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

Article Type: Full length article

Keywords: ants; body part specificity; infection intensity; Laboulbeniales; parasitism; prevalence

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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, Myrmica 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.

Title:

Distribution of the myrmecoparasitic fungus *Rickiawasmannii* (Ascomycota: Laboulbeniales) across colonies, individuals, and body parts of *Myrmicascabrinodis*

Running title:

Prevalence and distribution of *Rickiawasmannii* on ants

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Abstract

The ant species *Myrmicascabrinodis* 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 *Rickiawasmannii*, an enigmatic member of entomoparasiticlaboulbenialean 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, *Myrmicascabrinodis*, 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 above-ground part of nests (presumably older ones acting as foragers) were moreinfected than those from the below-ground part. Fungal thalli could be found all over the body of the hosts, the head and the abdomen being the most infected body parts. The fungi's distributionamong host body parts statistically differed between low- versus high-intensity infections: the initial dominance of the head decreased with advancing infection. These results may provide baseline data for future comparative or monitoring studies.

Keywords: ants, body part specificity, infection intensity, Laboulbeniales, parasitism, prevalence

No. of pages: 15

No. of figures: 4

No. of tables: 1

We are grateful for the reviewers' comments, they helped us improving the manuscript considerably. We addressed every question and carried out all the requested corrections. More specifically, we clarified all those aspects that were not addressed by us in the required manner in the previous version of the manuscript (e.g. methodological details in the Materials and Methods), and eliminated one GLMM analysis since the dataset behind it was very unbalanced, and the results of the analysis where anyhow non-significant. We added a more detailed paragraph on host specificity to the discussion and generally corrected all mistakes and errors that were mentioned by the reviewers. In the end the general message of the manuscript has not changed. A detailed list of responses was also prepared further on.

Detailed answers

REVIEWER #1:

General comments

R: The 'entomopathogenicity' role of Laboulbeniales in the genus Rickia is not clear, so this word should be carefully used in sentences as "an enigmatic member of entomopathogeniclaboulbenialean fungi". Nevertheless, I think that the biological interaction between Laboulbeniales and hosts has scientific relevance for fitting in the scope of the Journal of Invertebrate Pathology.
A: Indeed, generally, it is not yet clear to what extent Laboulbeniales fungi are pathogenic. Therefore we changed everywhere in the text pathogenic to parasitic when referring to Laboulbeniales. However, at least in *Rickiawasmannii* just recently some studies have demonstrated reduced longevity of infected ants and other behavioural effects as well, that lead us to conclude that a mild pathogenicity could be exerted from the part of the fungus (see Csata et al., 2014; Báthori et al., 2015). Such negative effects are also documented in other Laboulbeniales fungi: according to Riddick (2010) laboulbenian infection reduces the winter survival of *Harmonia axyridis*, while other Laboulbeniales species could reduce the mobility of their hosts (Gemeno et al., 2004), and decrease their lifespan (Strandberg and Tucker, 1974; Gemeno et al., 2004).

R: The major problem is related to the sampling methodology and methodological description. It should be more clear and subdivided by every analysis the authors performed: colony-level; within-colony infection; within-individuals; to clearly understand how the study was carried out. Especially the analysis about the habitat influence, wet vs dry, it is clear that the dry habitat influences the proportion of ant colonies, but not influencing the infection.
A: We sub-divided the methodological section and also relocated some sentences in order to have a more clear structure. As for the influence of the habitat conditions see our responses later on.

Specific comments:

R: Although English is good, it should be revised, some grammatical errors are consistent over the manuscript and some sentences are a bit confused. Example: "In most cases (e.g. all but 1 of the 11 known sites in Romania), its only or at least its primary host is *M. scabrinodis* (Csata et al., 2013)."
A: The manuscript has been carefully revised and corrected again. The sentence mentioned by the reviewer has been corrected as follows: "*Rickiawasmannii* 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)."

R:Abstract - should be revised in order to include the revised suggestions
A: We corrected the abstract. Thus we reformulated the reference to body part specificity and corrected it to "The fungi's distribution among host body parts..." Also, we applied other smaller corrections, but the message of the abstract did not change, since the basic results remained unchanged after the correction of the manuscript.

Highlights

R: The proportion of infected ant colonies is higher in wet habitats than in dry ones. - Is this really supported by this work? or simply, the proportion of colonies is higher in wet habitats?
A: Yes, it is supported statistically as well. We performed a Fisher's exact test to confirm this. Thus we added an explanatory sentence on this matter to the end of the first paragraph of the 3.1. subchapter of the Results. This part of the paragraph is as follows: "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, $p< 0.001$)."

R: We provide the first evidence of body part specificity in R. wasmannii. - Does it has specificity for body parts? It grows in all parts of the ant, including in the eyes! I think this highlight should be carefully rewritten!
A: Based on our analysis the head of an infected ant is more infected in case of light infections, than in advanced stages of infection. But indeed the emergent pattern is not an evidence for body part specificity in the narrow sense. Thus we corrected everywhere in the text the wording and now refer to this pattern as a specific distribution pattern among body parts. As we state in the Discussions: "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 *Rickiawasmannii* exhibits some kind of non-random distribution pattern among body parts." However, the fungus indeed occurs on other body parts of the host, thus whether this specificity in the pattern occurs due to some sort of body part preference (probably not) or due to other ecological-behavioural factors (e.g. due to increased frequency of head-to-head contacts in ants) (more probably) that remains to be studied. This problem is treated though in the discussion. In order to avoid confusion we reformulated the highlight point as follows: "The distribution of *R. wasmannii*shows biases among body parts."

