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MYRMECOPHILOUS LABOULBENIALES (ASCOMYCOTA) IN BELGIUM

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Summary
This paper presents the first record of *Rickia wasmannii* (Laboulbeniales) from *Myrmica sabuleti* in Belgium. Aspects of prevalence and thallus density of *R. wasmannii* are discussed, and a description and illustrations are given. Screening of the oldest colony of *Lasius neglectus* in Belgium (Ghent University Botanic Garden) did not reveal infections with *Laboulbenia formicarum*.

Samenvatting
Deze bijdrage meldt de eerste Belgische vondst van *Rickia wasmannii* (Laboulbeniales) op *Myrmica sabuleti* (Zandsteekmier). Aspecten met betrekking tot frequentie en thallusdensiteit van *Rickia wasmannii* worden besproken, en een morfologische beschrijving en illustraties worden gegeven. Screenen van de oudste gekende kolonie van *Lasius neglectus* (Plantentuin Universiteit Gent) leverde geen infecties met *Laboulbenia formicarum*.

Keywords: *Rickia*, Hymenoptera, *Myrmica*.

Introduction

Worldwide six species of Laboulbeniales (Fungi, Ascomycota) have been reported on ants (Hymenoptera, Formicidae). Based on the distribution of ants in Belgium (Dekoninck *et al.* 2012), two species of myrmecophilous Laboulbeniales could be expected to occur in Belgium: *Laboulbenia formicarum* Thaxt. and especially *Rickia wasmannii* Cavara.
Laboulbenia formicarum has been reported from Spain, Madeira (Herraiz & Espadaler 2007), and France (Espadaler & Santamaría 2012) on *Lasius neglectus* Van Loon *et al.,* 1990. This invasive ant produces super-colonies and was accidentally introduced in Belgium about 20 years ago (Dekoninck *et al.* 2002). At this time only a few localities are known for this ant (Dekoninck *et al.* 2012), one of which was screened in this study.
Rickia wasmannii is the type species of *Rickia* Cavara, a very diverse genus of Laboulbeniales counting 161 described species worldwide (Santamaría *et al.* 2016). Although representatives of *Rickia* can be found on a wide variety of phylogenetically very different hosts, most species are host specific. *Rickia wasmannii* only grows on ants and has been reported from nine species, all within the genus *Myrmica* Latreille, 1804 (Haelewaters *et al.* 2015a).
Since its description *R. wasmannii* has been reported from sixteen European countries (Haelewaters *et al.* 2015a), but so far not from Belgium. After the recent reports on *Rickia wasmannii* in the Netherlands (Haelewaters 2012, Haelewaters *et al.* 2015a, 2015b) just across the Belgian border, we decided to sample *Myrmica* spp. in a few Belgian localities. This paper presents the first record of *Rickia wasmannii* in Belgium and an update on the data available from a Dutch study site (Gulpen-Wittem) studied by Haelewaters *et al.* (2015a, 2015b).

Materials and methods

Lasius neglectus and *Myrmica* spp. ant workers were collected in several sites in Belgium and one site in the Netherlands. Nests were detected at sight and workers were collected either with a mouth-operated aspirator, or with flexible tweezers (Leonard type). Individuals were immediately stored in 96% denaturated ethanol.
Screening for infection and removal of thalli was done at 50× magnification using an Olympus SZ61 stereomicroscope. Thalli were mounted in Amann medium (Benjamin 1971) and slides were sealed with transparent nail varnish. Drawings and measurements were made using an Olympus BX51 light microscope with drawing tube, digital camera and AnalySIS (Soft Imaging System GmbH). Identification of ants was done using Seifert (2007). Microscope slides are deposited at the Herbarium of the Botanic Garden Meise, Belgium (BR). Ant specimens are stored in RBINS collections (Brussels).

Results

1. *Laboulbenia formicarum* Thaxt.

