Dominikia lithuanica, a new arbuscular mycorrhizal fungal species from the Curonian Spit dunes

Arbuscular mycorrhiza, what is it? Why it is so important?

Arbuscule inside cortical cell of root

Although arbuscular mycorrhizal fungi (AMF) are visible only under microscope and are difficult to research, scientists proved that their role in the ecosystem is very important. These microorganisms create a kind of symbiosis in which fungal hyphae penetrate the cortical cells of the plant roots. The hyphae spread forming a branched and coiled network inside the root and in the surrounding soil. In exchange for carbon compounds, the fungi provide phosphorus, nitrogen, and other micronutrients to plant that are difficult to reach for the plant roots. The AMF also increase the plant's resistance to negative influences of nonliving factors (abiotic stress) or of other living organisms (biotic stress). It is a win–win situation. Arbuscular mycorrhizal fungi owe their name to arbuscules (from the Latin "arbusculum", meaning bush or little tree) that are highly branched structures placed inside cortical cells and are responsible for nutrients exchange between the partners of the symbiosis. The vast majority of vascular land plants form symbiotic associations with AMF, including most important crops worldwide and many endangered species. Scientists have proven that this association had formed at least 460 million years ago and suggest that arbuscular mycorrhiza-like symbiosis should have helped plants to colonize the land environment. Plants probably would not occupy many of the "difficult" habitats without the arbuscular mycorrhiza symbiosis. The AM fungi help to stabilize the ground and support the processes of soil formation. We can use them to introduce plants to new environments or for ecosystem restoration. Possibly, they might help us in the future in colonization of other planets, by increasing the chance of survival of the plants in difficult conditions

Curonian Spit, why have we chosen this place?

The AMF are particularly important for plants growing in poor soils, like the Curonian Spit dunes located in Lithuania. Due to strong winds, continuous backfilling of sand, and low content of nutrients in the soil many plants cannot survive under these conditions. The AMF can restrain the plants strongly to the ground, increase their resistance to salinity

Drifting sand on Curonian Spit dune

and help plants to acquire low mobility nutrients from the soil. Studies show that the presence and species diversity of AMF community is lower in the fertile soils than in infertile. Therefore, biodiversity research of AMF on shifting dunes is a good idea and Curonian Spit is famous for the highest shifting sand dunes in Europe.

Biodiversity research, how we do it?

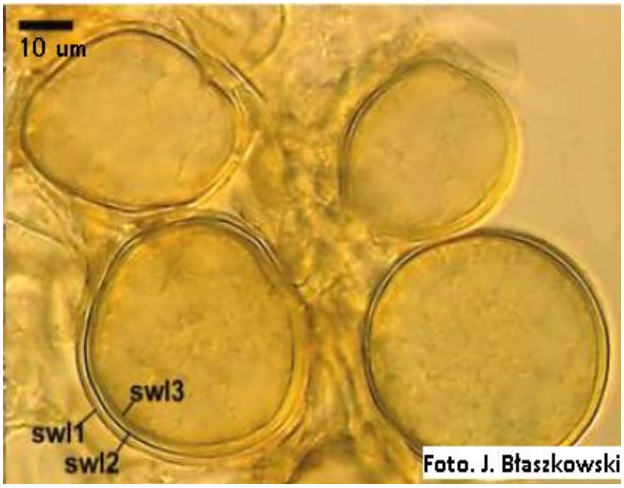
What is the best way to study microscopic fungi hiding partially in plants roots and partly in the surrounding soil? How to distinguish the different species of fungi, which at first glance appear as chaotic, loose tangle of hyphae? Biodiversity research can have various approaches. One of the methods used is culture technique. Fungal spores or mycelium found in soil samples are used to establish cultures, in which they initiate symbiosis with a host plant in a sterile environment. We can grow AMF on previously sterilized soil with a whole plant. It can also be done in a container with suitable growth medium in association with the excised roots. In that case, it is called *in vitro* cultivation of AMF in root organ culture (ROC). It is important to choose the host carefully, because AMF are obligate symbionts and that means, they cannot live and grow without a host plant. Properly cultivated fungi can produce large number of spores and they may be used in identification procedures, based on the characters visible under the light microscope. To distinguish the different species, we study the microscopic construction of spores, their shape, number of spore wall layers, color, and histochemical properties. But, to prove that what we have found is a new species, morphological description should be complemented by molecular data, as single-celled spores of each species can be very similar to each other. We acquire the molecular data by isolating genomic DNA from spores or mycelium and then by amplifying and sequencing the genomic fragment. As a result of the abovementioned processes and data handling, we obtain the nucleotide sequences denoted by the abbreviations A, T, C, or G representing the four nucleotide bases of a DNA strand. Suitably selected short genetic sequence will allow us to distinguish each species in the way a supermarket scanner distinguishes products using the bar codes. As a string of broader and narrower lanes in the bar code is specific to each commodity, so a nucleotide sequence is specific for a particular species. The DNA barcode is used by taxonomists for the classification of species. By comparing our results with nucleotide sequence database like NCBI, we can determine if the species we found is new and whether it was recorded somewhere else in the world. The disadvantage of this approach is that the establishment and upkeep of cultures requires lot of work and is time-consuming. Also, currently not all AMF species are culturable. The advantage of this approach is that each received sequence can be assigned to the species of known morphology and can determine whether a species is able to enter into symbiosis with the host plant selected by a researcher. Knowledge of whether a species can be brought into cultivation in artificial conditions is important for practical reasons. It can help in the selection of species for a high quality AMF inoculum, which we would like to grow on a larger scale to exploit for the purposes mentioned before.

Metagenomic approaches allow us to explore the whole population of a given group of microorganisms, omitting the cultivation procedure. Sequences representing a particular group of microorganisms in the tested environment are captured by amplifying the DNA obtained directly from environmental samples, and with the help of the "next-generation" sequencing methods. We get a huge amount of molecular data (sequences) that make up the profile of biodiversity in the surveyed environment. This approach allows us to compare the biological diversity of different environments and determine whether a species represented by the sequences is common or it is

associated only with a specific type of habitat. But metagenomic methods, as a result, give us thousands of sequences that cannot be attributed to a specific genus to research further, because no researcher has yet managed to grow and describe them. However, those species represented only by sequence help us to estimate the so-far-unknown number of the AMF species.

***Dominikia lithuanica*, what is so special about this species? Why is it named so?**

Sequences obtained from *Dominikia lithuanica* were compared with other sequences deposited in NCBI Database; as a result of this action, we can say that the Curonian Spit in Lithuania is the only place where this species has been found so far. Hence, it is named so, in honor of the country of origin. *Dominikia lithuanica* probably creates a symbiosis with *Ammophila arenaria*, because it was found among the roots of those plant species. This grass commonly occurs on the dunes of the Curonian Spit. *Dominikia lithuanica* sporulates with a 3-layered spore wall that arises in clusters and are globose to subglobose, as we can see on the photo.
An interesting observation was the ability of this fungus to bind to organic matter, which so far has not been seen by us on such scale in other species of AMF.
This property causes lumps of soil and probably is very important in stabilizing the dunes. It is amazing how this simple microorganism can have a serious impact on the landscape and the environment of Curonian Spit dunes.



Spore wall layers (swl) of *D. lithuanica*

Detailed species description can be found in an article by Janusz Błaszkowski, Gerard Chwat, and Anna Kozłowska entitled "*Dominikia lithuanica* and *Kamienskia divaricata*: new species in the Glomeromycota". Botany 94: 1075–1085 (2016).

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