the ant colony.  
187 Thus, disease prevalence at the population level is 100%.

188 For the long-term study, during these first six consecutive months (Dec 2010-May  
189 2011), we identified 347 newly dead ants, killed by *O. camponoti-rufipedis* surrounding  
190 the four colonies (Fig. 1, Movie S1). The number of dead ants is month-dependent  
191 (Mixed-model:  $X^2_5$ : 60.877;  $P < 0.0001$ ). December 2010 had the highest density of  
192 parasitized ants: 146 dead ants attached to leaves were found in the census for that month  
193 (Mixed-model:  $X^2_1$ : 18.052;  $P < 0.0001$ ) (Fig. 2). The lowest occurrence of dead ants was  
194 in March 2011, when we recorded a total of 12 dead ants; but it did not differ statistically  
195 from February (24 dead ants) (Mixed-model:  $X^2_1$ : 2.0164;  $P = 0.1556$ ) (Fig. 2). November,  
196 December and January receive 75% of the yearly precipitation [19], which is likely an  
197 important determinant of abundance for fungal parasites. In January 2012, after we left  
198 the plots for seven months, we found a total of 39 new dead ants within the 4 plots (that  
199 is, after seven months, each nest had freshly killed cadavers attached to leaves). Finally,  
200 when we returned in July 2012 and established that, even after 20 months, each of those  
201 four nests had ants manipulated to die in the immediate vicinity of both the nest and  
202 trails, demonstrating the long-term persistence of disease surrounding these colonies.

203 Over the 20-month period we measured disease in eight months (i.e. months 1-6,  
204 7, and 20) surrounding four colonies. Only once and for one colony we did not find new

205 records of *O. camponoti-rufipedis* surrounding host colonies (Nest C) (Fig. 2). This was  
206 the month that the density of new cadavers was lowest for all colonies (March 2011) (Fig.  
207 2). However, in the following month (April 2011), we did find newly manipulated killed  
208 ants outside Nest C, demonstrating that the colony was not disease free.

209         Because we measured and mapped the position and abundance of ant trails, we  
210 also investigated the role of host activity on the disease dynamics. We calculated the  
211 number of trails for each month, which represent the healthy ants susceptible to new  
212 infections, and related it with the number of dead ants of each month. We would expect  
213 to find more dead ants when the ants were more active (activity was measured by number  
214 of trails). Surprisingly, the number of infected ants attached to leaves surrounding the  
215 colony was not related with the number of susceptible hosts (Mixed-model:  $X^2_1$ : 2.1078;  
216  $P=0.1466$ ).

217

## 218 ***Discussion:***

219         Our results support the previously well-established theory of social immunity  
220 operating inside the nest of social insects, as we have shown that the *Camponotus rufipes*  
221 ants removed most of the *Ophiocordyceps camponoti-rufipedis* parasitized cadavers  
222 placed within the nest. Additionally, however, we also found that this specialized fungal  
223 parasite, when placed inside a nest without ants, cannot grow to the stage suitable for  
224 transmission. Previous studies on disease in ant societies have shown that corpses are  
225 removed by nestmates [20-22] and sick ants experience social isolation [9,23]. Both of  
226 these behaviors are interpreted as a class of behavioral immunity that prevents diseases  
227 spreading among nestmates [9,20,23]. However, we showed that simply being within the

228 nest reduces the fitness of the specialized parasitic fungus *O. camponoti-rufipedis* to zero,  
229 whether the nest is inhabited by ants or not. It may be that the removal of corpses and,  
230 more importantly, dying in social isolation (outside the nest) actually increases the  
231 opportunity for the parasite to complete its development and be transmitted to the next  
232 host. From the perspective of the colony, the ability of nestmates to destroy cadavers  
233 before the fungus can become infectious means that remaining inside the nest might  
234 better serve the colony compared to ants dying in social isolation outside the nest where  
235 fungal growth can occur. The same may apply for generalist pathogens, such as  
236 *Metarhizium* (used in the majority of studies on social immunity), that have a broad range  
237 of hosts [24]. These do not necessarily need to be transmitted from ant to ant and can be  
238 transmitted to others insect groups outside the nest.

239 Not all parasites of ants use the same strategy of manipulating the host as  
240 *Ophiocordyceps* that we studied. To place our results within the wider context of  
241 parasites evolved to infect ant societies we examined the mode of transmission for other  
242 specialized parasites of ants (Table S1). It was striking that, as with *Ophiocordyceps*, the  
243 majority of parasites of ants require the infected ant to leave the nest to continue the life  
244 cycle. Social isolation mediated by parasites may be a widespread strategy in parasites  
245 that attack ant societies. These parasites only can only be within the nest when they are  
246 invisible to the nestmates, internal to the infected ant body. The life stage that requires  
247 them to either exit from or protrude from the host body occurs outside the nest, where the  
248 social immunity does not act.

