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The first record of *Laboulbeniales* (Fungi, Ascomycota) on Ants (Hymenoptera, Formicidae) in The Netherlands

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Summary: *Laboulbeniales* (Fungi, Ascomycetes) are obligate ectoparasites of arthropods, mostly true insects. 80% of all *Laboulbeniales* parasitize beetles, 10% flies. Also other groups of insects are known to support *Laboulbeniales* infections. This paper gives data and microscopic illustrations on *Rickia wasmannii* from an ant, a *Myrmica scabrinodis* worker, collected in The Netherlands. It appears to be the first Dutch record of a myrmecophilous fungus. Information on specificity and geographical distribution is given.

Keywords: Ant-specific fungi, *Rickia wasmannii*, Hymenoptera, Formicidae, ants.

Introduction

Laboulbeniales (Fungi, Ascomycota) include some 2.050 species in about 140 genera (Santamaría, 1998; Weir & Blackwell, 2005; 146 genera according to Kirk *et al.*, 2008) of obligate ectoparasitic fungi that live associated with arthropods, mostly true insects. Amongst insect-associated fungi they are of excellent use in estimating the diversity of fungi associated with arthropods. *Laboulbeniales* have no mycelium; their thalli are small and of determinate growth, bearing antheridia and perithecia on a receptacle with appendages (Tavares, 1985). Only sexual stages are known. Most *Laboulbeniales* are known to be fairly to extreme host-specific (Thaxter, 1896; Scheloske, 1969; Tavares, 1985; Majewski, 1994; De Kesel, 1996, 1997). Each species seems to have its own host range, i.e. infecting either one host species (stenotopic) or several species (eurytopic). The wider host spectrum is always composed of related taxa (congeneric). In case the hosts of a single parasite species are not related, i.e. from a different family, order or subphylum, they always occupy the same micro-habitat (ants nest, termite mound), suggesting that the parasite's success is affected by this specific environment. This is where the nature of the relationship between host, habitat and parasite is not yet fully understood. Also strong morphological variability within a number of genera such as *Chitonomyces* Peyr. (Santamaría, 2001; De Kesel & Werbrouck, 2008; De Kesel & Haelewaters, 2012) and *Laboulbenia* Mont. & C.P. Robin (Benjamin & Shanor, 1952; Rossi & Kotrba, 2004; De Kesel & Van Den Neucker, 2005) and failing classical tools (biometry/allometry and statistics) present many challenges to resolving the group's both ecology and taxonomy, all this explaining the unrelenting need for molecular tools in addition to the traditional morphological approach.

Hosts of Laboulbeniales

Laboulbeniales occur almost exclusively on adult hosts, infections of pre-imaginal stages are excessively rare and only observed on particular hosts (cockroaches, termites and ants; Benjamin, 1971). The majority of the *Laboulbeniales* parasitizes representatives of the subphylum Hexapoda, often Coleoptera (beetles); representatives of ten orders are known as hosts (table 1; Weir & Hammond, 1997).
The greater part of the beetle hosts are members of the two families Carabidae (ground beetles) and Staphylinidae (rove beetles). It is interesting that the diversification of laboulbenialean genera within Coleoptera is greatest within Staphylinidae, i.e. 49 genera, with relatively few species per genus. In contrast, Carabidae host only 15 genera of *Laboulbeniales*, sometimes with hundreds of species in a single genus (*Laboulbenia*) (Tavares, 1979).
Within the Hymenoptera only ants are known to serve as hosts of these fungi. In this paper the first record for The Netherlands of an ant inhabiting member of the order is described. So far, in Europe, three laboulbenialean species associated with ants (Hymenoptera, family Formicidae) have been reported: *Rickia wasmannii* Cavara, *Laboulbenia formicarum* Thaxt. and *Laboulbenia camponoti* S.W.T. Batra (Herraiz & Espadaler, 2007).

Material and methods

Host

The infected ant specimen was collected in 'Zure Dries', a nature reserve near the city of Maastricht in the southeastern part of The Netherlands. The specific site is a very small, yet centuries old woodland clearing on a steep south facing slope. Currently the grassland is managed as a pasture and shows a strong vegetation gradient ranging from acid nutrient poor dry grassland (*Violion caninae*) at the top, through well-developed limestone grassland (*Mesobromion*) in the middle part, to more productive grassland on loamy soil (*Arrhenatherion*) at the basis of the slope. In the 20th century the open area of the clearing has gradually shrunken by wood encroachment, but because of its rare and for Dutch standards thermophilous plant species, the limestone grassland part has always been kept open and in recent years the

