

1 **Title:**

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

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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 2,000m² each (100x20m), covering
122 44,000m². 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 2,000m² 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^3 (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 ($x=500\text{cm}$,
145 $y=500\text{cm}$) and on the forest floor ($z=0\text{cm}$). 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

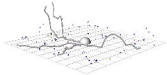
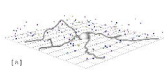
480 **Figure S1: *Camponotus rufipes* ants infected by *Ophiocordyceps camponoti-***
481 ***rufipedis*.** (A) Ant recently killed by the specialized parasite *Ophiocordyceps camponoti-*
482 *rufipedis*. (B) Mature *O. camponoti-rufipedis* stage, suitable to transmission. The arrow
483 points to the frutification 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 ●

