Journal of Invertebrate Pathology 136 (2016) 74–80

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Distribution of the myrmecoparasitic fungus *Rickia wasmannii* (Ascomycota: Laboulbeniales) across colonies, individuals, and body parts of *Myrmica scabrinodis*

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ARTICLE INFO

Article history:
Received 10 June 2015
Revised 7 September 2015
Accepted 9 March 2016
Available online 9 March 2016

Keywords:
Ants
Body part specificity
Infection intensity
Laboulbeniales
Parasitism
Prevalence

ABSTRACT

The ant species *Myrmica scabrinodis* plays a markedly important ecological role through much of the humid grasslands of Eurasia. It hosts a species-rich community of pathogens and parasites, including *Rickia wasmannii*, an enigmatic member of entomoparasitic laboulbenialean fungi. This study provides a descriptive ecology of *R. wasmannii* by characterizing its prevalence and distribution across several hierarchical levels: colonies, individuals, and anatomic body parts. Infections were restricted to a single ant species, *M. scabrinodis*, and infected colonies occurred predominantly in wet habitats. Infections tended to be highly prevalent within infected colonies, often reaching 100% sample prevalence among workers. Individual infections exhibited an aggregated distribution typical to host-parasite systems. Workers from the aboveground part of nests (presumably older ones acting as foragers) were more infected than those from the belowground part. Fungal thalli could be found all over the body of the hosts, the head and the abdomen being the most infected parts of the body. The fungi's distribution among host body parts statistically differed between low versus high-intensity infections: the initial dominance of the head decreased with advancing infection. These findings may provide baseline data for future comparative or monitoring studies.

1. Introduction

Pathogens and parasites constitute only a small proportion of the total biomass. However, they exert a major influence on every life form, making parasitism a very successful way of life (Hudson et al., 2006). Among their potential host organisms, eusocial insects, and ants in particular, offer a promising nutrient source, as they globally represent a huge amount of biomass and live together in highly aggregated groups of genetically homogenous individuals (Schmid-Hempel, 1998). Not surprisingly, ants have developed a plethora of anti-parasitic defenses that act both at individual and colony levels. They produce fungicidal secretions, practice auto- and allogrooming, pathogen avoidance, nest hygiene, carcass removal, and exclusion or emigration of infected individuals from the colonies (Schmid-Hempel, 1998; Poulsen et al., 2002; Fernández-Marín et al., 2006; Roy et al., 2006; Walker and Hughes, 2009; Heinze and Walter, 2010; Nielsen et al., 2010; Walker and Hughes, 2011; Konrad et al., 2012; Csata et al., 2014).

While some ant parasites have become iconic due to their ability to manipulate host behavior or their aesthetic beauty (myrmecophilous butterflies offer a good example of both, see Thomas and Settele, 2004; Witek et al., 2014), unfortunately, the ecology of less charismatic ant pathogens and parasites, such as microscopic fungi, is not well understood. This creates a major gap in our ecological thinking, because dominant ant species often participate in particularly strong interspecific interactions. They also structurally alter their habitat (the soil) and thus act as keystone species (Mills et al., 1993) and as ecosystem engineers (Folgarait, 1998; Underwood and Fisher, 2006), while at the same time they host various fungal parasites.

Rickia wasmannii is a myrmecophilous fungal symbiont that is widespread in Europe (Espadaler and Santamaria, 2012), including Central-Eastern Europe (Csata et al., 2013). Though usually considered non-pathogenic to its primary host, *Myrmica scabrinodis*, it recently was demonstrated to exert certain levels of virulence

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http://dx.doi.org/10.1016/j.jip.2016.03.008
0022-2011/

(Csata et al., 2014; Báthori et al., 2015). However, we still do not have basic information on its prevalence and distribution.

Our aim in this study is to provide information on the prevalence and distribution of this fungus across different spatial scales, namely colonies, individuals, and body parts of individuals. Moreover, we compare infection levels between different habitat types (wet versus dry) and also between different parts of the infected colonies (aboveground versus belowground) to determine whether infected ants occur under specific environmental conditions or in specific age or task classes of ants residing in different parts of the nest (e.g. older individuals and foragers, who are usually located on the outer perimeter). Throughout our inquiry, we only take into consideration infection among members of the worker caste. The general assumption of our study is that in order to obtain a fairly accurate view of a parasite's distribution within eusocial hosts one needs to extend investigations to several different levels of organizational hierarchy.