Phylum Arthropoda	Common name
Subphylum Cheliceriformes	
Class Chelicerata	
Subclass Arachnida	
Order Acari	Mites
Subphylum Myriapoda	
Class Diplopoda	Millipedes
Subclass Chilognatha	
Order Callipodida	
Order Julida	
Order Sphaerotheriida	
Order Spirostriptida	
Subphylum Hexapoda	
Class Pterygota	
Subclass Exopterygota	
Order Hemiptera	True bugs
Order Mallophaga	Bird lice
Order Blattodea	Cockroaches and allies
Order Thysanoptera	Thrips
Order Orthoptera	Crickets and allies
Order Dermaptera	Earwigs
Order Isoptera	Termites
Subclass Endopterygota	
Order **Hymenoptera**	**Bees, wasps and ants**
Order Diptera	True flies
Order Coleoptera	Beetles

Table 1. Distribution of arthropod hosts parasitized by *Laboulbeniales* (based on Weir & Hammond, 1997). About 80% of the described species of *Laboulbeniales* parasitize Coleoptera (beetles) (Weir & Blackwell, 2005).

clearing has been enlarged once again (Smits & Schaminée, 2004).

The fungus-infested ant, a *Myrmica scabrinodis* worker, was sampled by hand in the limestone zone, where according to an earlier ant survey (De Boer, 1983) *M. scabrinodis* appears to be one of the most abundant ant species. Although *M. scabrinodis* is somewhat thermophilous (Seifert, 1984), it has a rather broad ecological amplitude (Elmes *et al.*, 1998) and is not characteristic for limestone grasslands (Deconinck *et al.*, 2007). As it is highly tolerant to humidity differences *M. scabrinodis* is often abundant or even dominant in wet biotopes like bogs (Seifert, 1984).

As the ant was collected as a possible contribution to an ongoing Dutch DNA barcoding project conducted by NCB Naturalis (http://science.naturalis.nl/dnabarcoding), the specimen was preserved in 90% ethanol in order to enable DNA analysis. Yet NCB Naturalis was not interested in the specimen as fungi do not belong to its target species. Moreover DNA extraction of *Laboulbeniales* is extremely difficult (Hanson, 1992; Goodwin & Lee, 1993; Haugland *et al.*, 1999; Weir & Blackwell, 2001). Eventually the specimen was sent to the author.

Laboulbeniales

Thalli were removed from the hosts and mounted in permanent microscope slides for identification. Thalli were removed from the host using an entomological pin (size 2 or 3) and immediately embedded in glycerine to which was added a small amount of a saturated alcoholic solution of eosin for permanent mounting (Thaxter, 1896). Cover slips were ringed with nail varnish.

Microscopic photographs of thalli were taken in the lab of Dr. Alex Weir at the State University of New York College of Environmental Science and Forestry, Syracuse (NY), using a Nikon Eclipse E800 light microscope equipped with differential interference contrast optics and Spot Edition 4.5 imaging software (Digital Camera Systems, Sterling Heights, MI). The microscope slide collection is deposited in FH (Farlow Herbarium, Harvard University Herbaria, Cambridge, MA).

Description

Rickia wasmannii Cavara, *Malpighia*, 13 : 182, pl. VI (1899) – Fig. 1.

Total length of thallus average 153 µm, maximum 175 µm. **Receptacle** comprising a single (non-septate) basal cell surmounted by three series of cells: a posterior series consisting of five to six cells, with secondary appendages or antheridia, terminated by a single primary appendage; an anterior series similar to the posterior, consisting of four to five cells, and ending in a perithecium; and a median series consisting of about six cells. **Antheridia** 7–15 × 2.5–4.8 µm, compound, subtended by a blackened septum, irregularly disposed, numbers varying in different individuals, flask-shaped, the inflated venters about twice as long as the narrow necks. **Perithecium** 44–53 × 14–21 µm, hyaline, normally solitary, somewhat asymmetrical, its distal half ending in the somewhat irregular blunt, or truncate, hardly differentiated tip.

Studied material: (female) *Myrmica scabrinodis* Nylander, 1846 (fig. 2). The Netherlands: Savelsbos, Zure Dries (180.3-312.6), 23.VIII.2011, *leg*. I. Raemakers, FH-DH40 (fig. 3).

Specificity and geographical distribution

Rickia wasmannii was originally described from Germany by Cavara (1899) on *Myrmica rubra* (Linnaeus), collected in Linz on the Rhine by the renown formicologist Wasmann (as *M. laevinodis*), but is known to also appear on several other species of *Myrmica* (Espadaler & Santamaría, 2012): *M. sabuleti* Meinert, *M. scabrinodis* Nylander, *M. slovaca* Sadil, *M. specioides* Nylander, *M. spinosior* Bondroit, and *M. vandeli* Bondroit.

