

Studies of Laboulbeniales (Fungi, Ascomycota) on *Myrmica* ants: *Rickia wasmannii* in the Netherlands

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Abstract

An important group of fungal insect parasites is the Laboulbeniales (Ascomycota). These are microscopic in size and live attached to the cuticle of their arthropod hosts. *Rickia wasmannii* is a common European species limited to the ant genus *Myrmica* (Hymenoptera, Formicidae). We present new records of *R. wasmannii* in the Netherlands on three host species: *Myrmica ruginodis*, *M. sabuleti*, and *M. scabrinodis*. Our data show a mass infection of *M. sabuleti* by *R. wasmannii*. The average parasite prevalence is 38% (n = 3,876). The prevalence was much lower on the other *Myrmica* species. So far, *R. wasmannii* infections have been found only on *Myrmica* species in the *rubra*-group and the *scabrinodis*-group. We provide possible explanations for this observation. To date, *Rickia wasmannii* is known on nine *Myrmica* species in sixteen European countries; an overview is included in tabulated form.

Keywords

Ant-associated fungi, ectoparasites, Formicidae, host shift, Laboulbeniales, parasite prevalence

Introduction

The order Laboulbeniales (Ascomycota: Laboulbeniomycetes) consists of microscopic ectoparasites of Arthropoda, mostly true insects. Within the subphylum Hexapoda representatives of nine orders are known as hosts (Weir and Hammond 1997): Blattodea, Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Mallophaga, Orthoptera, and Thysanoptera. [Note that the termites, previously ranked in the order Isoptera, were recently included in the order Blattodea, based on phylogenetic data (Beccaloni and Eggleton 2013).] In addition, Acari (subphylum Cheliceriformes) and Diplopoda (subphylum Myriapoda) are known to host Laboulbeniales (Haelewaters et al. 2012, Weir and Hammond 1997). Laboulbeniales are unusual in their determinate growth pattern and lack of hyphae. Diversity in the group is largely underexplored and many questions related to the taxonomy and biology of these fungi remain unresolved.

Within the order Hymenoptera, only ants (family Formicidae) host Laboulbeniales. To date, six species have been reported from ants: *Dimorphomyces formicicola* (Speg.) I.I. Tav., *Laboulbenia camponoti* S.W.T. Batra, *Laboulbenia ecitonis* G. Blum, *Laboulbenia formicarum* Thaxt., *Rickia wasmannii* Cavara, and the recently described *Rickia lenoirii* Santam. (for a short review, see Santamaría and Espadaler 2014; fungal names updated to meet most recent revisions).

Rickia wasmannii is widely distributed in Europe, with reports from Austria, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Luxembourg, Romania, Slovakia, Slovenia, Spain, Switzerland, the United Kingdom (Espadaler and Santamaría 2012), and from the Netherlands (Haelewaters 2012) and most recently Poland (Witek et al. 2014). It was originally described from Germany on *Myrmica rubra* (Linnaeus, 1758) [as *Myrmica laevinodis*], and is also known to infect eight other species of *Myrmica*, i.e. *M. gallienii* Bondroit, 1920; *M. ruginodis* Nylander, 1846; *M. sabuleti* Meinert, 1861; *M. scabrinodis* Nylander, 1846, *M. slovacica* Sadil, 1952; *M. specioides* Bondroit, 1918; *M. spinosior* Santschi, 1931; and *M. vandeli* Bondroit, 1920 (Csata et al. 2013, Espadaler and Santamaría 2012).

In the Netherlands, a single worker of *Myrmica sabuleti* Meinert, 1861 (as *M. scabrinodis*) infected with *Rickia wasmannii* is known (Haelewaters 2012). Its discovery has initiated the search for Laboulbeniales on ants in this country. For this research project, ants were collected with many pitfall traps and subsequently screened for the presence of *R. wasmannii*.

Methods

The study site spans somewhat less than 1 km², situated east of Maastricht (Limburg, the Netherlands) near the border with Belgium. This area has a rolling landscape and calcareous soil. Many different habitats were sampled: calcareous grassland, thicket, moist forest, forest edge, felling area, agricultural field edge, and hollow road.