2. Materials and methods

2.1. Study species

The order Laboulbeniales (Ascomycota) contains entomoparasitic fungi (Santamaria, 2001; Espadaler and Santamaria, 2012), including *Rickia* species that parasitize mites (Acari), millipedes (Diplopoda), mole crickets (Orthoptera: Gryllotalpidae), beetles (Coleoptera) and ants (Weir and Blackwell, 2005). *R. wasmannii* Cavara (1899) is the most common among the myrmecophilous *Rickia* species in the Holarctic (Santamaria and Espadaler, 2015), and it obligatorily exploits *Myrmica* ants (see Espadaler and Santamaria, 2012; Csata et al., 2013; Witek et al., 2014 for reviews). Like other Laboulbeniales, this fungus has no mycelium and thus the thallus develops from a bicellular ascospore, while only sexual stages are known (Haelewaters, 2012). The thalli attach to the outer layer of the cuticle and appear on the surface of the hosts as clubbed setae-like structures under the stereomicroscope. Highly infected hosts appear to be unusually 'hairy' even to the naked eye. Infections are usually regarded as neutral (García et al., 2010; Espadaler and Santamaria, 2012), though recent studies have demonstrated increased allogrooming, increased water-consumption, and reduced longevity of infected ants (Csata et al., 2014; Báthori et al., 2015). *R. wasmannii* has been reported in many European countries and in several *Myrmica* host species, but its primary host is *M. scabrinodis* Nylander, 1846 (Tartally et al., 2007; Espadaler and Santamaria, 2012; Haelewaters, 2012; Csata et al., 2013; Haelewaters et al., 2015). *M. scabrinodis* is a widely distributed Euro-Siberian ant species inhabiting moderately humid open habitats. It tolerates high soil moisture but needs high solar insolation and thus often occurs in peat bogs in the temperate region. Nests are mostly built in the ground, in grass or in moss tufts. Colonies are monogynous or have only a few queens, and they contain up to 2500 workers (Radchenko and Elmes, 2010).

2.2. Study sites and period

Collections were carried out at two locations in Cluj County, Romania (Luna de Jos: N 46.921961, E 23.734032, 430 m a.s.l., and Fânațele Clujului: N 46.842599, E 23.641898, 550 m a.s.l.) from April 17 to June 03, 2010. Both sites are meadows of northern exposure of >20 ha, consisting of a mosaic of meso-xeric and wet patches, which clearly differed based on their vegetation; e.g. the presence of *Molinia caerulea* was characteristic for moist patches. The first site near Luna de Jos is mostly covered by meso-xeric grasslands (dominated by *Festuca rupicola*, *Brachypodium pinnatum*, *Agrostis tenuis*, *Poa angustifolia*) rich in dicotyledonous species (e.g. *Dorycnium herbaceum*, *Filipendula vulgaris*, *Salvia pratensis*). Wet patches within the grassland are dominated by *Festuca pratensis*, *M. caerulea*, *Calamagrostis epigeios* or *Poa pratensis*, with *Serratula tinctoria*, *Cirsium rivulare*, *Sanguisorba officinalis*, *Iris sibirica* and *Pastinaca sativa* as characteristic species. This area was traditionally used as a hayfield and pasture. The other site at Fânațele Clujului is a meso-xeric basiphilous grassland dominated by *F. rupicola*, *B. pinnatum*, *Elymus hispidus*, *Agrostis capillaris*, *Carex michelii*, and a high representation of *F. vulgaris*, *Adonis vernalis*, *S. pratensis*, *Clematis recta*, *Plantago media*, *Lotus corniculatus* and *Trifolium montanum*. A mesic vegetation type appears in small wet pits embedded within this grassland, in which *Sanguisorba officinalis*, *M. caerulea*, *Iris sibirica* and *Scirpus sylvaticus* are frequent. This site is mowed occasionally, and the surrounding areas are intensively grazed by sheep.

