Hindawi Publishing Corporation
Psyche
Volume 2015, Article ID 364967, 4 pages
http://dx.doi.org/10.1155/2015/364967

Hindawi

Research Article

The Ant *Cardiocondyla elegans* as Host of the Enigmatic Endoparasitic Fungus *Myrmicinosporidium durum*

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Received 25 March 2015; Accepted 4 June 2015

Academic Editor: Abraham Hefetz

Data on host species and the distribution of the endoparasitic fungus *Myrmicinosporidium durum* increased continuously in recent decades. Here, we add the ant *Cardiocondyla elegans* as new host species. Colonies of the monogynous species were found infested in the region of Languedoc-Roussillon (South France). Samples from the nest indicate high infection rates. All castes and sexes were infected by the spores. Variations of infection rates between sampling methods and species are discussed.

1. Introduction

Ant-infesting parasitic fungi have been studied by scientists for more than a century. While the life cycles and transmission have been revealed for some fungal parasites, the endoparasitic fungus *Myrmicinosporidum durum* is only incompletely understood [1].

M. durum was first discovered in workers of *Solenopsis fugax* and *Temnothorax tuberum* and described as a haplosporidian protist [2, 3]. Only much later it was identified as a fungus [4]. Research over the last decades has broadened our knowledge about host species and global distribution but still little is known about its life cycle [3–5]. Up to now, almost 40 host ant species of the subfamilies Myrmicinae, Formicinae, and Dolichoderinae have been described [6, 7]. Infections have been reported from Western Europe to Asia and also from America (see [6]).

In France, *M. durum* has been reported first in 1982 by Espadaler and to date it has been found parasitizing four host species: *Temnothorax recedens* [8], *T. unifasciatus* [8], *Pheidole pallidula* [9], and *Solenopsis fugax* [9]. Generally, infested ants contain plenty of dark brown spores, which are lensoid in shape and measure up to 65 μm in diameter [4, 10]. Due to the characteristic shape of the spores, Hölldobler [3] described the infection as "Näpfchenkrankheit" (small bowl sickness). Spores initially occur in the gaster and later extend to other parts of the body [6, 7, 10] but never infest vital organs. Infected individuals therefore can still be long-lived [3, 4]. Most infected individuals were captured in pit fall traps or by hand in the field, indicating that infected workers forage. In addition, *M. durum* has been found in young swarming queens of *S. fugax* and a male of *P. pallidula* [11]; that is, both castes and both sexes of ants can be infested [10, 11] and at least workers and swarming queens continue to show their typical caste-specific behavior [3, 10]. The absence of detectable negative effects on the numerous hosts [3, 10] was interpreted as evidence for a long common evolutionary history of host and parasite [12].

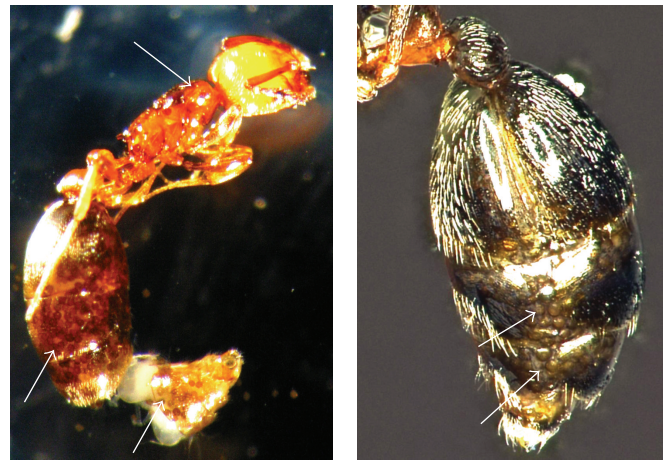
Here, we report on the first finding of *M. durum* in workers, queens, and males of the ant *Cardiocondyla elegans* at the banks of Le Gard du Rhône, France. Colonies of the ant *C. elegans* are small with less than 500 workers, one queen, and in summer also numerous female sexuals and a few wingless (ergatoid) males [13, 14]. In our study area they nest in cavities in the soil. Sexuals mate in late summer in their natal nest and after hibernation young mated queens disperse on foot to found new colonies nearby [13, 14]. Our addition of a new genus to the already quite extensive list of host species supports the hypothesis that *M. durum* is a nonselective generalist with a large host range species [5–7].

2. Material and Methods

Study samples were collected at six different sites in the region of Languedoc-Roussillon (South France) from 19 to

Table 1: *Myrmicinosporidium durum* (endoparasitic fungus) infection rates in populations, colonies, and individuals of the ant *Cardiocondyla elegans*.