This is the first report of *Rickia wasmannii* for The Netherlands, the second for the Benelux, where in Luxembourg it was recorded on *Myrmica rubra* (Linnaeus) by Huldén (1985). Elsewhere in Europe, *R. wasmannii* has been reported in France, Switzerland, Austria, Slovenia, Spain and Italy (Espadaler & Santamaría, 2012), and more recently in the United Kingdom (Pontin, 2005; «Sifolinia's AntBlog», 2009), Hungary, Romania (Tartally *et al.*, 2007), Bulgaria (Lapeva-Gjonova & Santamaría, 2011), Czech Republic (Bezděčková & Bezděčka, 2011) and Slovakia (Bezděčka & Bezděčková, 2011). *Rickia wasmannii* seems likely to be widespread in Europe.

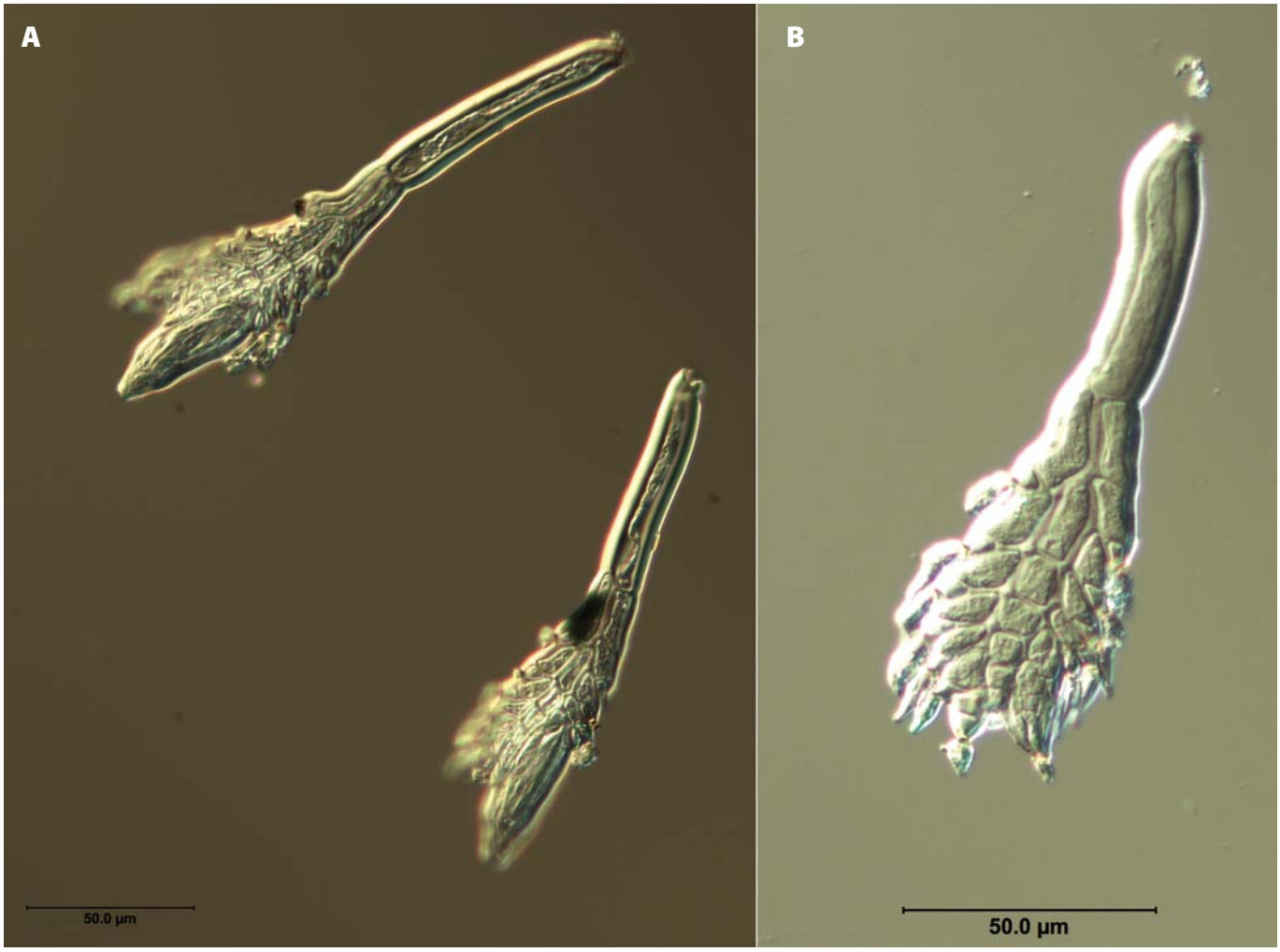

Fig. 1 – *Rickia wasmannii*
A, B. Specimens of *Rickia wasmannii*. Collection FH-DH40h. Pictures: D. Haelewaters.