2.3. Sampling methods

Since *R. wasmannii* is known to prefer wet host habitats (Csata et al., 2013), we subdivided both sites into 'wet' versus 'dry' habitats prior to collections on the basis of vegetation characteristics (see previously). Thus patches dominated by the purple moor-grass *M. caerulea*, a grass known to prefer habitats with high water table and humid conditions, were labelled 'wet', while surrounding meso-xeric meadows lacking this species and associated plants were handled 'dry' habitats. Several sampling patches (circles of 2 m radius used generally for *Myrmica* species [see Elmes et al., 1998] as known host ants of *R. wasmannii*) were established randomly within each habitat type, located >2 m from one another in order to ensure independent sampling. We (BM, EK, EN, ZC) searched systematically for ant nests (whatever the species) in these patches and collected workers from each nest in order to confirm the ant species specificity of the fungus. At Luna de Jos, 5 and 6 sampling patches were selected in wet versus dry habitats, while 5–5 wet versus dry patches were chosen at Fânațele Clujului. We collected a mean of 26.66 (SE ± 1.75, N = 92 nests) individuals by *Myrmica* spp. nests and a mean of 6.86 (SE ± 0.57, N = 72 nests) individuals per nest for other ant species, all in vials filled with 96% ethanol.

In order to determine the within-nest localization of infected ants, we also sampled the upper (aboveground part, called solaria) and the lower part of the nest (the belowground part, around the brood chambers) separately in 18 randomly selected infected *M. scabrinodis* colonies in the wet habitat patches at Luna de Jos. In this case, we used a 30× hand magnifying glass to identify the species and infection status of colonies in the field. Infected ants are easy to recognize for the myrmecologist, as they appear unusually hairy.

All collected samples were screened for fungal thalli using an Olympus SZ51 stereomiscroscope at 80× magnification in laboratory conditions. Ants were identified at the species level with the use of various keys (Seifert, 2007; Czechowski et al., 2012; Czekes et al., 2012) in laboratory conditions with the same stereomicroscope.

2.4. Statistical measures and analyses

2.4.1. Colony-level measures

The colony-level prevalence of the fungus was calculated as the proportion of infected colonies among all *M. scabrinodis* colonies examined. Each colony which contained at least one infected individual with at least one mature thallus on the cuticle was considered infected. Sterne's method was applied to construct confidence intervals (Sterne, 1954; Reiczigel, 2003). Fisher's exact test was used to compare colony-level prevalence between the two sites and then between different habitat types (wet versus dry).

2.4.2. Within-colony measures

Within-colony prevalence was expressed as the proportion of infected individuals among all individuals in a sample representing a particular colony. Uninfected individuals were excluded from all further analyses.

In order to quantify the intensity of infection (number of thalli/host individual), random sub-samples of infected ant workers were taken from all infected nests, and the number of fungal thalli were counted on the right side of each individual (N = 527, mean 12.25 ants/nest, SE ± 0.84) separately for each major body part (head, antennae, thorax, 1st, 2nd and 3rd legs separately, petiole and postpetiole together, and abdomen) with an Olympus SZ51 stereomicroscope at 80× magnification, while an ocular micrometer was used to set the axial line through the ant's body to separate the right and left sides. We applied Poulin's (1996) discrepancy index (the most widespread index to quantify levels of parasite aggregation) to characterize the distribution of fungi among host individuals.

In order to establish whether there is a within-colony spatial bias in infection intensity, sub-samples of infected workers from the aboveground (mean 8.83 ants/nest, SE ± 0.15), and from the belowground (mean 8.61 ants/nest, SE ± 0.27) parts of the nests were taken into account separately in case of the 18 colonies (N = 314 ants) in which collections were spatially divided. Poulin's discrepancy index was used to characterize the distribution of fungi among host individuals. The Generalized Linear Mixed Model approach (GLMM, negative binomial, maximum likelihood) was applied to compare infection intensities between the aboveground and belowground subsamples: location was included as factor, while colony code was introduced as a random factor to handle dependencies.