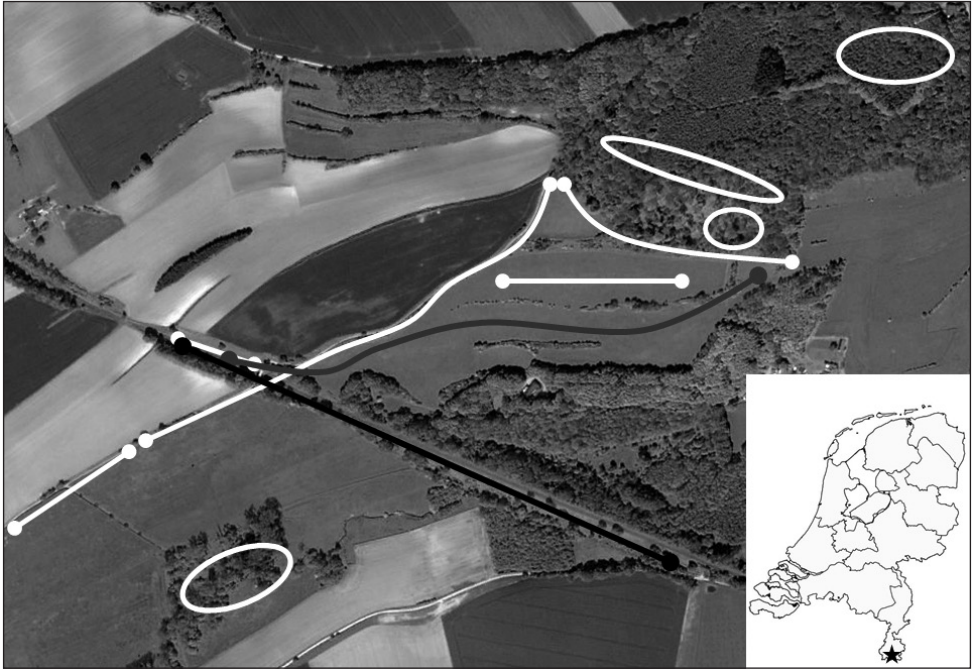


Figure 1. The study site with all the sampling localities indicated. Trajectories (lines) and areas (circles) were sampled with pitfall traps during three inventories. The separate inventories that are mentioned in the text are given: (i) in black (45 pitfall traps in nine series), (ii) in white (55 pitfall traps in eleven series), and (iii) in grey (324 pitfall traps in nine series). Inset: map of the Netherlands with location of the study site in Limburg.

Pitfall traps were filled with a formol solution (3%) or with a saturated salt solution. A lid was placed above each trap to exclude rainfall. Pitfall trapping was performed during three inventories for which in total 424 pitfall traps were placed in 29 series: (i) a year-round inventory of the insect fauna with nine trap series (each with five pitfall traps) at a railroad verge with semi-natural grassland and thicket, (ii) a two-month-long inventory with eleven trap series (each with five pitfall traps) of the arthropod fauna of a nature restoration parcel with a species-rich hay-meadow (dominated by *Arrhenatheretum elatioris* Braun-Blanq.), and (iii) a four-day-long inventory of nine different habitats (nine traps series, one series per habitat, each with 36 pitfall traps) during a student course. For an overview of the study area, see Figure 1.

Screening for thalli of Laboulbeniales was done at 45× magnification with a Euromex Z-1740H stereomicroscope (Arnhem, the Netherlands). Infected ants were sent to Harvard University for study of their associated fungi. Thalli were carefully removed from the host integument using a Minuten Pin (BioQuip #1208SA) and embedded in Amann solution (Benjamin 1971) or PVA Mounting Medium (BioQuip #6371A). Cover slips were ringed with transparent nail varnish. Morphological analyses, measurements, and identifications were done using an Olympus BX40 light microscope

with Olympus XC50 digital camera and MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH). Voucher specimens are deposited at the Farlow Herbarium, Harvard University (FH).