Introduction

R: Line 56: "Our aim here is to provide a descriptive ecology of this species, or more specifically," - not clear!
A: Corrected as follows: "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."

R: Line 61-64: "The general assumption beyond our study is that one can obtain a fairly accurate view of a pathogen's distribution within eusocial hosts only by extending investigations to several different levels of organizational hierarchy, in parallel with each other." - Does this sentence makes sense?
A: Corrected. Now the sentence is as follows: "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."

Material and methods

R: Line 91: "2.2. Study sites and periods" - periods?
A: Corrected to "period"

R: Line 93-94: "from 17.04 to 03. 06., 2010" - correct date format
A: Corrected to "from April 17 to June 03, 2010"

R: Line 110: "to prefer wet host habitats" - what is a "wet host habitat"?;"we sub-divided both sites into wet versus dry habitats prior to collections." - how?
A: Indeed, we missed to specify that. In fact in both cases wet patches were characterized by the exclusive presence of the purple moor-grass *Moliniacaerulea* (and some other associated plants), a species known to prefer high water table, and humid conditions. In order to clarify this we added an explanatory sentence to the beginning of subchapter 2.3., which is as follows: "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 *Moliniacaerulea*, 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"

R: Line 114: how was the species specificity confirmed? Slide preparations? Or just disregarding that other species of ants did not had any Laboulbeniales?
A: There is no need for slide preparations in order to confirm the presence of *R. wasmannii*. The fungal thalli is very conspicuous, and, compared to some related myrmecoparasitic fungi, it's quite large (see e.g. photo in graphical abstract). Thus collected samples were screened for fungal infection with the use of an Olympus stereomicroscope in laboratory conditions. In order to clarify this we inserted the following paragraph at the end of the 2.3. subchapter: "All collected samples were screened for fungalthalli 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."

R: Why 5 to 6 patches where selected in first site and in the other site 5-5?
A:The number of infected colonies was very low in dry habitats, and we found them only in one site, the site, where we surveyed 6 plots in dry habitats. We added the 6^{th} plot in order to make sure that the pattern that was emerging from the previous 5 was not an accidental pattern. Since 6plots were surveyed in dry habitats only, where anyhow *M. scabrinodis* occurrence was lower (and that of infected hosts even lower), and not wet (where the opposite pattern was valid), this additional 6^{th} plot did not boost up by any means the data referring to the host *M. scabrinodis*, it merely rounded the composition of the ant assemblage of dry habitats, which is also valuable addition for any further studies inquiring the ecological conditions that infected hosts live in. In the other site no *M. scabrinodis* was found in dry habitats, so no additional survey was thought to be necessary.

R: Line 116: "individuals for" - individuals by?
A: corrected

R: Line 116-117: Why using different numbers? How many colonies of ants have you survey for this study?
A:The number of surveyed ant colonies is featured in Table 1, but we introduced them in the text as well after the featured averages in order to specify the sample size. In all previous publicationson *R. wasmanniiMyrmica* species were mentioned as sole hosts. Also our previous collections confirmed this (see Csata et al. 2013). However, none of these collections were as systematic, as the work presented here. Given these, from the start we knew that we had to concentrate on *Myrmica* species if we want to evaluate infection intensity, but in order to do that higher number of workers was needed, than generally. On the other hand, given the high prevalence of *R. wasmannii* (from own field experience and from unsystematic collections of other authors) we were aware that a lower number of ants was also enough to show datawise whether a species was infected or not combined with a relatively high number of colonies sampled. We refer to this in the 2nd paragraph of the discussion.

R: Line 141: "Ndry = 15 ants" - only 15 ants, compared to Nwet = 512?
A:Indeed, the data structure is very unbalanced, thus makes statistical analysis questionable. Therefore, although the GLMM analysis anyhow yielded non-significant results, we decided to remove this analysis from the manuscript and instead of it we included the following sentence in the Results: “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.”

R: "Site, Sampling patch and colony IDs" - remove caps; ID - for identification?
A: Corrected, caps removed. Colony ID refers to the identification number/code of the colony. We corrected it to “colony code”.

R: Line 151: these are results and should be in the results section of the manuscript!
A: Corrected, we removed the part referring to results.

R: Line 155: in line 135 author´s say that they have count only in the right side of the animal and now they are considering thalli on the head/thalli on the abdomen and total number of fungal thalli - is this only in the right side or total thalli? - please provide clear methodological information.
A: We introduced a clarification in the first part of the paragraph. Thus the paragraph starts now in the following way: “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.” Also in the sentence (later on in the same paragraph) mentioned by the reviewer we added “(right sides only)”.

Results

R: Line 175-176: "In the larger region, former studies have shown M. gallienii to be occasionally infected (see Csata et al., 2013), while M. schencki has never been found to be infected (see Witek et al., 2014)." - this is discussion, not results!
A:Corrected, the sentence has been relocated in the discussion to the end of the second paragraph.

R: Line 192: with only 2 infected colonies in "dry habitat", present only in one of the study sites, how can the statistical analysis be applied and significant?

A:We removed the GLMM analysis, which anyhow yielded non-significant result – see our previous response

R: Figure 1 and 4 – is the total number of thalli concordant in both figures? Does this represents total number of thalli, or thalli counted in the right side of the ant?
A: Corrected. In the figure captions of both figures clarifications have been inserted.
Thus the caption for fig 1. is modified as follows: "**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."
The caption for fig. 4. is modified as follows: "**Fig. 4.** Relationship between the proportion of thalli on the head / thalli on the abdomen and the total number of thallibased on the number of fungal thalli counted on the right side of each individual."