2.4.3. Within-individual measures

The differences in the intensity of infection among body parts of the hosts were tested with GLMM (negative binomial, maximum likelihood, N = 527). As mentioned above, the number of fungal thalli on the right side of each individual was taken into account. Colony code and individual ID were introduced as nested random factors. All body parts were considered separately (see above). To assess potential changes of the distribution of thalli, we created an index (thalli on the head/thalli on the abdomen), since the head and the abdomen were the two most heavily infected body parts (see below). Then we explored the relationship between this index and the total number of thalli (right sides only). Only individuals which carried at least one thallus both on the head and on the abdomen were included in the analysis (N = 487).

Statistical procedures were carried out using Quantitative Parasitology 3.0 (Rózsa et al., 2000) and the R 3.1.1 Statistical Environment (R Development Core Team, 2014). GLMMs were performed using *glmer.nb* function in *lme4* package (Bates et al., 2014), while the exact significance values of input variables were retrieved with the use of *Anova* function in *car* package (Fox and Weisberg, 2011). *Relevel* function was used in order to carry out sequential comparisons among factor levels when performing GLMM analyses in case of body part specificity. We applied table-wide sequential Bonferroni-Holm correction to reveal the exact significance levels among different factor levels in these cases. Whenever relevant, statistical significance (p) refers to two-sided probabilities, and confidence intervals (CI) refer to 95% probabilities.

3. Results

3.1. Colony-level comparisons

Eleven ant species were collected altogether (Table 1), including three *Myrmica* species that are potential hosts to *R. wasmannii*.

Table 1
A list of ant species and the number of their colonies occurring at the two study sites in wet versus dry habitats. The number of *Rickia wasmannii*-infected colonies (if any) are given in brackets.

Species and sites	Fânațele Clujului		Luna de Jos	
	Wet	Dry	Wet	Dry
Formica rufibarbis Fabricius, 1793	0	0	0	2
Lasius alienus (Förster, 1850)	7	0	1	5
Lasius flavus (Fabricius, 1782)	4	1	5	7
Lasius niger (Linnaeus, 1758)	5	20	0	2
Lasius paralienus Seifert, 1992	0	0	2	3
Myrmica gallienii Bondroit, 1920	15	0	0	0
Myrmica scabrinodis Nylander, 1846	11 (5)	0	49 (35)	16 (2)
Myrmica schencki Viereck, 1903	1	0	0	0
Solenopsis fugax (Latreille, 1798)	0	2	0	3
Tapinoma subboreale Seifert, 2012	0	0	0	2
Tetramorium cf. *caespitum*	1	0	0	0

However, only *M. scabrinodis* was infected (Table 1). Out of 76 *M. scabrinodis* nests examined, 42 were infected, thus colony-level prevalence was 0.55 (CI: 0.44–0.67) (Table 1). Since the prevalence was similar between the two sites (QP3.0, Fisher's exact test, $p = 0.53$), we united the two datasets. Colonies in wet habitats were significantly more likely (0.67, CI: 0.53–0.78) to harbor infection than colonies in dry habitats (0.13, CI: 0.02–0.37) (QP3.0, Fisher's exact test, $p < 0.0001$). This was not a side-effect of *M. scabrinodis*' general preference for moist conditions, however, as infected *M. scabrinodis* colonies preferred wet habitats over uninfected ones (Fisher's exact test, $p < 0.001$).

3.2. Comparisons of within-colony measures of infection

Once we excluded uninfected colonies, within-colony prevalence varied from 0.03 to 1.00 (mean 0.79, SD ± 0.26) among the 42 infected colonies. The only 2 infected colonies of dry habitats did not exhibit markedly different prevalences (0.60 and 0.80) from infected colonies of wet habitats, however, the low number in the former category disallowed any statistical comparisons.

Maximum intensity on the right side of individuals was 439 thalli, but the majority (76.85%) of infected individuals bore <100 thalli (Fig. 1). The distribution of fungi among infected hosts showed a clearly aggregated pattern, as indicated by Poulin's discrepancy index (D = 0.52). Since the number of infected individuals from dry habitats was too low (N = 15) compared to those coming from wet habitats (N = 512), no statistical comparisons could be reliably made.