Screening of over 100 *Lasius neglectus* workers from the Ghent University Botanical Garden (51°2’7.35”N, 03°43’19.62”E) revealed no infection with *Laboulbenia formicarum*. Although *Lasius neglectus* is being reported in an increasing number of sites in Europe (Mabelis *et al.* 2010), infected populations are reported only from Madeira, Spain and France (Espadaler & Santamaria 2012). The reported prevalence of *L. formicarum* on *L. neglectus* fluctuates between 28.8-88% (Herriaz & Espadaler 2007, Espadaler et al. 2011). An extensive study in Hungary (Tartally & Báthori 2015) revealed the absence of *L. formicarum* in all 21 known populations of *Lasius neglectus* in Hungary. In that study and also in ours, a sample of 100 specimens per locality was used to determine presence or absence of the parasite. Based on our observations we think it is safe to conclude that the parasite is most probably not present in the population sampled in Ghent. It would be interesting to keep monitoring this population and other large *L. neglectus* populations in Belgium for Laboulbeniales.

2. *Rickia wasmannii* Cavara
Malpighia 13: 182 (1899)
Select. icones: Cavara 1899 (Tav. VI); Santamaría 1989 (Lám. LIII d-e); Espadaler & Santamaria 2012 (Fig. 4a-b). **Fig. 1a-e.**

Description: thallus hyaline, except for the black foot and the basal septa of appendages and antheridia, 148-195 µm long. Receptacle one-layered, unbranched, triseriate; basal cell very long, 60-91 µm. Ventral (anterior) series composed of 4-5(-6) receptacle cells, each with one smaller apical cell. Median (axial) series composed of 5-7 squarrish receptacle cells, the lowermost distinctly higher than broad. Dorsal (posterior) series composed of 6-8 receptacle cells of variable shape, the lower two always higher than broad. Appendages short and unicellular, 20-35 µm long, quickly deteriorated, often only its dark basal septum remaining. Antheridia flask shaped, with fine tapering neck, 10-13 × 3-5 µm, solitary, more frequent in the upper part of the receptacle, with constricted dark basal septum. Perithecium relatively small, 1-2(-3) per thallus, 48-55 × 19-25 µm, elongate ovoid, becoming asymmetric at maturity, the anterior margin mostly free, the posterior margin halfway free from the receptacle, with rounded apex and inconspicuous ostiolar lips. Trichogyne not seen. Spores fusiform, 1-septate, hyaline, 32-35 × 3-4 µm.

Studied material:
BELGIUM, Prov. Limburg, Moelingen (50°44’57.41”N, 05°43’24.49”E), on *Myrmica sabuleti* Meinert, 1861 (Hymenoptera, Formicidae), pasture on a steep slope, 13.ix.2015, coll. & leg. A. De Kesel, slides: *De Kesel 6270a, b*. THE NETHERLANDS, Prov. Nederlands-Limburg, Gulpen-Wittem (50°49’38.30”N, 05°54’35.37”E), on *Myrmica scabrinodis* Nylander, 1846, calcareous grassland on a steep slope along a railway track, 08.ix.2015, coll. & leg. A. De Kesel, slides: *De Kesel 6271*.