Chi-square tests were performed to analyze whether infection rate of the infected ant species was different from a hypothesized even distribution of *Rickia wasmannii* presence over the infected species. In social insects, it sometimes seems better to analyze the number of infected nests instead of the number of infected workers. However, the infected *Myrmica* species are very abundant in our study area, are polygynous and occur in clusters of mini-populations. They are hardly territorial and contact between workers of different nests is likely (Garnas et al. 2007, Wilson 1971). Each pitfall trap (and trap series) thus likely samples ants from many nests. Our sampling effort with 424 pitfall traps is so widespread over the study area and combines workers from so many nests, that we performed our test on worker individuals.

Results

Twenty-seven ant species were recorded during this study (Table 1). Only three species in the genus *Myrmica* bore thalli of *Rickia wasmannii*.

Within the current study, the highest parasite prevalence was found on *Myrmica sabuleti*: 38% (n = 3,876; Figure 2). For the other two species, parasite prevalence was considerably lower: 11% on *M. scabrinodis* (n = 643) and 0.55% on *M. ruginodis* (n = 182). These infection rates differ significantly when an even proportional distribution of *Rickia* over the three species is presumed, with *M. sabuleti* a significantly higher infection rate as would be expected by chance (observed number of infected ants: 1479; expected: 1279; $\chi^2=46.82$, df=2, $p<0,001$), and a significantly lower infection rate for both *M. scabrinodis* (observed number of infected ants: 71; expected: 212; $\chi^2=140.11$, df=2, $p<0,001$) and *M. ruginodis* (observed number of infected ants: 1; expected: 60; $\chi^2=68.64$, df=2, $p<0,001$). When we look at *Rickia* prevalence over the 29 traps series instead of at the level of individual workers, the same pattern emerges: infected *M. sabuleti* workers in 15 series, infected *M. scabrinodis* in five series, and infected *M. ruginodis* in only one series.

Only workers were found infected; neither gynes nor males were found infected, not even in the highly infected *Myrmica sabuleti*. *Myrmica ruginodis* and *M. scabrinodis* are reported as hosts of *Rickia wasmannii* in the Netherlands for the first time.

Discussion

Rickia wasmannii is mentioned in the literature to occur on nine host species (Table 2), most of which are widespread across Europe (Radchenko and Elmes 2010). It is reported from sixteen European countries, and expected to occur in Belgium, Denmark, Ireland, and Portugal (Espadaler and Santamaría 2012, Haelewaters 2012, Witek et al. 2014).

Table 1. Total number of ant species studied for infection with *R. wasmannii*, over all three series of pitfall traps.

Genus	Species	Author, Year	Workers			Sexuals	
			Total	Infected	Parasite prevalence	Gynes	Males
<i>Formica</i>	<i>cunicularia</i>	Latreille, 1798	589	0	0	0	0
<i>Formica</i>	<i>fusca</i>	Latreille, 1798	193	0	0	0	0
<i>Formica</i>	<i>polyctena</i>	Foerster, 1850	0	0	0	1	0
<i>Formica</i>	<i>rufibarbis</i>	Fabricius, 1793	92	0	0	0	0
<i>Lasius</i>	<i>brunneus</i>	(Latreille, 1798)	148	0	0	0	0
<i>Lasius</i>	<i>flavus</i>	(Fabricius, 1782)	983	0	0	22	8
<i>Lasius</i>	<i>fuliginosus</i>	(Latreille, 1798)	267	0	0	19	0
<i>Lasius</i>	<i>mixtus</i>	(Nylander, 1846)	6	0	0	8	0
<i>Lasius</i>	<i>niger</i>	(Linnaeus, 1758)	> 3,400	0	0	5	0
<i>Lasius</i>	<i>platythorax</i>	Seifert, 1991	39	0	0	0	0
<i>Lasius</i>	<i>sabularum</i>	(Bondroit, 1918)	0	0	0	4	0
<i>Lasius</i>	<i>umbratus</i>	(Nylander, 1846)	1	0	0	5	0
<i>Myrmecina</i>	<i>graminicola</i>	(Latreille, 1802)	328	0	0	13	0
<i>Myrmica</i>	<i>rubra</i>	(Linnaeus, 1758)	974	0	0	1	3
<i>Myrmica</i>	<i>ruginodis</i>	Nylander, 1846	182	1	0.55	0	1
<i>Myrmica</i>	<i>rugulosa</i>	Nylander, 1849	7	0	0	0	0
<i>Myrmica</i>	<i>sabuleti</i>	Meinert, 1861	3,876	1,479	38	41	3
<i>Myrmica</i>	<i>scabrinodis</i>	Nylander, 1846	643	71	11	11	0
<i>Myrmica</i>	<i>schencki</i>	Viereck, 1903	632	0	0	63	1
<i>Ponera</i>	<i>coarctata</i>	(Latreille, 1802)	2	0	0	0	0
<i>Solenopsis</i>	<i>fugax</i>	(Latreille, 1798)	1	0	0	0	0
<i>Stenamamma</i>	<i>debile</i>	Foerster, 1850	236	0	0	6	1
<i>Stenamamma</i>	<i>westwoodi</i>	Westwood, 1840	0	0	0	1	0
<i>Tapinoma</i>	<i>erraticum</i>	(Latreille, 1798)	12	0	0	0	0
<i>Temnothorax</i>	<i>affinis</i>	(Mayr, 1855)	3	0	0	0	0
<i>Temnothorax</i>	<i>nylanderi</i>	(Foerster, 1850)	25	0	0	1	0
<i>Tetramorium</i>	<i>caespitum</i>	(Linnaeus, 1758)	33	0	0	0	0
		Total Myrmica	6,314	1,551		116	8
		Total ants	> 12,675	1,551		201	17