Within-colony prevalence showed clear spatial bias within nests. Infections exhibited a slightly more aggregated frequency distribution in the belowground samples according to the index of discrepancy ($D_{above} = 0.478$ and $D_{below} = 0.501$). The GLMM analysis also indicated that infected individuals from the belowground part of the colony bore significantly less fungal thalli than those from the aboveground solaria (GLMM $t = -3.25$, $p < 0.001$; Fig. 2).

3.3. Comparisons of within-individual measures of infection: distribution across body parts

R. wasmannii was present on the surfaces of all major body parts, from the mandibles and antennae to the abdomen, and in some extreme cases even the eyes were invaded. Its frequency distribution showed a bias to the head and abdomen in particular (Fig. 3). Significant differences were revealed among all body parts in the number of fungal thalli with the exception of the three legs that carried infections similar to one another. As we were unable to measure the surface areas of different body parts, we could not determine whether the detected pattern differed from the one

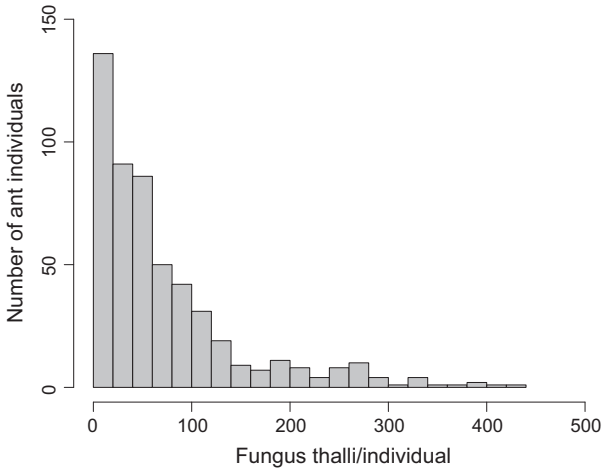

Fig. 1. The distribution of ant individuals among infection classes based on the number of fungal thalli counted on the right side of each individual.

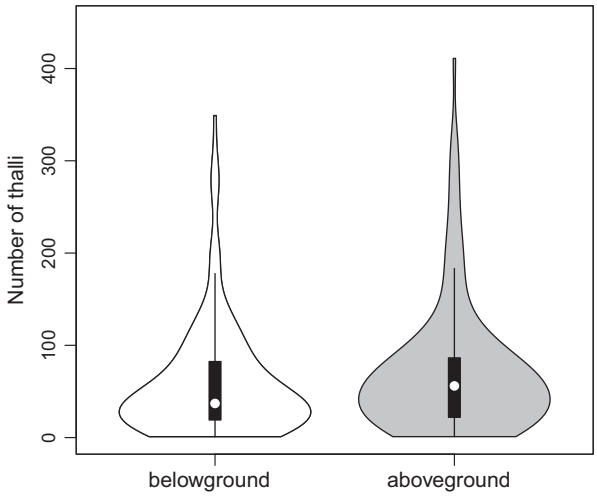

Fig. 2. Differences in the intensity of infection between ant workers from belowground, and aboveground parts of the ant nests (median, quartiles, min-max values).

expected by chance. The proportion (thalli on the head/thalli on the abdomen) was significantly negatively influenced by the intensity of the infection ($F = 4.36$, $R^2 = 0.0089$, $p = 0.03$; Fig. 4).

4. Discussion

The natural history of *R. wasmannii* and – speaking more generally – of myrmecoparasitic Laboulbeniales fungi is rather poorly understood compared to our understanding of Laboulbeniales fungi that parasitize other insects (e.g. De Kesel, 1996). In this study, infections were restricted to a single host species, *M. scabrinodis*, and infected colonies were mostly concentrated in moist habitats. Individual infections exhibited the aggregated (biased) distributions typical of host-parasite systems. Within infected colonies, workers collected from the belowground part of nests carried less fungi than those collected from the aboveground solaria. The distribution of fungi among host body parts statistically differed between low-intensity versus high-intensity infections. Since the present study is the first to outline a descriptive ecology for the occurrence of *R. wasmannii* in natural habitats and across wide ranges of hierarchical levels (habitats, colonies, individuals, body parts), we can make no comparisons with other studies of this myrmecoparasitic fungi (we can only draw comparison with research carried out on other Laboulbeniales). Rather, we hope that our findings will provide baseline data for future comparative or monitoring studies.