Discussion (*R. wasmannii*)
- Thallus development and distribution
Spores of *Rickia wasmannii* are able to develop on any part of the host integument, including hairs, eyes and mouth parts. Spores also attach on mature thalli. Judging from thallus distributions and remains of the black foot on the hosts’s integument, we assume that there is virtually no place on the host where *Rickia wasmannii* cannot develop.
Our observations clearly show that poorly infected *Myrmica sabuleti*, i.e. workers without remains of the black foot on their body, carry thalli only on the frontal part of the head. This suggests that infections with *Rickia wasmannii* most often start in this particular area, possibly in the nest and as a direct result of contacts with previously infected, older ants. Transmission of spores of Laboulbeniales is not fully understood, but theoretical considerations and experimental results with Coleoptera show that it follows a few basic patterns, all directly related to the host’s behavior (Scheloske 1969, De Kesel 1996, Riddick 2006). In the case of infections with *Rickia wasmannii* we assume that once the sticky spores germinate and produce mature thalli, the ant can further infect itself (autogrooming) and others by direct contact (allogrooming) (Haelewaters *et al.* 2015b). In this respect there is little or no difference with Laboulbeniales from beetles (Coleoptera). However, the gender related infection patterns we often observe in Laboulbeniales from beetles (De Kesel 1993, 1995; Goldman & Weir 2012) are evidently absent in worker ants infected with *R. wasmannii*. In fact, all heavily infested ants (see further) exhibit a similar infection pattern.
- Prevalence and sampling methods
The infected specimens we found in Gulpen-Wittem belong to *Myrmica scabrinodis*. From this site *R. wasmannii* was reported from *M. sabuleti*, *M. scabrinodis* and *M. ruginodis* Nylander, 1846 (Haelewaters *et al.* 2015a, 2015b). Thallus density and parasite prevalence was based on pitfall data and found highest on *Myrmica sabuleti*, followed by *M. scabrinodis*, especially after winter (Haelewaters *et al.* 2015b, pg. 226). Our data from this locality show that a sample of 50 specimens taken in a single nest of *M. scabrinodis* was entirely infected (parasite prevalence of 100%, September 2015). Virtually all *M. scabrinodis* workers showed a high thallus density (> 200 thalli / individual), with juvenile and mature thalli spread over the entire body.
We think that this extremely high thallus density is due to the short life cycle of *R. wasmannii*, combined with an efficient and continuous transfer of spores between ants from the same nest. We also think that the summer months (July and August), prior to our sampling, may correspond with the optimal period for transmission and development of *Rickia wasmannii*. This hypothesis is supported by results from Baumgartner’s (1934) experiments, demonstrating that thallus development of *Rickia wasmannii* takes 12 to 15 days. He also showed that the introduction of 4 infected *Myrmica rubra* (Linnaeus, 1758) [as *Myrmica laevinodis*] in an experimental and parasite-free colony of 50 specimens results in an almost 100% parasite prevalence on workers in 4 weeks time. He also discovered that *Rickia wasmannii* can infect the larvae and the nymphs, i.e. boosting the inoculum present in the ant nest. We have neither data on the number of contacts between ants from different nests nor on how these contacts promote transfer from Laboulbeniales between nests. In spite of the impact caused by introducing inoculum in an ant nest (see Baumgartner 1934), we suspect there still may be differences in parasite prevalence between nests from the same area. In order to understand the impact of the habitat on Laboulbeniales from ants, one should sample or monitor several nests in each habitat, eventually combined with pitfall trapping. This way the impact of soil and vegetation on parasite prevalence and thallus density can be assessed 1) throughout the year, 2) at the level of the habitat, and 3) per individual nest.

Conclusion
The mechanisms that govern prevalence and infection patterns of *Rickia wasmannii* cannot be compared with those affecting Laboulbeniales from Coleoptera. This is mainly due to the fact that population density, copulation, and contact between generations – all factors deeply governing transmission of Laboulbeniales – is fundamentally different between ants and beetles.
For non-social insects, such as beetles, yearround pitfall trapping is a good approach to assess the impact