Fig. 2 – Head of *Myrmica scabrinodis*, heavily infested with *Rickia wasmannii*
Picture: I. Raemakers.

Fig. 3 – Site where *Myrmica scabrinodis* with *Rickia wasmannii* has been found in The Nether-

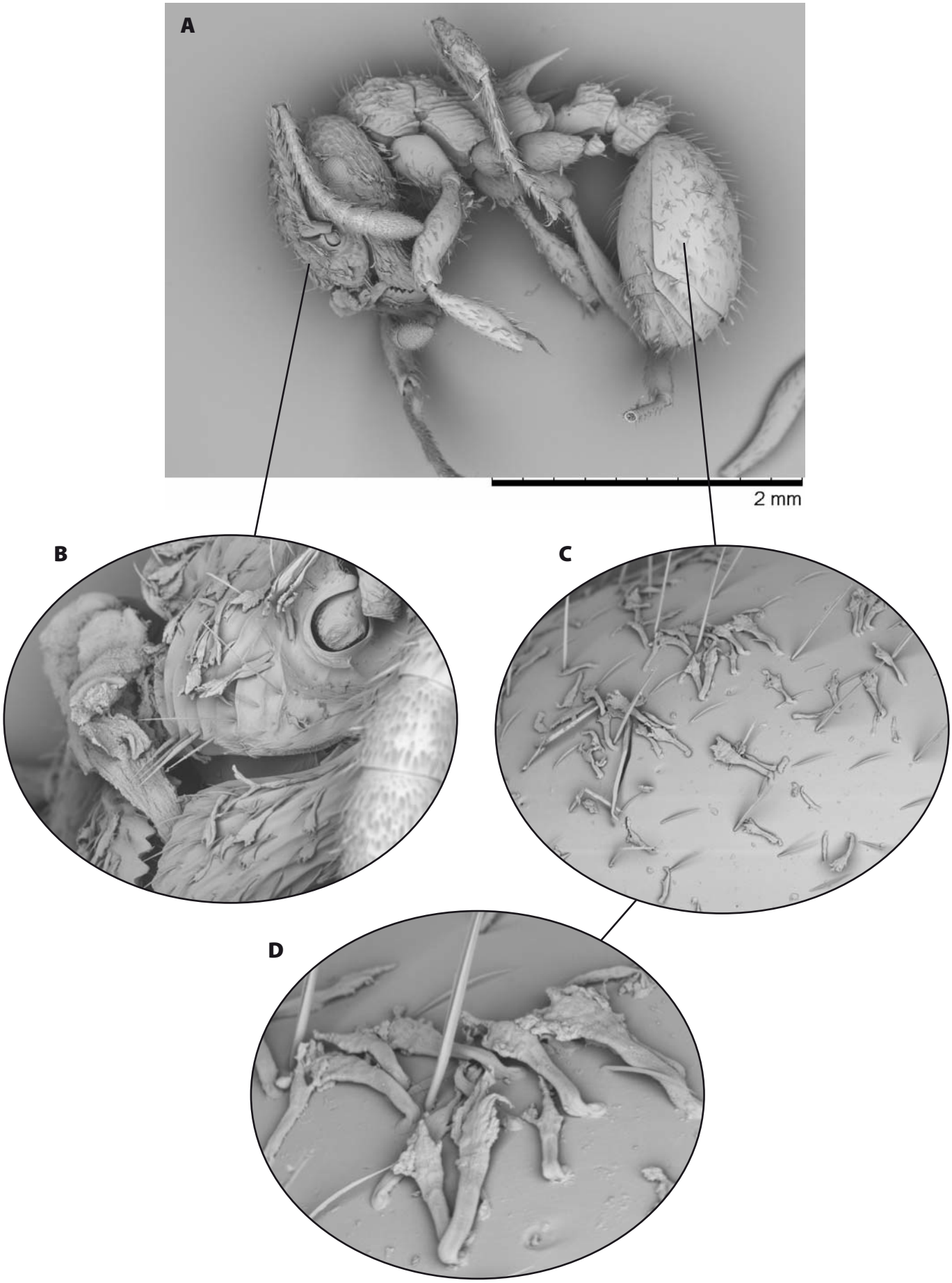

Fig. 4 – *Rickia wasmannii* - SEM
A. Overview of the fungus-infested *Myrmica scabrinodis* ant. B. Detail of the head and mandibles. C, D. Details of the metasoma. Pictures: P. Martin.

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