Our results support the view according to which the primary host of *R. wasmannii* is *M. scabrinodis*, at least in the wider study region. Other studies (e.g. Espadaler and Santamaria, 2012; Haelewaters, 2012; Csata et al., 2013; Haelewaters et al., 2015) have also shown that the fungus is restricted to *Myrmica* species. Thus it is not surprising that co-occurring ant species from other genera were not infected. Fungal infection, when it occurred, was demonstrable in the case of other species, in spite of the fact that fewer individuals were collected. The relatively high number of colonies sampled helped compensate for this, as did the available data from previous field studies (see Csata et al., 2013). Alternatively, the low number of *M. gallienii* and *M. schencki* colonies (15 and 1) that were found may also explain why these species appeared to be free of infection. In the larger region, former studies have shown *M. gallienii* to be an occasional host (see Csata et al., 2013), but *M. schencki* has never been found to be infected (see Witek et al., 2014).

Several *Myrmica* species are known to bear *R. wasmannii* infection in the wider region (Csata et al., 2013), so we have to consider the possibility that the host specificity observed here could be mediated by environmental conditions, as already proven in the fungus *Laboulbenia slackensis* ectoparasite of ground beetles (De Kesel, 1996). Tragust et al. (2015) also demonstrated that, despite its rather strict host specificity manifested in the field, the myrme-

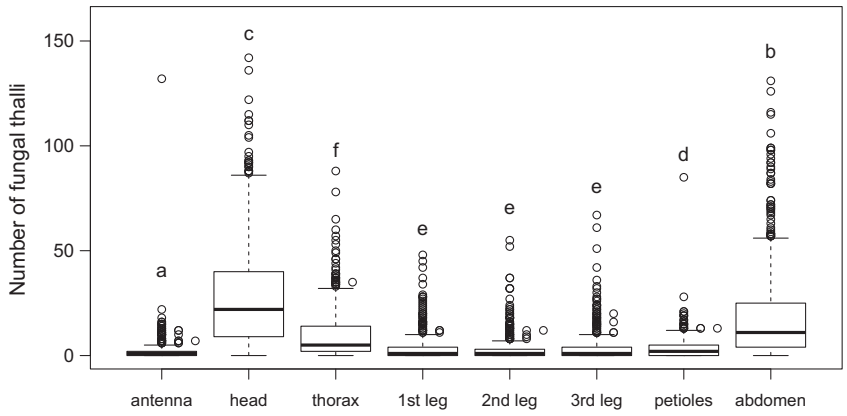

Fig. 3. Infection intensity on different body parts of ant workers (median, quartiles, min-max values) (GLMM, $\chi^2 = 7538.3$, $p < 0.0001$). Different letters indicate significant differences among groups ($t \geqslant 3.72$, $p < 0.001$).

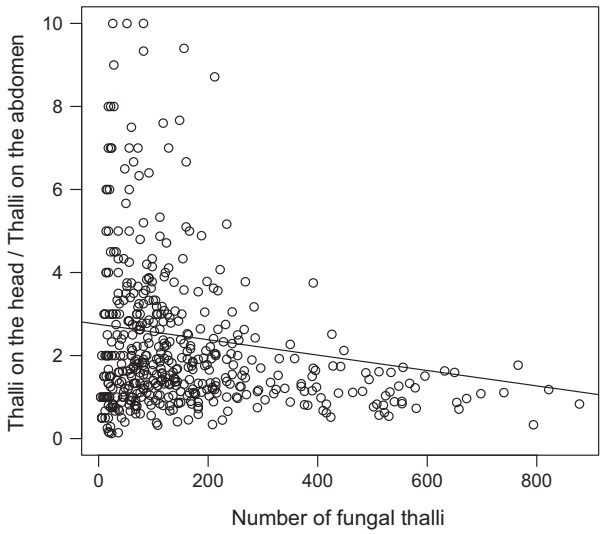

Fig. 4. Relationship between the proportion of thalli on the head/thalli on the abdomen and the total number of thalli based on the number of fungal thalli counted on the right side of each individual.

