Scanning electron microscopical analysis of the infection of *Rickia wasmannii* on *Myrmica scabrinodis* ants

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Summary

It is well known in the scientific literature, that the *Ascomycota Laboulbeniales* species, including the subject of our research, the *Rickia wasmannii* fungi are very host specific, parasitic fungi. Since this order of fungi contains over 2000 different subspecies it provides an interesting field of research. We worked with the *Rickia wasmannii* species infected *Myrmica scabrinodis* ant. We determined the level of parasitism using a scanning electron microscope, to measure the area of infestation on the host ants, and to determine the differences between the morphology of the fungi on the ant, depending on the region that it is infected.

The specimens were killed by chloroform overdose, and prepared for scanning electron microscopy. The preparation included the specimens' fixation on SEM specimen holders, air drying and gold sputtering to make them observable in our SEM.

The next step included the observation of the infected ants and the counting of the fungi hyphae on the regions of interest. According to our preliminary research these regions included the antennae, and the ocular regions of the ants. The antennae and eyes are the main communication and observation anatomical parts of the ants, so the level of infestation possibly adversely affects the survivability of the host in several ways. We observed several broken talli on the surface of the specimens. Our hypothesis was that the ants are not cleaning themselves from the fungus, so the cause of the braking of the fungi must be the movement of the ant, or the movement of the specific anatomical parts. This widened our research interest so we included in our examination the post-petiole region of the ants. Because this is a really important and very often moving part of the ant, and in preliminary research under a stereomicroscope we did not see much talli. Because of this we decided to examine this under SEM.

Figure 1. *Myrmica scabrinodis* worker

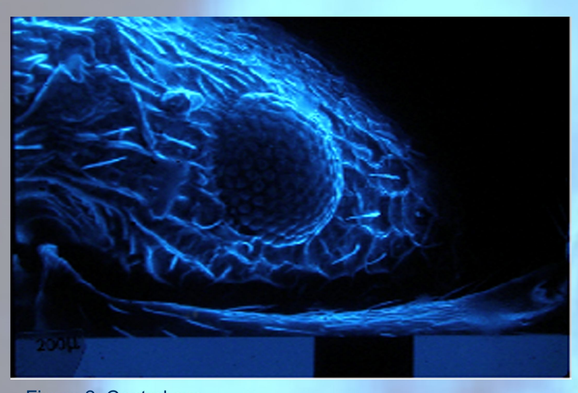


Figure 2. Control eye

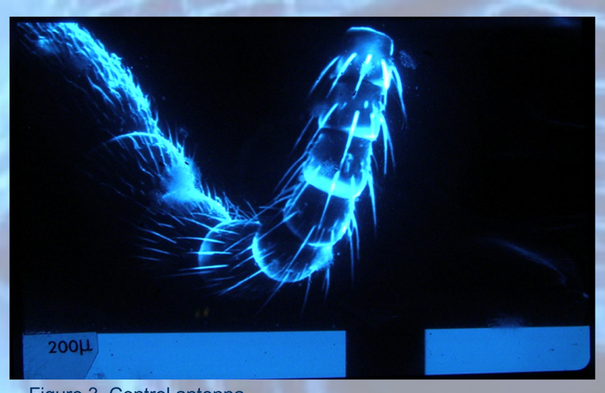


Figure 3. Control antenna

Figure 4. Infected antenna

Figure 5. Infected antenna

Methods

Specimens:

The subject of our research was the ant species Myrmica scabrinodis. These ants can be found in Hungary. The specimens were collected from North-East Hungary.

Preparation of the specimens:

We collected 20 ants for the experiment, that were killed using chloroform and fixated. Our main concern was to place the ant on the adhesive in a way that the antennae and the ocular region can be observed under the SEM.

Gold sputtering:

The specimen holders with ants were put in a gold sputtering device to cover them with a thin gold layer, so they can be observed under the SEM. This procedure was conducted according to the standard gold sputtering protocol, in argon atmosphere.

SEM observation and analysis of the specimens:

After this step, the holders were put in our Cambridge Stereoscan 604 scanning electron microscope and were observed on low magnification (20x) to gather information about the state of the specimen and the overall fungal infection on the surface of the ant.

