

Final publishable summary report: “AntLab”

Interactions between ANTs and LABoulbeniales fungi

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Introduction

The order Laboulbeniales (Fungi, Ascomycota; Fig. 1) is a little-studied group of microscopic ectoparasites of invertebrates, mostly insects. Laboulbeniales are understudied by European myrmecologists (ant researchers), and the effects of Laboulbeniales species on their hosts are mostly unknown. Based on the relative ease of culturing ants in the laboratory and the large proportion of infected individuals within an infected ant nest, ants provide an excellent model organism to study the interaction of Laboulbeniales with their arthropod hosts. The main aim of my project is therefore to provide hitherto missing basic knowledge about the ant-Laboulbeniales system, and to foster deeper study of this interaction.

Methods

To get knowledge about the presence and distribution of the ant-parasitic Laboulbeniales fungi in the Carpathian-Basin, the ant collection of the Hungarian Natural History Museum was checked thoroughly and several regions of the Carpathian-Basin were visited. We checked the presence of the fungi not only on ants but also on myrmecophilous arthropods. Light, scanning and transmission electron microscopy were used to study the mode of attachment and the presence of penetrating structures in four of the six currently recognized ant-parasitizing Laboulbeniales. It also was examined whether AgNPs (silver nanoparticles) are able to exterminate *Rickia wasmannii* fungus efficiently from its *Myrmica scabrinodis* host ant. We tried several isolation techniques to enable efficient and reliable genetic analyses of these fungi. The effect of this fungus on the survival rate and water consumption of *M. scabrinodis* was investigated in a laboratory experiment. To enhance the results, environmental stresses were simulated by depriving the ants of water and food.

Results

Rickia lenoirii and *Laboulbenia camponoti* are new species for the Carpathian Basin and *R. wasmannii* has been recorded from new sites (Fig. 1). *Rickia wasmannii* thalli were found on larvae of the myrmecophilous hoverfly *Microdon myrmicae* (Fig. 2) and also on mites (Fig. 3) collected from *Myrmica scabrinodis* nests. Our observations have not given any indication of fungus penetration into the host tissues (Fig. 4). The AgNP topical treatment successfully eliminated *Rickia* infection from *M. scabrinodis* (Fig. 5). We were able to amplify phylogenetically important DNA sequences from more Laboulbeniales species (Fig. 6). By analysing infected in comparison with uninfected *M. scabrinodis* workers, the results showed that after 12 hours of water deprivation, infected individuals spent significantly more time consuming water (Fig. 7). The lifespan of the uninfected *M. scabrinodis* workers, after depriving of water and food, was significantly higher than that of the infected ones (Fig. 8).

Discussion

This project has shown that native ant-parasitic Laboulbeniales fungi are commoner than previously thought, and that their contact and relationship with the hosts is mysterious from several aspects. These new results confirm that these interesting and understudied fungi deserve more attention. Additionally, such basic questions are still open as how and from where these fungi get nutrition. Despite these, this unusual host-parasite system can help to understand host-parasite interactions in general.

Developing the new techniques and approaches in this project is an important milestone in my scientific career. It has helped my reintegration to the University of Debrecen where I received a permanent senior lecturer position and where I have started building my MYRMecological Team.

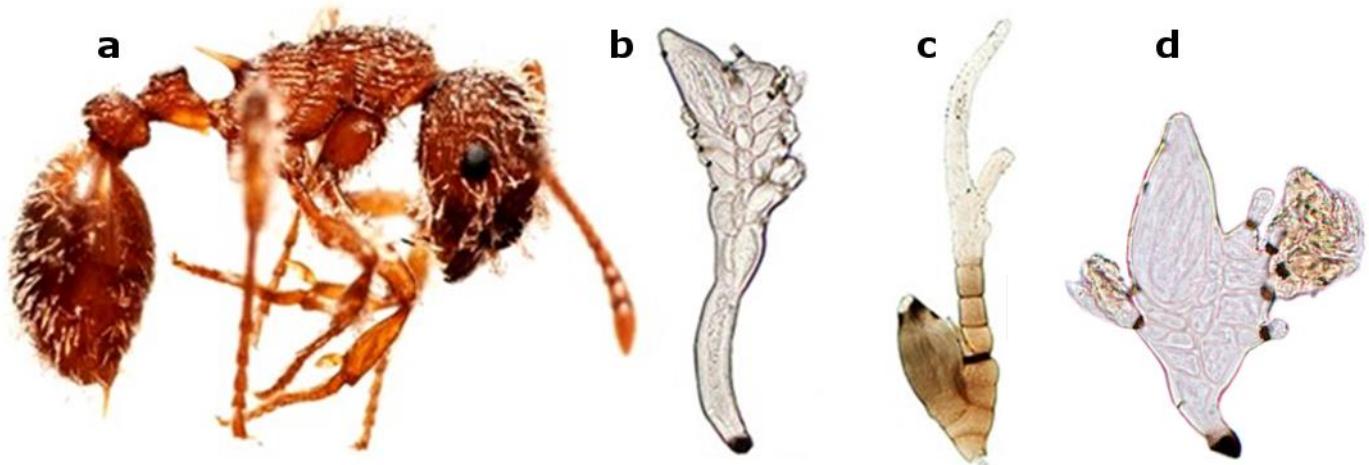


Fig. 1. *Myrmica scabrinodis* (a) infected with *Rickia wasmannii* (b); *Laboulbenia camponoti* from *Camponotus aethiops* worker (c); *R. lenoirii* from *Messor structor* worker (d) (by W.P. Pflieger)



Fig. 2. Two *Rickia wasmannii* thalli on the posterior respiratory process of a *Microdon myrmicae* larva (by M. Szarka).