coparasitic *Laboulbenia formicarum* can infect other closely-related ant species under appropriate laboratory conditions. According to the study of De Kesel (1996), suitable soil type is one of the major underlying factors that ensures successful transmission of and infection with *L. slackensis*. Structural properties of the soil, its composition, and probably its interaction with humidity, along with the appropriate physiological and anatomical features of the host, determine the persistence of this species (De Kesel, 1996). A close relationship between a parasite and its host should not tempt us to forget that a parasite may still display its own environmental preferences (De Kesel, 1996). The moist habitat type studied here, which is also suitable for *M. scabrinodis*, appears to match the habitat conditions needed by the fungus. Most probably, the interaction of soil properties with microclimatic conditions is the key to success for *R. wasmannii* in our case as well. Quite a number of closely related entomoparasitic laboulbenialean fungi tend to be restricted to insects living in wet habitats (e.g. De Kesel, 1993, 1996; Sugiura et al., 2010), and in the wider study region all known 11 *R. wasmannii* populations were found in wet meadows as well (e.g. Csata et al., 2013; pers. obs.). The fact that *M. gallienii* was not infected in our samples despite its known host status (Csata et al., 2013), while Haelewaters et al. (2015) found in the Netherlands that *Myrmica sabuleti*, which displays a preference for drier conditions, can be more infected by this fungus than *M. scabrinodis*, all likely indicate that *R. wasmannii* has regional variations in host specificity that are likely mediated by both host and environmental conditions.

Other Laboulbeniales fungi are also known for high prevalence on their hosts. *Hesperomyces virescens* can infect up to 95% of adult *Harmonia axyridis* (Kamburov et al., 1967; Riddick et al., 2005; Harwood et al., 2006; Nalepa and Weir, 2007), and the myrmecoparasitic *L. formicarum* can infect >80% of ants in the colony (Konrad et al., 2015; Tragust et al., 2015). Nevertheless, data on the prevalence of *R. wasmannii* (see e.g. García et al., 2010) was scarce, and there has been no information on the intensity of infection until now.

Data on the epidemiology of *R. wasmannii* bears a conservational relevance as well, since its host ant *M. scabrinodis* also nurses caterpillars of the socially parasitic *Maculinea* butterflies that are strictly protected all over Europe (see Witek et al., 2014). The reduced lifespan of infected host ants (Csata et al., 2013) might be relevant for the protection of *Maculinea*, since it could negatively influence the survival rate of parasitic *Maculinea* caterpillars as well.

The high prevalence and infection intensity of *R. wasmannii* within infected colonies documented by us could be a consequence of the fungus' low virulence combined with an efficient transmission strategy. The same strategy appears to characterize *L. formicarum*, which obtained a high prevalence and infection intensity in *Lasius neglectus* supercolonies within a decade (Tragust et al., 2015). Direct contact between hosts has already been demonstrated to be a major route of transmission for the related *L. slackensis* parasitizing carabid beetles (De Kesel, 1993, 1996), and for the myrmecoparasitic *L. formicarum* (Tragust et al., 2015) as well. Consequently, we presume that *R. wasmannii* may also rely on bodily contacts for transmission primarily among nestmates, but the importance of non-nestmate or even allospecific encounters cannot be ruled out, from this perspective (e.g. De Kesel, 1993, 1996; Tragust et al., 2015).

In ants, the secretion of several exocrine glands (e.g. metapleural gland, venom gland) is a highly efficient weapon in the fight against fungal infections (Poulsen et al., 2002; Fernández-Marín et al., 2006; Reber et al., 2011; Otti et al., 2014). Therefore, we hypothesize that *R. wasmannii*, like other myrmecoparasitic Laboulbeniales fungi, must be capable somehow of breaking this defensive line to obtain high infection intensity.