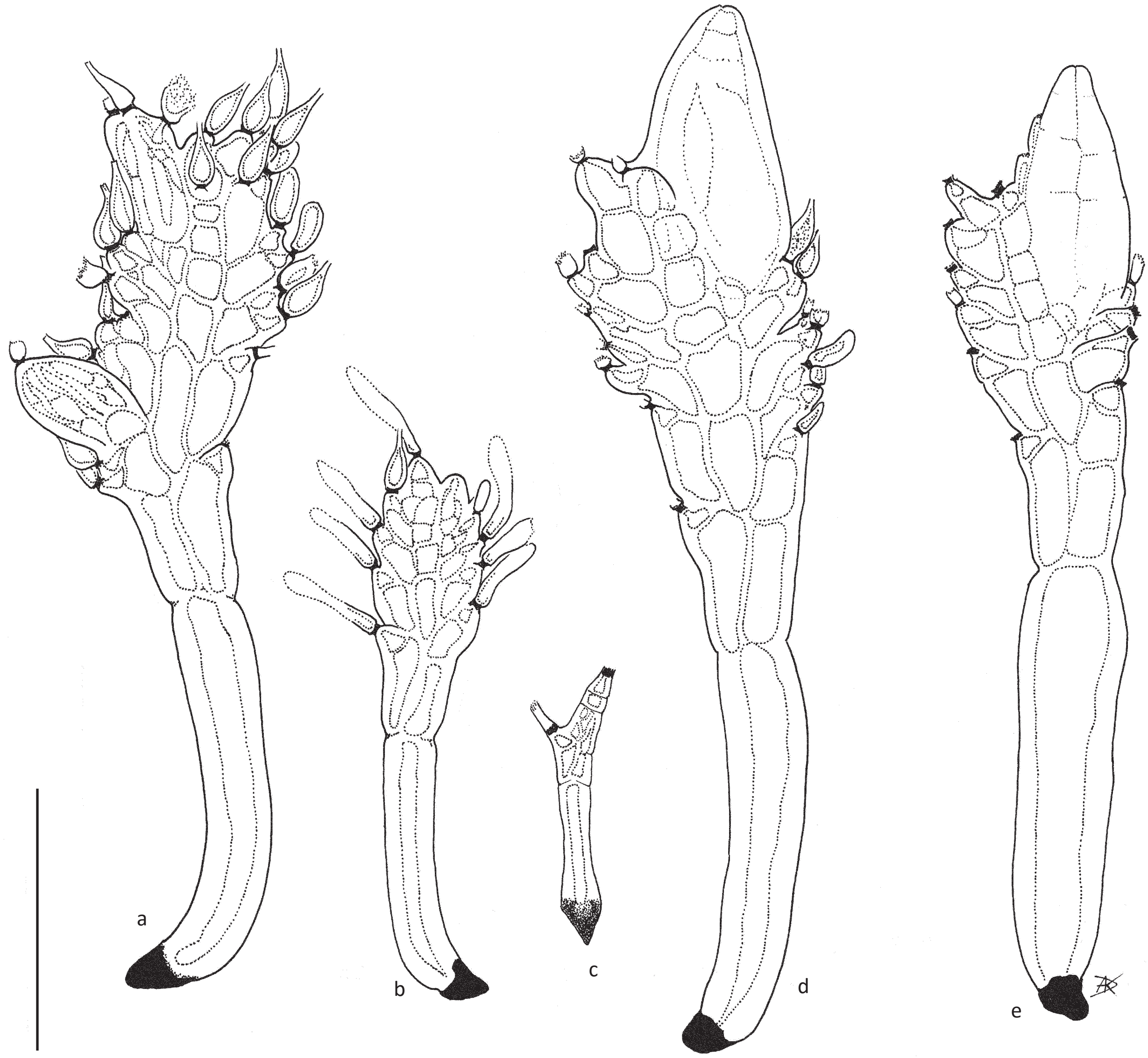

Fig. 1. *Rickia wasmannii* Cavara, taken from the head of *Myrmica sabuleti* (all from slides De Kesel 6270a, b). **a.** maturing thallus with two young perithecia and flask-shaped antheridia; **b.** young thallus with intact appendages; **c.** very young thallus; **d.** & **e.** mature thalli with typical development. Scale bar = 50 µm.

of habitat on parasite prevalence and thallus density. For Laboulbeniales from social insects we propose direct sampling in the nests.

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