Discussion

R: Line 213: the dot should be a comma?
A: Corrected to comma.

R: Line 213: Is this really statically supported and shown by the present work, or most of the colonies were only in wet areas of the two study sites (one of which didn't had M. scabrinodis colonies in the dry patches)?
A: Yes, it is supported statistically as well. We performed a Fisher's exact test to confirm this. Thus we added an explanatory sentence on this matter to the end of the first paragraph of the 3.1. subchapter of the Results (see in our previous answer referring the one highlight point). Thus the higher prevalence in wet habitats was not merely a side-effect of *M. scabrinodis*' preference for wet habitats, as the distribution of infected *M. scabrinodis* colonies between the two habitats significantly differed from the distribution of uninfected colonies between these two habitats (see in the previous answer at Highlights). *M. scabrinodis* is indeed known to prefer more humid habitats, however, this bias is very pronounced in infected colonies.

R: Line 220: But you can compare with ecological studies performed in other insects, that provided several insights on the ecology of Laboulbeniales, including on the habitat influence…
A: Indeed. We further on treat studies on other Laboulbeniales fungi throughout the Discussion. Here we wanted to simply underline that similar studies are not available for *R. wasmannii*. However, we added a phrase referring to this possibility and now the sentence is as follows: "Since the present study is the first to outline a descriptive ecology for the occurrence of *Rickiawasmannii* 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."

R: Line 235: laboulbeniales should be corrected to Laboulbeniales
A: Corrected

R: Line 244: high infection of Laboulbeniales on non-insects hosts have also been reported…
A: Corrected to: "Other Laboulbeniales fungi are also known for high prevalence on their hosts."

References

R: There is another key reference for the ecological study of Laboulbeniales not cited in the text, that I would recommend to the authors: De Kesel, A. (1996). Host specificity and habitat preference of Laboulbeniaslackensis. Mycologia, 565-573.
A: We thank the reviewer for the suggestion, we cite now the mentioned article.

R: Da Kesel 1993 is not cited in references and Da Kesel 1995 is not cited in the text, although this reference has a different year - please correct!
A: Corrected. The year was erroneously introduced in the reference, the correct year is 1993.

R: Line 342: "And" should be corrected to "Ant"
A: Corrected

References to answers (others than listed in the text)

Riddick E.W. 2010. Ectoparasitic mite and fungus on an invasive lady beetle: parasite coexistence and influence on host survival. Bull. Insectol. 63: 13-20

Gemeno C., Zurek L. and Schal C. 2004. Control of Herpomyces spp. (Ascomycetes: Laboulbeniales) infection in the wood cockroach, Parcoblattalata (Dictyoptera: Blattodea: Blattellidae), with benomyl. J. Invertebr. Pathol. 85: 132-135

Strandberg J.O. and Tucker L.C. 1974. FilariomycesforficulaeShanor occurrence and effects on the predatory earwig, Labidurariparia (Pallas). J. Invertebr. Pathol. 24: 357-364.

REVIEWER #2:

General comments

R: How did you identify the fungus? Is there only one species reported from ants so you could presume it was R. wasmannii. And how did you identify the ants?
A: The fungus was identified by Monica Hughes, specialist in *Rickia*, co-author of our former article in which we report the finding of *Rickiawasmannii* in many Romanian host ant populations, including the two populations that we studied in the frame of this paper. We did not mention this specifically in the manuscript since we refer to the article (Csata et al. 2013). However, now we introduced a sentence in the Acknowledgments: "We are grateful for the help of Monica Hughes, who provided assistance with the identification of the fungus". Indeed, there are more myrmecoparasiticLaboulbeniales species, and currently two more (*Rickialenoirii*, *Laboulbeniacamponoti*) are known as well in Eastern Europe (see mentioned in the text and included in the references). However, these species have hosts with entirely different habitat requirements than our studied habitats, and the fungi also look recognizably different from our species. Ants were identified based on several keys, and we corrected the text accordingly inserting a paragraph to the end of the 2.3. Sampling method subchapter: "All collected samples were screened for fungalthalli 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." Appropriate references were introduced in the References as well.

R: You collected a very low number of workers from most of the nests. Can you be sure that you did

not overlook infection in other species if you only collected less than 10 workers in a nest (especially if the prevalence among individual ants is low)- please address this point in the discussion.
A:We address this point in the discussion in the rewritten paragraphs on host specificity. In all previous publications on *R. wasmanniiMyrmica* species were mentioned as sole hosts. Also our previous collections confirmed this (see Csata et al. 2013). However, none of these collections were as systematic, as the work presented here. Given these, from the start we knew that we had to concentrate on *Myrmica* species if we want to evaluate infection intensity, but in order to do that higher number of workers was needed, than generally. On the other hand, given the high prevalence of *R. wasmannii* (from own field experience and from unsystematic collections of other authors) we were aware that a lower number of ants was also enough to show datawise whether a species was infected or not combined with the relatively high number of colonies sampled.

R: Add a section in materials and methods on how you transported ants to the lab and checked them for thalli distribution.
A: We added to the 2.3 Sampling method subchapter that ants were collected "in vials filled with 96° ethanol", and also added a paragraph to the end of the subchapter on fungus screening and ant identification (see in a previous answer)

R: The discussion on host specificity needs clarification. (line 223 to 239). How could moisture influence the infection and sporulation process (or the spread of the fungus within the colony) of the fungus (if moisture is indeed an important factor?)
A:We rewrote that entire paragraph referring to habitat mediated host specificity and other studies (De Kesel 1996) that specifically treat this question in other Laboulbeniales species.