	Population	Number of colonies	Number of infected colonies	Colony infection rate (%)	Number of dead individuals	Number of dead infected individuals	Individual infection rate (%)
	C1	8	5	62,5	279	43	15,4
	C2	3	3	100,0	74	24	32,4
	C3	8	3	37,5	223	24	10,8
	C4	5	3	60,0	205	24	11,7
	R1	9	1	11,1	486	10	2,1
	R2	9	1	11,1	216	1	0,5
Total	6	42	15		1483	126	



(a) (b)

Figure 1: Male (a) and gaster of a worker (b) of *Cardiocondyla elegans* infected with spores of the endoparasitic fungus *Myrmicinosporidium durum*. Spores are easier visible in the lightly pigmented male compared to the dark worker.

28 September 2014 (Remoulins, R1: N43°56′31.1″ E4°33′33.91″; R2: N43°55′45.296″ E4°34′4.883″; Comps, C1: N43°50′38.314″ E4°37′0.134″; C2: N43°50′56.364″ E4°37′10.326″; C3: N43°51′9.889″ E4°37′2.363″; C4: N43°49′34.561″ E4°38′16.858″). Nests were located in moist and earthy habitats with grass patches and one site also contained sandy soil. Colonies were located by following foragers to the nest entrance. Nests were excavated, and ants were collected with an aspirator and transferred to the laboratory in Regensburg, where they were housed in plastic trays in incubators at 12 h light/12 h dark cycles. Temperature was gradually decreased from 18°C/13°C (day/night) to 15°C/10°C (day/night) and finally to 12°C/8°C (day/night) until mid-December and maintained for three weeks to simulate a short hibernation period. Afterwards, temperature was again gradually increased until mid-January (20°C/16°C day/night) and remaining queens and workers were separated for colony founding experiments. Colonies were fed regularly twice per week with honey and fruit flies or parts of cockroaches.

Colonies were scanned twice per week and all dead ants were stored in 100% ETOH and subsequently investigated under a microscope. Infections were detected through the cuticle of dead ants with a microscope (magnification 35x). The dark spores of *M. durum* are easily detected in lightly pigmented species but hardly visible by eye in dark species [6, 7, 12, 15]. Female sexuals and workers have a dark coloration, while wingless (ergatoid) males, typical for *Cardiocondyla*, are yellowish [13, 14]. Hence, infections are much easier to recognize in males than in the female castes of *C. elegans* (see Figure 1).

3. Results and Discussion

Infected ants were found in all six investigated populations and in 15 of a total of 42 colonies (= 36.6%; for details see Table 1). Both, males and females (all castes) were found infested (see Figure 1). In most cases, spores were restricted to the gaster, but in several cases they also infested thorax and head. In our study, most of the infected individuals reared in the laboratory died before or during hibernation. After hibernation, only 4 of 137 (= 2.9%) dead ants were infected

with various developmental stages of the spores (observation period: mid-January until mid-March).

Our data suggest a rather high infection rate of *C. elegans* with the endoparasitic fungus *Myrmicinosporidum durum*. In previous studies, infection rates ranged between 0.17% and 66% [11, 15]. Furthermore, infection rates differ among species; for example, *Solenopsis fugax* had a much higher infection rate than *Temnothorax tuberum* [3]. This has been explained by the different colony size and behavior of the two species; in *S. fugax*, colonies are larger and, therefore, more workers are active and more likely to pick up spores and distribute them within the colony. In addition, workers of *S. fugax* are very active below ground, which may spread the fungal spores and cause a higher infection rate in the colony compared to the less active genus *Temnothorax* [3]. Generally, different infection rates may vary drastically, even within populations, dependent on the life cycle and the respective environmental conditions [6, 8], making it difficult to compare infection rates between and within species.

Interestingly, *C. batesii*, a close relative of *C. elegans*, was never infected although living in an area with other heavily infested ant species [6]. At the moment, we can only speculate about possible reasons. For example, colonies of *C. elegans* are larger and, hence, more individuals might forage and/or dig new nest chambers in the soil and thereby gather spores. Moreover, the special life history of *C. elegans* with an exchange of sexuals between colonies [13] might facilitate the spread of spores among colonies. Finally, different mechanisms of food filtration in the infrabuccal pocket of the ants might explain the interspecific variation in susceptibility [4, 16].

Unfortunately, it is still unclear whether *M. durum* is "[···] a generalist microparasite and not a mixture of differentiated host lineages" as suggested by Gonçalves and colleagues [6] or rather a fungus-complex [15] as it infests so many diverse ant subfamilies which are distributed over several continents [6, 7].

The increased mortality shortly after collection might be due to stress during excavation of the nest and transportation [15]. In contrast, under constant conditions, no reduction of lifespan has been reported [3, 4]. This finding is also supported by our study, as after hibernation, only a few infected individuals died.

4. Conclusion

Our study increases the geographic distribution and host range of *M. durum*. As the study species *C. elegans* can be easily maintained in the laboratory, it might be well suited for further investigations on the parasite's life cycle and transmission.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors thank three referees for their helpful comments to improve an earlier version of the paper.

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