Ant species with *Rickia* infection

Santamaría and Espadaler (2014) mention the “high host phylogenetic specificity” of Laboulbeniales, but caution is needed when interpreting support for this assertion. Although specific to the genus *Myrmica*, host species of *Rickia wasmannii* belong to two clades or so-called species groups that are not phylogenetically closely related: *rubra*-group and *scabrinodis*-group (Jansen et al. 2010).

The Palearctic species in the genus *Myrmica* are classified into 17 taxonomic species groups based on their morphology (Radchenko and Elmes 2010), three of which are represented in our study: the *rubra*-group, encompassing *M. rubra* and *M. ruginodis*; the *scabrinodis*-group, with *M. rugulosa*, *M. sabuleti*, and *M. scabrinodis*; and the *schlencki*-group, to which (in most parts of Europe) only *M. schencki* belongs. The



Figure 2. A worker of *M. sabuleti*, heavily infected with *R. wasmannii* on all body parts. Photograph: Theodoor Heijerman.

monophyly of these morphological species groups was confirmed using molecular data (Jansen et al. 2010). So far, only species of the *rubra*-group and *scabrinodis*-group have been found with *Rickia wasmannii* infection.

Our research confirms this finding: although six *Myrmica* species were screened ($n = 6,314$), *Rickia wasmannii* was only present on *M. ruginodis*, *M. sabuleti*, and *M. scabrinodis* (Table 1). Several researchers screened multiple species of *Myrmica* in Hungary/Romania (Tartally et al. 2007), Slovakia (Bezděčka and Bezděčkova 2011), and the Czech Republic (Bezděčkova and Bezděčka 2011), but found only species in the *scabrinodis*-group infected by *R. wasmannii*. In addition, our study in the Netherlands revealed a single lightly infested worker in the *rubra*-group (*M. ruginodis*). In all four studies, *R. wasmannii* was found only in the *rubra*- and *scabrinodis*-groups.

Inadequate sampling of potential hosts may (partly) explain this pattern, although recently in Europe several studies have been conducted on Laboulbeniales on *Myrmica*, including various studies in which different species groups were sampled and screened. To avoid taxon-sampling errors in these kinds of observation, we suggest that systematic collections in natural history museums be screened for *Rickia wasmannii* on *Myrmica*. Screening museum collections previously has yielded important contributions on patterns of host utilization (Weir and Hammond 1997: 80,000 insects screened) and the distribution of *Hesperomyces virescens* Thaxt. (Haelewaters et al. 2014: 4,000 ladybirds screened).