R: Line 246 Explain how the fungus and its interaction with the ant could be of importance for the Maculinea butterflies
A:We introduced an explanatory sentence, thus the section is as follows: "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."

R: Line 271. Are there any records of Queens being infected or do you just think they could act as vectors?
A: Yes, queens can be infected as well. In fact all infected colonies have at least one infected queen according to our unpubl. data, but in the vast majority of cases all queens of such colonies are highly infected (pers. obs.). There is a publication on queens being infected (Tartally et al. 2007), thus we added this to the references and to the text. Currently the corrected sentence is as follows: "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."

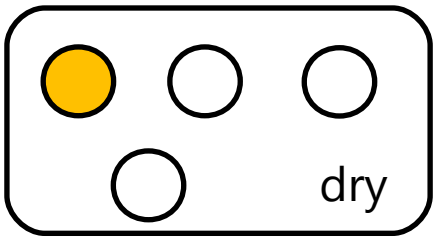
R: Line 271. Would ants invading nests of conspecifics or other ant species have a potential role in the spread of the fungus infection as well?
A:That is a possibility of course, that cannot be ruled out. However, other ant workers very rarely invade other nests, either conspecific or allospecific, unless they are social parasites as slave-maker

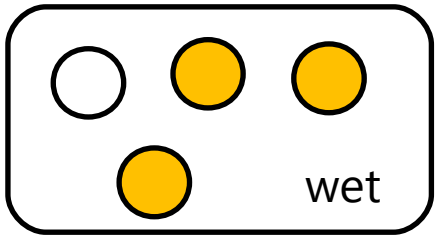
ants (e.g. amazon ants) or inquiline species. *Myrmica* ants, on the other hand, don't have slave-makers to invade them, only inquiline parasites, where parasitic queens infiltrate in the host colony. In the case of our populations it's safe to say that there are not any social parasites using our *Myrmica* species, since we have been continuously conducting studies in that area for many years now. Conflicts escalating between rival workers in the field, outside the colony, specifically between infected and uninfected foragers, could indeed lead to spore transmission. Thus, we added a phrase on this matter to the end of the 3rd paragraph of the discussion, where the matter was treated before: "Direct contact between hosts has already been demonstrated to be a major route of transmission for the related *Laboulbeniaslackensis* 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)."

*Graphical Abstract (for review)

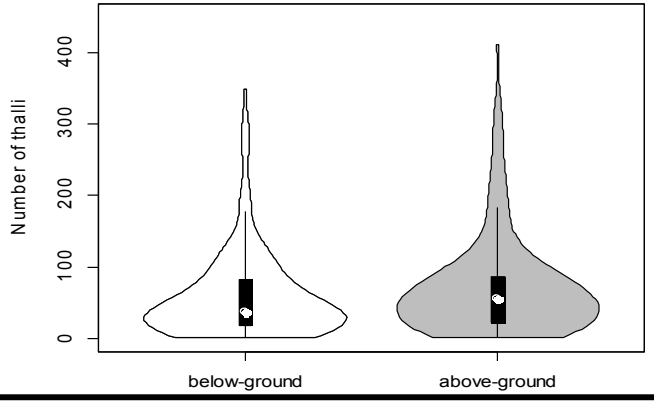
habitats

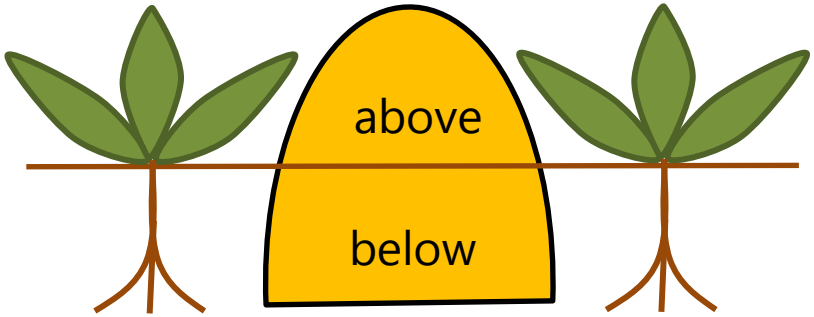
Distribution of *Rickia wasmannii* across several levels



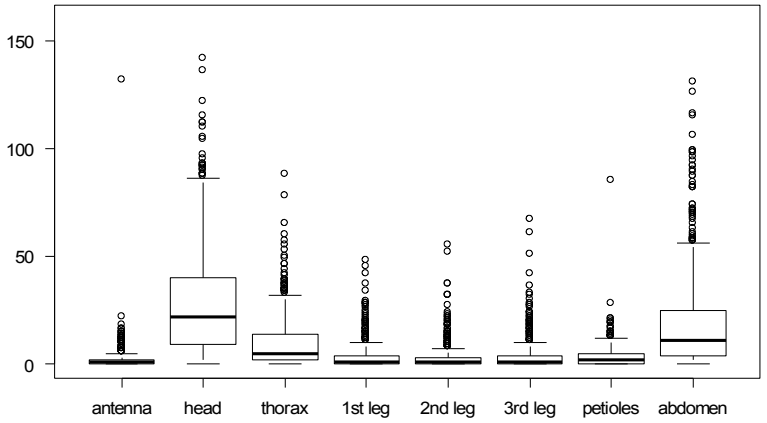


nests





body parts



***Highlights (for review)**

Title:

Distribution of the myrmecoparasitic fungus *Rickiawasmannii* (Ascomycetes: Laboulbeniales) across colonies, individuals, and body parts of *Myrmicascabrinodis*

Authors:Bálint Markó[1,2], Enikő Csata[1] , Katalin Erős[1], Enikő Német[1], Zsolt Czekes[1], Lajos Rózsa[3]

- This is the first study on the distribution of *R.wasmannii* across several levels.
- The proportion of infected ant colonies is higher in wet habitats than in dry ones.
- Ant workers are more infected in the above- than in the below-ground nest portion.
- The distribution of*R.wasmannii*shows biases among body parts.