249 Although social immunity is present in insect societies such as the ants studied  
250 here, and does function to prevent disease transmission within the nest, our full

251 appreciation of it may not be wholly realized because to date we have been biased by  
252 studies that have focused solely on ant behavior towards diseases inside the nest. But as  
253 we showed and as is reflected in the literature (Table S1) most parasites of ants transmit  
254 outside the nest. To date, studies of social immunity have not considered the importance  
255 of the environment outside the nest for transmission and diseases dynamics. By mapping  
256 the disease *in natura*, we were able to graphically simplify the environment without  
257 reducing or eliminating any component of the system, making it possible to study disease  
258 dynamics for an insect society. In what is the first survey of a specialized disease of an  
259 ant colony in a rainforest we established that disease is a permanent feature (across 20  
260 months) and it is present in 100% of the examined colonies (17 total).

261 ***The terminal host model of transmission.*** Social immunity is effective and prevents  
262 disease transmission within the nest. From a host-centric view this would appear to  
263 provide an advantage to the host within the assumed arms race between the two parties.  
264 However, we offer an additional viewpoint. The fungus *O. camponotini-rufipedis*, infects  
265 susceptible hosts (foraging ant workers) by means of large curved spores that fall directly  
266 down from the cadavers attached to leaves that will be picked up by a new host [14].  
267 Foraging for food is an indispensable task for the colony and workers must perform this  
268 task. Typically, it is a very risky job carried out by older workers, which are going to die  
269 sooner [25, 26]. In this scenario, where the older ants collect the food supplies required by  
270 the colony, there is a constant turnover of new susceptible ants on trails. We expect that the  
271 proportion of infected ants within a colony is low, since not all workers forage. As we have  
272 shown, the foragers are constantly being killed by the parasite, and new workers will take  
273 over the risky tasks that are done outside the nest, providing a continual stream of new  
274 hosts for the parasite that sits right on the doorstep of the colony. Probably over the long

275 term such a strategy has impact on the host demography and social interactions, although  
276 evidence remains lacking on this important question.

277 Targeting a specific group within a population or group of cells within a body is a  
278 widespread strategy in antagonistic interactions. For instance, many predators attack  
279 weak prey, which include old, sick and young individuals. These are easier to capture as  
280 they occupy peripheral positions on outside of the herd or simply lag behind in chases  
281 and because of their weak status are undefended. Considering within-body host-parasite  
282 interactions the papillomavirus uses the strategy of a high reproductive rate in terminal  
283 cells, which is considered advantageous because there is no immune surveillance in such  
284 cells [27]. This virus, which is transmitted by contact, forms warts on the most external  
285 surface of the host body - skin, enabling the transmission to a new potential host [27]. The  
286 trematoda *Euhaplorchis californienis*, a killifish parasite, has also evolved the strategy of  
287 making its host occupy external positions within a group: by changing host swimming  
288 behavior, the parasites increases the probability of predation which is advantageous as the  
289 parasite reaches its final host – the avian predator [28]. We suggest that the specialized  
290 parasite *O. camponotini-rufipedis* (and other *Ophiocordyceps* species infecting ants)  
291 specifically targets older individuals from ant societies and causes them to die on the  
292 doorstep of the colony. The advantage is that the parasite does not need to evolve  
293 mechanism to overcome the effective social immunity that occurs inside the nest, and at  
294 the same time, it ensures a constant supply of susceptible hosts.

295 An option for the host would be extending the social immunity to the outside nest  
296 environment. There are anecdotal observations of ants removing fungal manipulated and  
297 killed cadavers from the environment. In the Amazon rainforest, the turtle ant,  
298 *Cephalotes atratus*, which is arboreal, removes the cadavers from the bark of trees [29].

299 The wood ant, *Formica rufa*, which inhabits grasslands, remove the cadavers on the  
300 doorstep of the colony, surrounding their nest [30]. It would be of great interest to test  
301 how far out from the colony social immunity can extend. In the 20 months of fieldwork  
302 we did not see any ants destroying or removing the cadavers attached to leaves  
303 surrounding the nest, leading us to suspect that they do not display the same defensive  
304 behavior around the nest as they do inside. It is likely difficult or costly for ants to control  
305 the outside environment, where *O. camponoti-rufipedis* is strategically positioned.  
306 Although social immunity does not occur outside the nest in this case, it might be  
307 possible for adaptive changes in ant behavior to reduce the disease burden. The species of  
308 ant we studied builds their foraging trails using bridges and this might function to reduce  
309 contact with the soil and establish the permanent use of the same pathway, both of which  
310 might decrease the risk of infection [17,31]. We do know examples of how the foraging  
311 trail network of ants adaptively shift in response to changing food abundance [32,33] or  
312 to reduce the incidence of attack by predators [34,35,36] or competing colonies [37,38].  
313 There are also examples of trails shifting in presence of parasitoid females that lay eggs  
314 in workers [39-41]. Also, the presence of *O. unilateralis* in Thailand was suspected of  
315 causing the target ant, *Camponotus leonardi* to reduce the time spent near the forest floor  
316 [13]. Generally, ant trail behavior and its response to parasites are neglected but through  
317 our focus on within forest parasite-host dynamics we hope to encourage such work.  
318 However, because foraging ants tend to be older there may simply be little selection on  
319 the host to evolve strategies against the parasite. If this is the case then host and parasite  
320 may not be involved in a coevolutionary arms race (as is commonly assumed) at all. They  
321 may both be following quite stable evolutionary strategies.

