What is what in the phylum Glomeromycota?

Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota form a symbiotic association with ca. 70% of vascular land plants. The symbiosis is called arbuscular because the most important structures formed in root cells by fungi of 42 out of a total of 43 genera of this phylum are arbuscules, i.e. bushy branched hyphal tips that take part in a bilateral exchange of carbon, phosphorous, and other physiologically important particles. The symbiosis is of high interest to biologist and ecologist because AMF among others (*i*) regulate the cycling of carbon, nitrogen and phosphorous, (*ii*) influence soil structure and plant productivity, and (*iii*) enhance the tolerance of plants to heavy metals, water stresses, and pathogenic fungi and nematodes. Accurate identification and classification of AMF are crucial for the description and understanding of phylogenetic, functional, and trait diversity. In many studies it is important to know the AMF identities because their influence on plants usually differs substantially. However, the identification of AMF is difficult because the morphological diversity of their spores is low. Due to these reasons many species of AMF were systematically misplaced within the Glomeromycota. The phylogenies reconstructed from analyses of sequences of the SSU-ITS-LSU nuc rDNA region and the *RPB1* and *hsp60* genes, whose resolving power allows distinguishing even the most closely related species, remain unknown in ca. 62%, 75%, and 93% described species, respectively. No named species have been characterized phylogenetically using SSU-ITS-LSU plus *RPB1* plus *hsp60* sequences. Data from recent literature and our observations indicate that the Glomeromycota also includes undefined species that are morphologically almost identical to described taxa but differ considerably in molecular phylogeny. Finally, there are many species that still retain the same status as they were originally named because their natural phylogeny remains unknown and transferring of some species to newly erected genera based on DNA sequence data has not been accepted or is uncertain. The AM fungi affected by such problems are, e.g. the species originally described as *Acaulospora capsicula*, *A. gedanensis*, *A. polonica*, *A. thomii*, *Archaeospora trappei*, *Entrophospora infrequens*, *Glomus constrictum*, *G. drummondii*, *G. majewskii*, *G. przelewicensis*, *G. pustulatum*, *G. walkeri*, and *Gigaspora gregaria*.

The promotor of the principal investigator of the project keeps a living collection of AMF consisting of ca. 1000 pot trap and single-species cultures representing different regions of the world. In the collection, we have ca. 150 trap cultures that were inoculated with mixtures of rhizosphere soils and roots of five plant species dominating in maritime sand dunes of Poland and were sampled mainly in sites located in north-western Poland and along both coasts of the Hel Peninsula. Pilot studies of AMF extracted from the cultures indicated that they contain undescribed species. In addition, we established single-species cultures with many other morphotypes extracted during previous studies by J. Błaszkowski's research group. Thus, we are sure to describe at least some new species of the Glomeromycota during the realization of the project. Considering the data presented above, the aims of the project are: (1) to verify and, if needed, to correct or newly define the morphological and molecular characters, as well as the phylogenetic positions of at least 10 species previously found to dominate in maritime sand dunes of Poland and other regions of Europe based on (*i*) analyses of the phenotypic and histochemical features of their spore components using specimens freshly extracted from living cultures, (*ii*) studies of characters of their mycorrhizal structures, (*iii*) morphological comparisons of their features with those of other related species, and (*iii*) phylogenetic analyses of sequences of their SSU-ITS-LSU nuc rDNA region, *RPB1* and glomalin *hsp60* genes, and concatenated sequences of the three loci; (2) to construct new primers that will more efficiently amplify the molecular information contained in the *RPB1* and *hsp60* genes; and (3) to collect, analyze, and define morphological and molecular features of undescribed species originating from maritime dunes that are grown in living cultures of AMF harboured in the Plant Protection Laboratory of the West Pomeranian University of Technology (WPUT) in Szczecin.

The studies will be conducted using the following methods: (*i*) growth of pot trap cultures with rhizosphere soils and roots of dune plants and single-species cultures with morphotypes extracted from trap cultures in order to obtain a large number of living spores of AMF for morphological and molecular analyses, (*ii*) extraction and identification of spores of AMF; spores will be extracted by the method used by us and identified based on available literature and deposited or loaned vouchers, (*iii*) determination of molecular properties and phylogenetic positions of AMF based on analyses of sequences of the markers listed above; extraction of DNA from spores, its amplification, cloning and sequencing, as well as phylogenetic analysis of sequences will be performed according to the methods outlined in literature. Results of the project will (*i*) make identification of AMF easier and help understand their impact on ecosystem functioning, (*ii*) allow monitoring the AMF characterized during the realization of the project by the use of more precise molecular markers, and (*iii*) make the performance of further studies by scientists of different spheres easier, as well as enhance the effectiveness of application of AMF in practice.