Title:

Distribution of the myrmecoparasitic fungus *Rickiawasmannii* (Ascomycota: Laboulbeniales) across colonies, individuals, and body parts of *Myrmicascabrinodis*

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Abstract

The ant species *Myrmicascabrinodis* 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 *Rickiawasmannii*, an enigmatic member of entomoparasiticlaboulbenialean 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, *Myrmicascabrinodis*, 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 (presumablyolder 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 findingsmay 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 form of life, making parasitism a verysuccessful 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 manipulatehost 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.

Rickiawasmannii 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, *Myrmicascabrinodis*, it recently was demonstrated to exert certain levels of virulence (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 studyis 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 determinewhether 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 locatedon 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. Materialsandmethods

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). *Rickiawasmannii*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 bicellularascospore, 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 tothe 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). *Rickiawasmannii*has beenreported inmany European countries and inseveral *Myrmica*host species, butits 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). *Myrmicascabrinodis*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 2,500 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 FânațeleClujului: 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 more than 20 ha, consisting of a mosaic of meso-xericand wet patches, which clearly differed based on their vegetation; e.g. the presence of *Moliniacaerulea*wascharacteristic for moist patches. The first site near Luna de Jos is mostly covered by meso-xeric grasslands (dominated by *Festucarupicola,Brachypodiumpinnatum,Agrostis tenuis,Poaangustifolia*) rich in dicotyledonous species (e.g. *Dorycniumherbaceum,Filipendula vulgaris*, *Salvia pratensis*). Wet patches within the grassland are dominated by *Festucapratensis,Moliniacaerulea,Calamagrostisepigeios* or *Poapratensis*, with *Serratulatinctoria,Cirsiumrivulare*, *Sanguisorbaofficinalis*, *Iris sibirica*and*Pastinaca sativa* as characteristic species. This area was traditionally used as a hayfield and pasture. The other site at FânațeleClujului is a meso-xericbasiphilous grassland dominated by *Festucarupicola*, *Brachypodiumpinnatum*, *Elymushispidus*, *Agrostiscapillaris*, *Carexmichelii*, and a high representation of *Filipendula vulgaris*, *Adonis vernalis*, *Salvia pratensis*, *Clematis recta*, *Plantago media*, *Lotus corniculatus*and*Trifoliummontanum*. A mesic vegetation type appears in small wet pits embedded within this grassland, in which *Sanguisorbaofficinalis*, *Moliniacaerulea*, *Iris sibirica* and *Scirpussylvaticus* 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 collectionson the basis of vegetation characteristics (see previously). Thuspatches dominated by the purple moor-grass *Moliniacaerulea*, 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 oneanother in order to ensure independent sampling. We (BM, EK, EN, ZC) searched systematically for ant nests (whatever thespecies) 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 FânațeleClujului. We collected a mean of 26.66 (SE ±1.75, N = 92 nests) individuals

123 by*Myrmica* spp. nests and a mean of 6.86 (SE ±0.57, N = 72 nests) individuals per nest for other ant
124 species, all in vials filled with 96° ethanol.

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

131 All collected samples were screened for fungalthalli using an Olympus SZ51 stereomiscroscope at
132 ×80 magnification in laboratory conditions. Ants were identified atthe species level with the use of
133 various keys (Seifert, 2007; Czechowski et al., 2012, Czekes et al., 2012) in laboratory conditions
134 with the same stereomicroscope.
135

136 2.4. Statistical measures and analyses

137 *(a) Colony-level measures*

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

144

145 *(b) Within-colony measures*

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

149 In order to quantify the intensity of infection (number of thalli/host individual), random sub-
150 samples of infected ant workers were taken from all infected nests, and the number of fungal thalli
151 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 toseparate 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.

(c) 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 *Rickiawasmannii*. 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).Sincethe 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 indry habitats (0.13, CI: 0.02–0.37) (QP3.0, Fisher's exact test, $p < 0.0001$). This was not aside-effect of *M. scabrinodis*' general preference for moist conditions, however, as infected *M. scabrinodis*coloniespreferred 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 less than 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

Rickiawasmannii 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 between all body parts in the frequency 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 determinewhether the detected pattern differed from the one 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 *Rickiawasmannii* and – speaking more generally – of myrmecoparasiticLaboulbeniales fungi is rather poorly understood compared to our understanding of Laboulbenialesfungi thatparasitizeother insects (e.g. De Kesel, 1996). In this study, infections were restricted to a single host species, *Myrmicascabrinodis*,and infected colonies were mostly concentrated in moist habitats. Individual infections exhibited the aggregated (biased) distributions typical ofhost-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 *Rickiawasmannii* in natural habitats and across wide ranges of hierarchical levels (habitats, colonies, individuals, body parts), we can make no comparisons withother 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 *Rickiawasmannii* is *Myrmicascabrinodis*, 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 considerthe possibility that thehost specificity observed here could be mediated by environmental conditions, as already provenin the fungus *Laboulbeniaslackensis*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 myrmecoparasitic*Laboulbeniaformicarum* 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). Aclose relationship between a parasite and its host should not tempt us to forget that aparasite may stilldisplayits 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 neededby 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 entomoparasiticlaboulbenialean 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 inwet meadowsas well (e.g. Csata et al., 2013; pers. obs.).The fact that *M. gallienii* was not infected in our samples despiteits known host status (Csata et al., 2013), whileHaelewaters et al. (2015) found in the Netherlands that *Myrmicasabuleti*, 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 specificitythat are likely mediated by both host and environmental conditions.