322 **Conclusion.** The concept of the social insect colony as a “factory constructed inside a  
323 fortress” [42] does present a challenge for parasites “breaking into the fortress” [6]. The  
324 highly evolved social immunity system is the first line of defense that until now appeared  
325 highly effective. Such was the supposed efficacy that early genome studies were quick to  
326 point out honeybees had 1/3 the immune genes of other insect because behavior was  
327 considered so important, obviating the need for humoral immunity [43]. But the  
328 existence of many specialized parasites of ant societies (Table S1) demonstrates parasites  
329 can and do transmit despite this collective defense. Our focus on a co-evolved specialized  
330 parasite in a complex tropical rainforest environment has highlighted a weakness in what  
331 is an otherwise effective barrier: workers need to leave the confines of the nest to collect  
332 food. This means susceptible hosts are predictable in both time and space and parasites  
333 have evolved to exploit this. As we emphasized throughout, the view that insect societies,  
334 such as ants, are paragons of effective collective defense against disease transmission  
335 [44] has largely been developed from studies conducted under artificial conditions. We  
336 have shown that within the complex arena of a rainforest, specialized diseases of ants  
337 have evolved effective methods to constantly transmit to new hosts by controlling worker  
338 ants to die on the doorstep of the colony. Since the majority of specialized parasites do  
339 require a transmission step outside the colony (Table S1), we would expect they also  
340 exploit the vulnerability of colonies where workers must leave to forage. Taken all the  
341 results together this implies that while social immunity is effective within the nest, it does  
342 not function against specialized parasites because they have evolved strategies to transmit  
343 outside the nest, consequently not encountering the social immunity.

344

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350

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466 **Figure Legend:**

467

468 **Figure 1: 3D maps of foraging trail and monthly-infected ants surrounding ant**  
469 **colonies in Atlantic rainforest, Brazil.** The infected ants represent accumulated dead  
470 ants in 7 months (Dec 2010-May2011 and Jan 2012). Distinct colors represent different  
471 months. The lines show trails were recorded in December 2010. (A) Nest A. (B) Nest B.  
472 (C) Nest C. (D) Nest D.

473

474 **Figure 2: Disease dynamics surrounding four nests of *Camponotus rufipes* in an**  
475 **Atlantic rainforest fragment, Brazil.** Different lines represent each analyzed nest (A, B,  
476 C or D). The numbers show the total of dead ants recorded for each of the six months.

477

478 **Supplementary Figures Legend:**

479

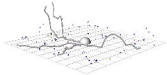
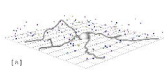
480 **Figure S1: *Camponotus rufipes* ants infected by *Ophiocordyceps camponoti-***  
481 ***rufipedis*.** (A) Ant recently killed by the specialized parasite *Ophiocordyceps camponoti-*  
482 *rufipedis*. (B) Mature *O. camponoti-rufipedis* stage, suitable to transmission. The arrow  
483 points to the frutification body from where the spores are shot. (C) Collected ant recently  
484 killed by the fungus parasite before on the experiment. Fungal presents initial  
485 development (arrow). (D) Same sample after 10 days inside the host nest. The fungal did  
486 not developed as it normally does outside the next (arrow).

487

488 **Movie 1: Spatiotemporal dynamics of the specialized fungal parasite attacking an**  
489 **ant colony across six consecutive months.** The data was collected in Atlantic rainforest,  
490 southeastern Brazil. The fungal parasite species is *Ophiocordyceps camponoti-rufipedis*  
491 that attacks the ant host *Camponotus rufipes*. The red dots represent the new ants killed  
492 by the parasite in the respective month. The grey dots represent the sum of dead ants  
493 from previous months. The red lines represent the forage trail on the ant host for each  
494 studied month.

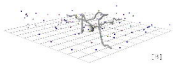
495

496 **Table S1: Overview of co-evolved parasites in ant societies.** Transmission can be  
497 between ants (direct) or also include another host (indirect). The final environment,  
498 where the sexual reproduction of the parasite occurs, can be in the environment  
499 surrounding the nest (Outside the nest), within the colony (Inside the nest) or final host  
500 (Vertebrate host). The effect of parasitism is often death of the infected, either directly  
501 attributable to the parasite (Direct death), or indirectly via a behavioral change that leads  
502 to the host being eaten by the final host (Predation) or jumping in water, to allow the  
503 parasite to enter water for mating (Drowning). Additional details of each group in  
504 Schmid-Hempel (1998) [6].

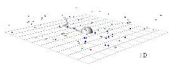


(C)

December 2018 ●  
January 2019 ●  
February 2019 ●  
March 2019 ●  
April 2019 ●  
May 2019 ●  
January 2020 ●



(E)



(G)