Within the ant nests, a spatial bias in the distribution of a Laboulbeniales fungi was documented for the first time in *R. wasmannii*. In ants, young workers are known to occur more often in the central part of nests around larval chambers (in our case, the belowground portion of the nest), while older and thus more experienced workers that act mostly as foragers are restricted to the outer perimeters (the aboveground level in our case) (Hölldobler and Wilson, 1990). Perhaps this difference is mirrored in our finding that the latter part of the colony is characterized by heavier (more advanced?) levels of infection. Lapeva-Gjonova and Santamaria (2011) showed that *R. wasmannii* was absent on lightly pigmented workers which were probably recently eclosed, but young carabids also show lower levels of infection with *L. slackensis* (De Kesel, 1993). As in *Myrmica* ants the young gynes spend several days in this aboveground part of the colony (among the more infected workers) before the nuptial flight (Radchenko and Elmes, 2010; pers. obs.) and queens often carry infection as well (Tartally et al., 2007; pers. obs.), it is fair to assume that perhaps they can acquire infective spores here before their mating flight, thus enhancing the transmission of the parasite either into a newly founded colony or to a preexisting colony that is adopting a new queen.

Several entomoparasitic laboulbenialean fungi have been shown to be more or less specific to certain body parts of the hosts (Benjamin and Shanor, 1952; Scheloske, 1976; Arndt and Desender, 2002; Garcés and Williams, 2004; Riddick and Schaefer, 2005; Harwood et al., 2006). In contrast, *R. wasmannii* appear to invade the host body surface as a whole, although some body parts may be affected more frequently and more dramatically than others. Indeed, rough data indicate that the head and abdomen are by far the most infected. This pattern, however, might have been the result of several different factors. First, these are the body parts (in addition to the thorax) with the largest surface areas, thus a random distribution of thalli would most probably yield the same result. Having no reliable information on the surface areas of each body part, we do not claim that this in itself proves a deviation from an expected random pattern. Therefore, we also showed that the proportion (thalli on the head/thalli on the abdomen) exhibits a highly significant dependence on intensity. In cases of light infections, the head is proportionally more infected than the abdomen, while in cases of heavier infections the dominance of infection of the head diminishes. This is the first evidence

so far that *R. wasmannii* exhibits some kind of non-random distribution pattern among body parts.

This pattern may arise due to several different factors. First, the low-intensity infections may be relatively new, and presuming that frequent head-to-head contacts (e.g. due to trophallaxis) are a major route of within-colony infections, one can expect that these infections would be more focused on the head. Second, spore attachment success may differ across body parts and also depend on infection intensity. Finally, differences in ant grooming and allogrooming activities across body parts and intensity levels may also cause deviation from random distribution across the host body surface, since the head is less accessible for autogrooming.

Overall, *M. scabrinodis* is an ant species abundant in a large proportion of humid grasslands all over Europe. We hope that the hierarchically structured epidemiological information outlined above may serve as a baseline for future comparative or monitoring studies, and we hope our inquiry will contribute to a better understanding of the ecology of *R. wasmannii* and laboulbelian fungi as a whole.

Acknowledgments

We are grateful for the help of Monica Hughes, who provided assistance with the identification of the fungus, and for the contributions of Annamária Fenesi and Eszter Ruprecht, who helped us characterize the habitats. The authors are indebted to Zsófia Szőke and Anna-Mária Szász-Len for their considerable help in the course of the fieldwork. The critical comments of Simon Tragust, and of two anonymous reviewers, and the linguistic corrections of Thomas Cooper helped us improve the manuscript considerably. During preparation of the manuscript, Bálint Markó's work was supported by the Bolyai János scholarship of the Hungarian Academy of Sciences. Katalin Erős and Enikő Csata benefited from scholarships provided by Collegium Talentum (Budapest, Hungary). The work of Katalin Erős and Enikő Csata was possible due to the financial support of the Sectoral Operational Program for Human Resources Development 2007–2013, co-financed by the European Social Fund under project no. POSDRU/159/1.5/S/132400, bearing the title "Young successful researchers professional development in an international and interdisciplinary environment". Enikő Német was supported by a scholarship in the framework of the POSDRU/159/1.5/S/133391, entitled "Doctoral and postdoctoral excellence programs for training highly qualified human resources for research in the fields of Life Sciences, Environment and Earth". Lajos Rózsa was supported by the Grant OTKA K108571 from the Hungarian National Research Fund.

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