Other Laboulbeniales fungi are also known for high prevalence on their hosts.*Hesperomycesvirescens*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*Laboulbeniaformicarum*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 *Laboulbeniaformicarum*, which obtained a high prevalence and infection intensity in *Lasiusneglectus*supercolonieswithin a decade (Tragust et al., 2015). Direct contact between hosts has already been demonstrated to be a major route of transmission for the related *Laboulbeniaslackensis*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-nestmateor even allospecificencounters 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) isa 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 myrmecoparasiticLaboulbeniales fungi,must be capable somehow ofbreaking 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-Gjonovaand 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 *Laboulbeniaslackensis* (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 assumethat 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 entomoparasiticlaboulbenialean fungi havebeen shown to be moreorless 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,*Rickiawasmannii* 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 ofseveral different factors. First, these are the body parts (in addition to the thorax)withthe 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 *Rickiawasmannii* 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-headcontacts(e.g. due to trophallaxis) are a major route of within-colony infections, one can expect that these infections wouldbe 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, *Myrmicascabrinodis* is an ant species abundant ina 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 *Rickiawasmannii* and laboulbelian fungi as a whole.

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Figure captions

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 below-, and above-ground parts of the ant nests (median, quartiles, min-max values).

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 ($t \geq 3.72$, $p < 0.001$).

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.

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

Figure 1.

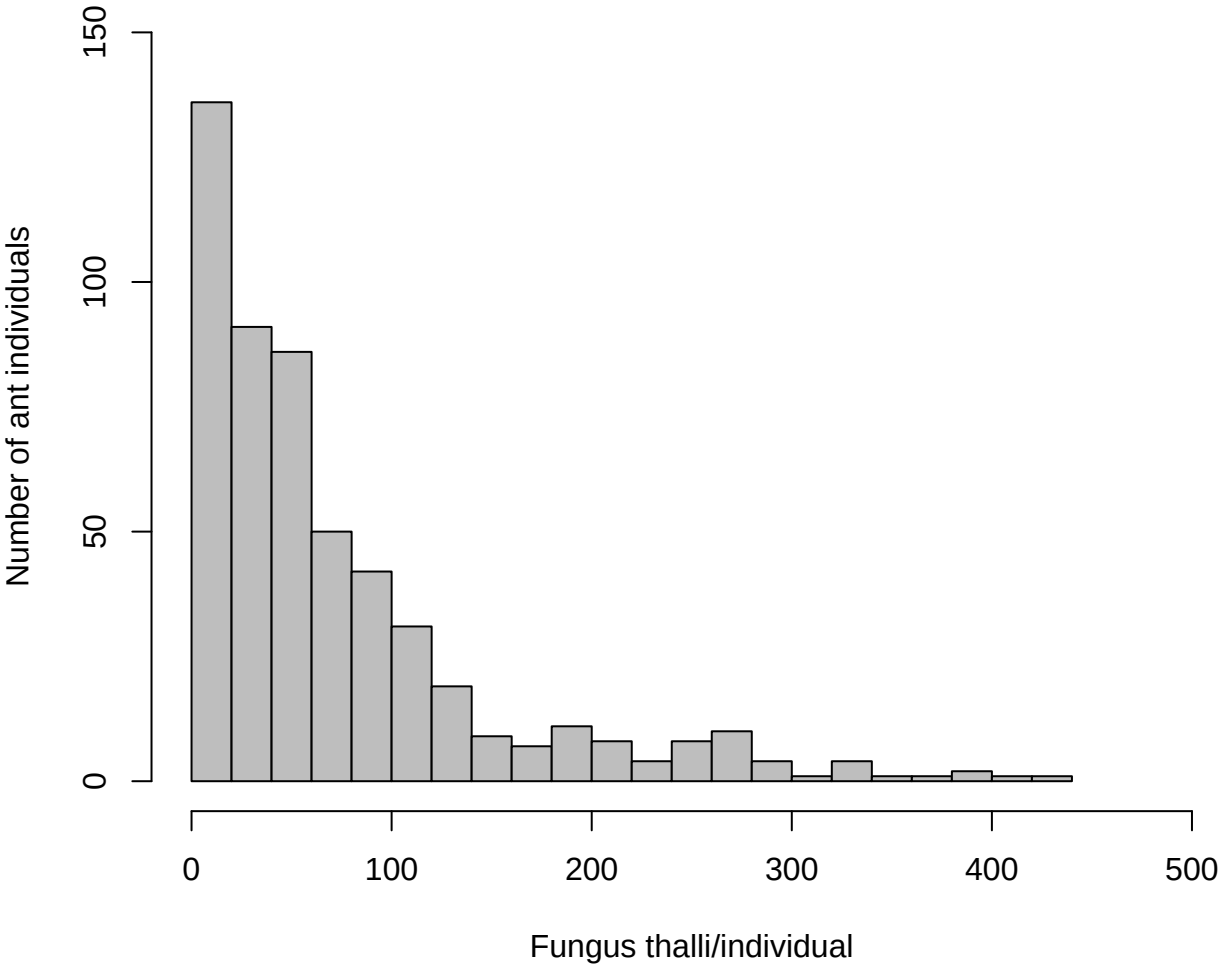

Number of ant individuals
0
50
100
150
0
100
200
300
400
500
Fungus thalli/individual

Figure 2.

