

A case of mistaken identity: fine endophyte (*Glomus tenue*) phylogenetically align with Mucoromycotina, not Glomeromycota.

Suzanne Orchard¹, Dr Sally Hilton², Prof Gary Bending², Prof Ian Dickie³, Dr Rachel Standish⁵, Dr Deirdre Gleeson¹, Mr Robert Jeffery¹, Prof Jeff Powell⁴, Dr Christopher Walker¹, Dr David Bass⁶, Dr Jana Monk³, Dr Anna Simonin⁴, Dr Megan Ryan¹

¹University Of Western Australia, ²University of Warwick, ³Lincoln University, ⁴Hawkesbury Institute for the Environment, University of Western Sydney, ⁵Murdoch University, ⁶Natural History Museum

Abstract

Arbuscular mycorrhizal (AM) fungi are important plant symbionts which exist at the interface between host-plant and soil, aiding nutrient uptake in exchange for carbon. Fine endophytes (FE) are morphologically distinct mycorrhizal fungi observed in stained plant roots. While FE may colonise numerous plant-hosts with a global distribution, they are prolific within Australia and New Zealand, especially in pastures colonising the roots of grasses and clovers. The presence of arbuscules led to FE being classified as *Glomus tenue*, an AM fungus in the phylum Glomeromycota. However, support for this classification was weak and lacking molecular evidence.

We designed a novel method to maximise FE colonisation within roots of *Trifolium subterraneum* by sieving and dilution of a field soil to remove other AM fungi (Enrichment experiment). Roots were visually assessed for FE and AM fungal colonisation, and community composition was determined by 18S rRNA gene sequencing. Roots from another experiment containing mixed AM fungal species, including FE, were also examined (Contrast experiment).

The percentage of the colonised root length which comprised FE was 90.2 ± 2.5 and 28.9 ± 6.3 in the Enrichment and Contrast experiments, respectively; other AM fungi were $<10\%$ in the former and $41.8 \pm 8.9\%$ in the latter. The percentage of sequences that matched Mucoromycotina was $88.4 \pm 1.8\%$ and $24.3 \pm 4.7\%$ in the Enrichment and Contrast experiments, respectively. The percentage of Mucoromycotina sequences in the Enrichment experiment was correlated to FE colonisation ($R^2=0.91$).

Our results, demonstrate that FE are not glomeromycotan fungi, but belong in the sub-phylum Mucoromycotina. These results are significant as they demonstrate that arbuscules are produced by fungi belonging to the Mucoromycotina and is the first report of arbuscules outside the Glomeromycota. Further, molecular studies of AM fungal communities using primers which target glomeromycotan fungi are overlooking a significant component of the soil mycorrhizal community.