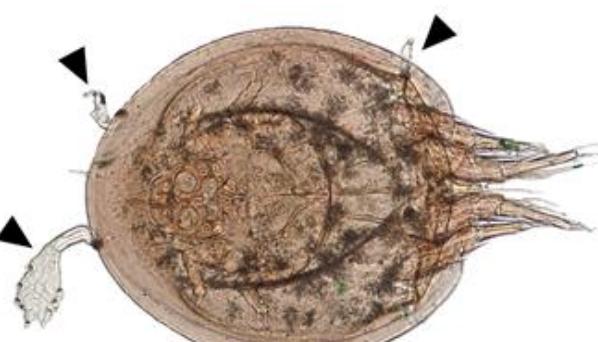


Fig. 3. Infected Acaridae deutonymph with three immature *R. wasmannii* thalli attached (marked) (by W.P. Pflieger).

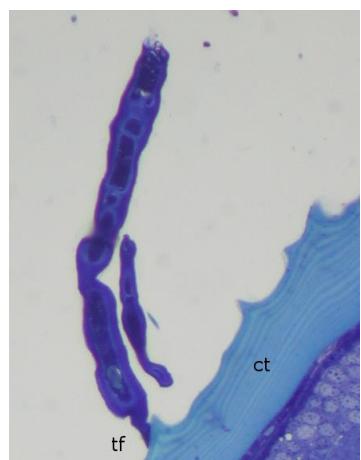


Fig. 4. Examination of sectioned parasitized ants under light microscopy did not reveal any indication of penetration structures originating from the thallus foot structure (tf) attached to the cuticle (ct). (by J. Billen)

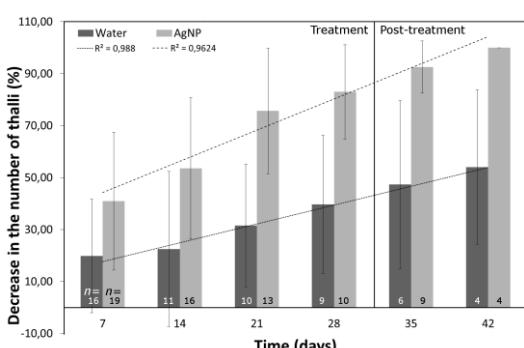


Fig. 5. The effect of AgNP topical treatment on *R. wasmannii*.

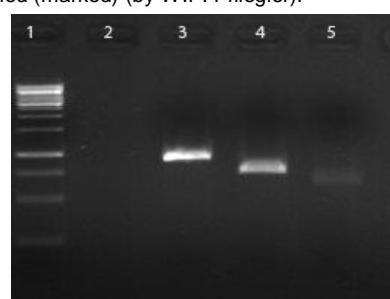


Fig. 6. Example of PCR-reactions carried out following our modified DNA-isolation method. 1: size marker, 2: negative control, 3-5: positive samples from *R. wasmannii* (by W.P. Pflieger).

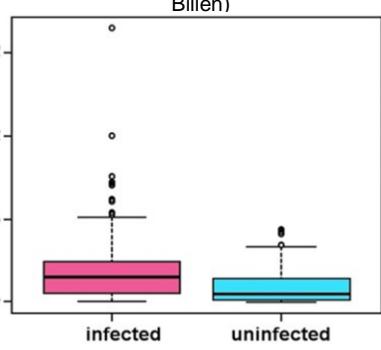
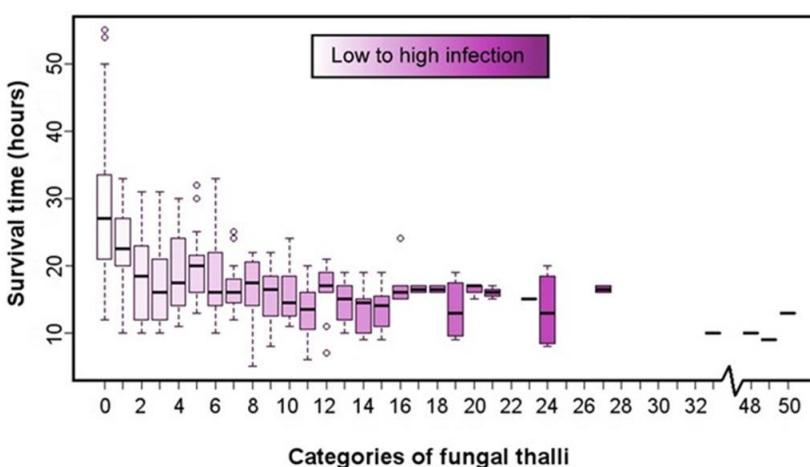


Fig. 7. Individual time (min) spent on water consumption in infected and uninfected populations.

Fig. 8. Survivorship of all the analysed *M. scabrinodis* workers ordered into 51 categories of infection (uninfected: "category 0"; infected: "category 1": 1-20 thalli, "category 2": 21-40 thalli and so on up to "category 50": 981-1000 thalli).