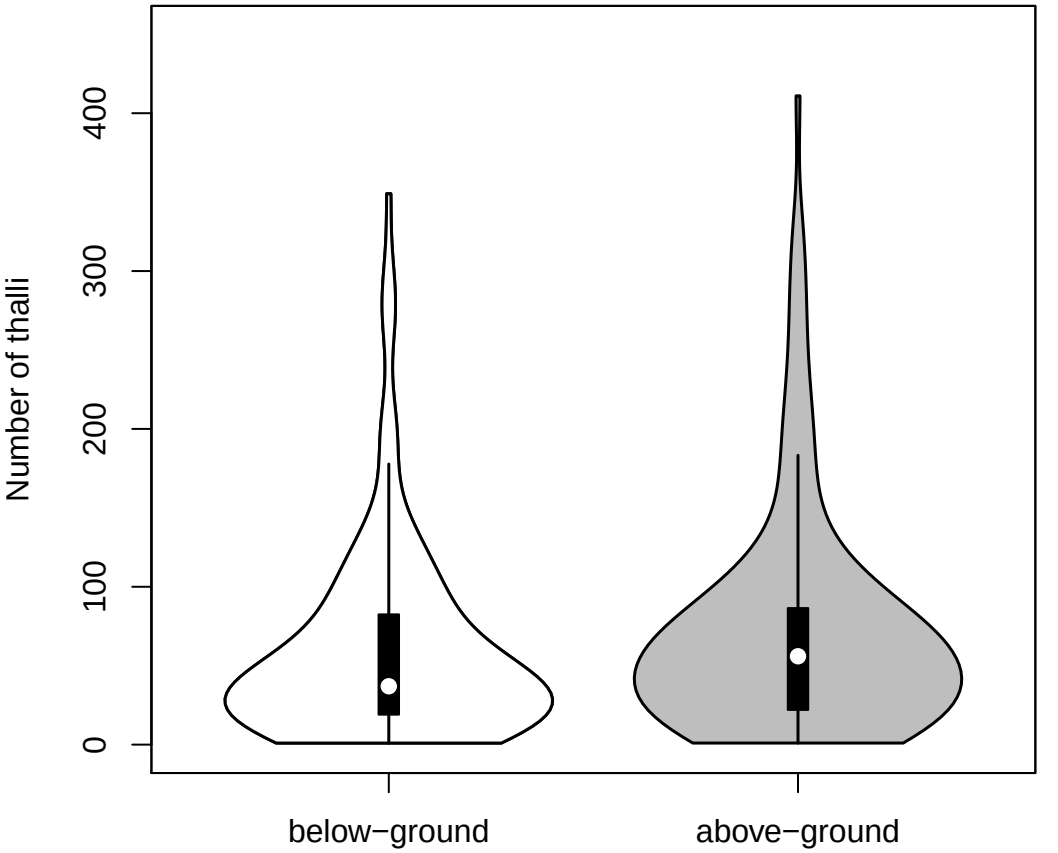


Figure 3.