The next step was to examine the level of infection on the antennae of the ants. We decided that the best choice is to divide the antennae of the ants into 3 regions, instead of the usual anatomical 2 regions, to make it easier to count the talli. In these examinations we used 500 to 1000 times magnification, and counted the full number of talli on these parts, than counted the broken talli, to prove our hypothesis.

Digital imaging:

The images were taken with a Olympus digital camera.

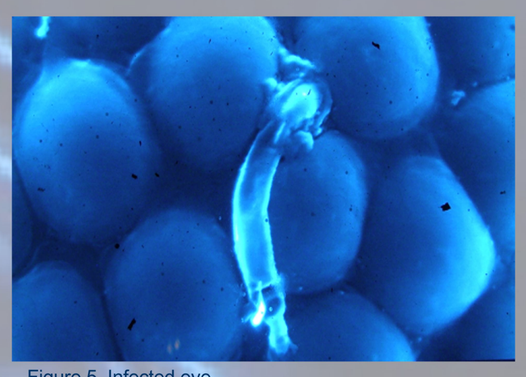


Figure 5. Infected eye


Figure 6. Infected eye

Results

Antennae:

The first part was the tip of the antennae and the following two internodes. This was the "tip" region in our nomenclature. On the tip part, we observed the signs of severe fungal infection. Around 75 to 90 percent of the talli on the tip were broken, and it is possible that it is affecting the inner working of the antennae.

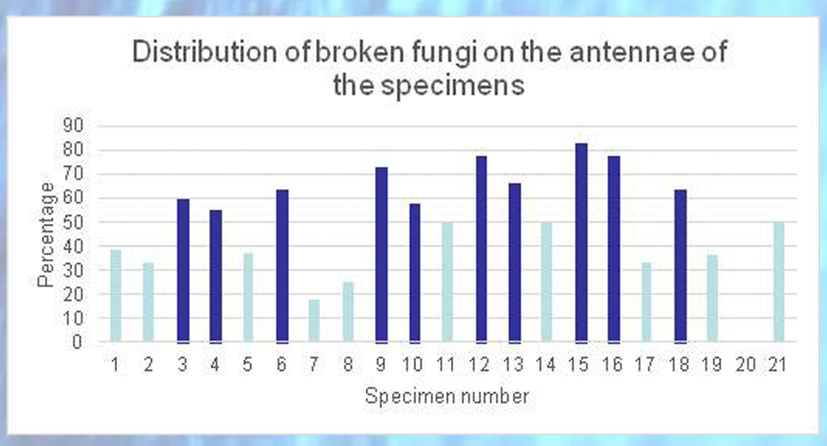
The second part was the middle of the antennae.During our examination of the middle part we proved our hypothesis, that there were some talli in these regions, and most of these was broken. This means the movement of this anatomical region adversely effects the growth of the fungi.

The third part of the antennae was the scape. It has limited movement capabilities, and we hypothesized that the relative unbroken talli coverage here will be the highest.

According to our measurements our assumptions were right, because from 3 and 2.9 talli per antennae there were only 6 to 17 percent were broken.

Eyes:

We observed that in most cases there is fungal infection on the eyes, mostly around the eye, but in several cases the tallus grew out of the ommatid, possibly blinding that spot of the field of vision of the ant.



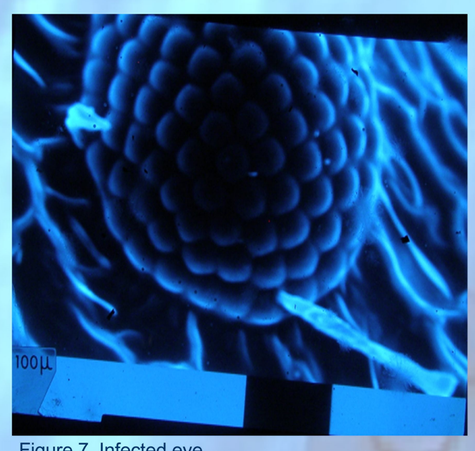


Figure 7. Infected eye

Conclusion

According to other researches in this field, the myrmecologists concluded that the fungal infection reduces the overall survivability of the infected host. In addition to these results, our research also provided some proof, that the infection -anatomically and physiologically speaking- adversely effects the ant. Since the talli of the *Rickia wasmannii* usually grows out of the eyes of specimens, and grows on the antennae. These facts are proving for us, that the *Rickia wasmannii* fungi are in a parasitic relationship with the host ant.

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