Host shift could be another explanation for the restricted presence of *R. wasmannii* on two species groups that are not sister clades. Host shifts have been suggested for morphologically similar *Laboulbenia* species between Cicindelinae and other Car-

Table 2. All published records of *R. wasmannii* on different *Myrmica* hosts (Bezděčka and Bezděčkova 2011; Bezděčkova and Bezděčka 2011; Csata et al. 2013; Espadaler and Santamaría 2012; Haelewaters 2012; Tartally et al. 2007; Witek et al. 2014), completed with the current findings from the Netherlands. *In Hungary and Romania, *M. scabrinodis* is the most common host of *R. wasmannii* (Csata et al. 2014; Tartally et al. 2007). In our study area in the Netherlands, *M. sabuleti* is the most commonly infected host species.

Country	<i>rubra</i> -group		<i>scabrinodis</i> -group						
	<i>M. rubra</i>	<i>M. ruginodis</i>	<i>M. gallienii</i>	<i>M. sabuleti</i>	<i>M. scabrinodis</i>	<i>M. slovacica</i>	<i>M. speciooides</i>	<i>M. spinosior</i>	<i>M. vandeli</i>
Austria	X				X				
Bulgaria					X				
Czech Republic					X	X			
France					X				
Germany	X								
Hungary*					X	X	X		X
Italy					X				
Luxembourg	X								
The Netherlands		X		X	X				
Poland					X				
Romania*	X	X	X		X	X			
Slovakia					X				
Slovenia				X					
Spain							X	X	
Switzerland	X								
United Kingdom				X					

abidae living in the same habitat (Arndt et al. 2003, Rossi 2011), and De Kesel and Haelewaters (2014) provide morphological and ecological data to support the hypothesis that a species of *Laboulbenia* shifted between Carabidae and Staphylinidae. To explain the extremely small size of *Rickia lenoirii* on *Messor* spp. ants (Hymenoptera, Formicidae), Santamaría and Espadaler (2014) suggest that *Laboulbeniales* can shift between myrmecophilous Acari and their ant hosts.

Geographical variation

Parasite prevalence (= infection frequency) is often used to quantify differences in populations of *Laboulbeniales* in a given host community (De Kesel 2011, Haelewaters et al. 2012). It is beyond doubt that in our study area *Myrmica sabuleti* is the main host of *Rickia wasmannii*, considering the actual prevalence of *R. wasmannii* on *M. sabuleti* workers infected, compared to the low parasite prevalence on *M. ruginodis* and *M. scabrinodis* (Table 1).

In our study site, parasite prevalence was high in the *scabrinodis*-group (Table 1). *Myrmica sabuleti* and *M. scabrinodis* occur in other habitats; the former species gener-

ally lives in drier areas than the latter. While in our study area in the province Limburg both species live in sympatry, we observed noticeable differences in occurrence and parasite prevalence. In the *rubra*-group, only one specimen of *M. ruginodis* was found with *Rickia wasmannii*. The fact that we did not find any infected *M. rubra* out of 974 workers raises questions, since this species is the second-most commonly found infected species in Europe after *M. scabrinodis*, with examples in Austria, Germany, Luxembourg, Romania, and Switzerland (Espadaler and Santamaría 2012, Table 2).

Also in Romania, so far, infection by *Rickia wasmannii* only occurs in the *rubra*-group and *scabrinodis*-group (Csata et al. 2013, Tartally et al. 2007). In Romania, *Myrmica scabrinodis* often is the only or main host; in a single population *R. wasmannii* was found only on *M. rubra*, although thalli were also observed in populations of *M. scabrinodis* and *M. slovacica*; *M. gallienii* and *M. rubra*; and *M. ruginodis* (Csata et al. 2013).

Myrmica scabrinodis is the main host in Romania (studied areas, Csata et al. 2013), as is *M. sabuleti* in our studied site in the Netherlands. These observations suggest (1) that *R. wasmannii* has a considerable host choice plasticity within the genus *Myrmica* and (2) that infection rates of *Rickia wasmannii* show geographical variation, potentially with different dominant host species across regions. It is not known what factors cause this geographical variation. Future research in the Netherlands is needed to confirm that *M. sabuleti* is the main host in a wider region.

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