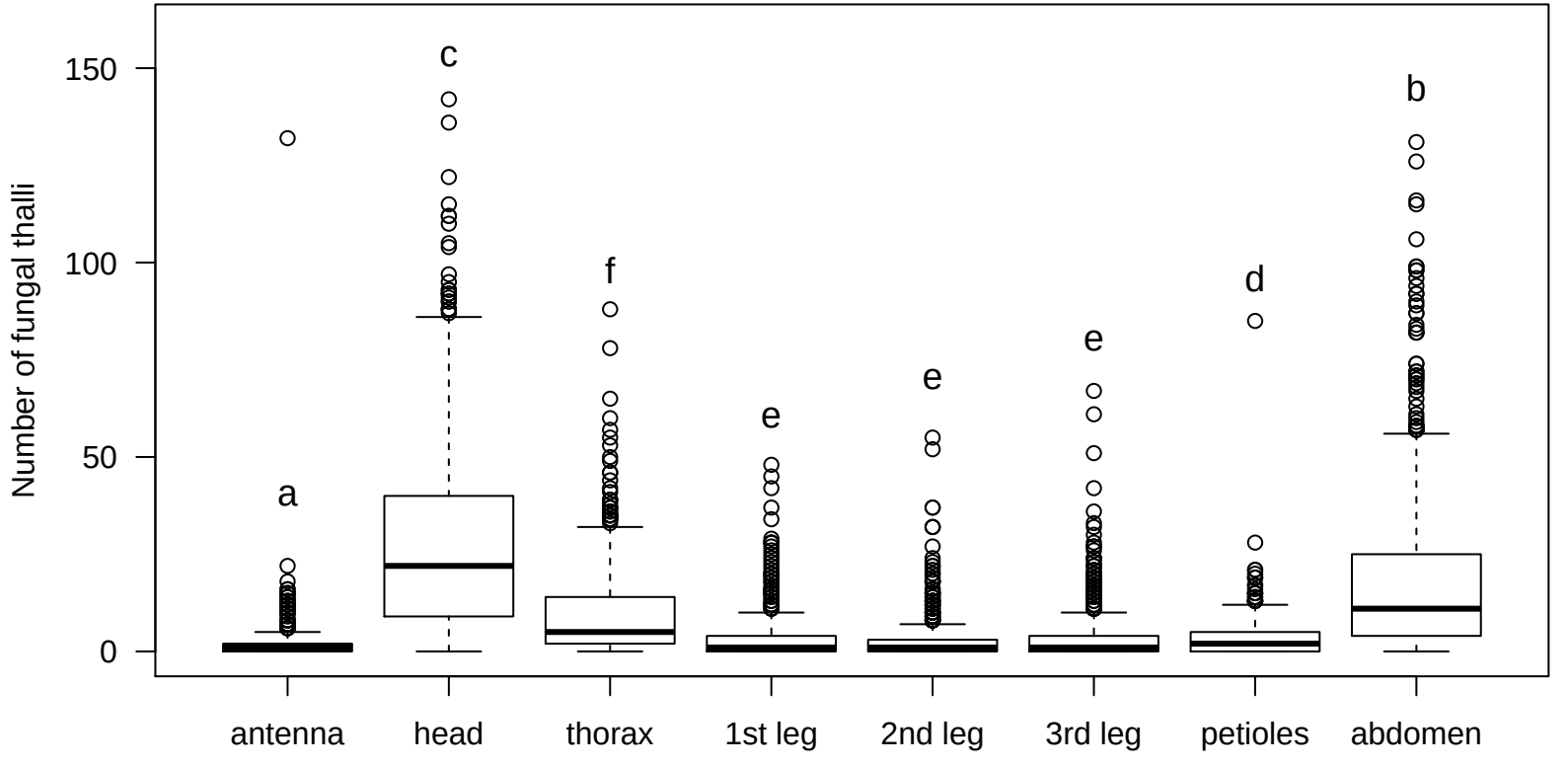
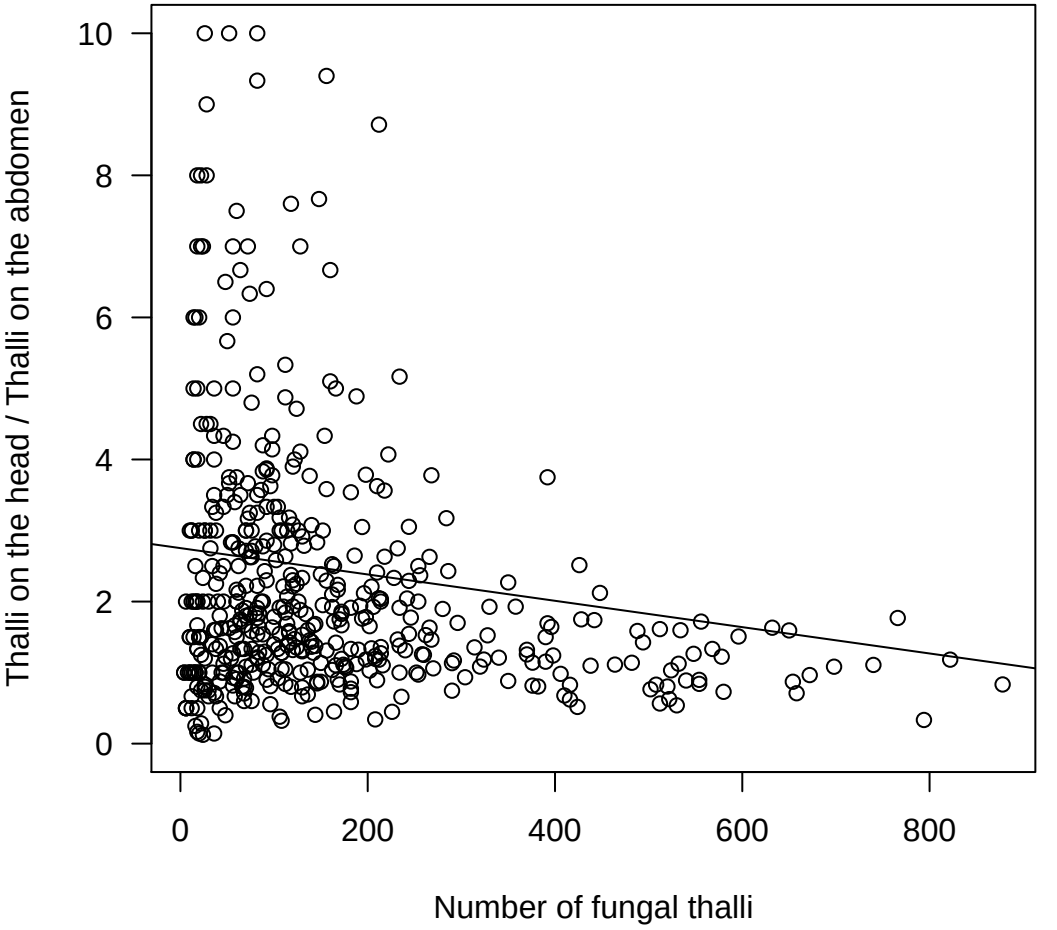

Number of fungal thalli
150
100
50
0
a
c
f
e
e
e
d
b
antenna
head
thorax
1st leg
2nd leg
3rd leg
petioles
abdomen

Figure 4.


Thalli on the head / Thalli on the abdomen
10
8
6
4
2
0
0
200
400
600
800
Number of fungal thalli

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