Title: A floristic treatment of *Russula* diversity in the spruce-fir ecosystem of the Southern Appalachian Mountains.

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Abstract: The spruce-fir ecosystem of the Southern Appalachian Mountains is considered highly endangered and receives the highest amounts of nitrogen deposition in this region, which negatively impacts the growth, composition, and function of soil organisms. *Russula* is an important genus of ectomycorrhizal fungi that associates with red spruce (*Picea rubens*) and Fraser Fir (*Abies fraseri*); however, no systematic molecular analysis of the genus has been completed in the region. We compared *Russula* collected from spruce-fir habitats of the Southern Appalachian Mountains with collections in the TENN herbarium and reference material from North America and Europe, using an in-depth morphological study and multilocus phylogenetic analysis. Additionally, we sampled 71 soil cores to understand the molecular diversity of *Russula* belowground as well as species' ecological preferences. We analyzed the predicted occurrence of the genus and species present using the random forest machine learning algorithm. Preliminary phylogenetic analysis has tentatively identified 49 species-level clades from basidiomata in the spruce-fir ecosystem. Eight of these species are distributed across eastern North America, including *R. peckii, R. paludosa, R. rugulosa, R. fragilis, R. betularum, R. dissimulans, R. granulata,* and *R. puellaris*. Four species appear to have intercontinental distributions that extend into spruce-fir habitats of Europe, including *R. claroflava, R. montana, R. aquosa,* and *R. vesca*. The remaining species require further investigation. Additionally, 51 *Russula* OTUs were identified using ITS2 sequences from soil samples, indicating that belowground diversity is twice as high as the observed aboveground diversity (27 species found aboveground). Results indicate that some of the *Russula* species studied may be undescribed based on unique morphological characters and distinct molecular composition. We determined that pH and carbon content are important soil factors that influence the community structure and diversity of *Russula* within this system. Finally, we found heavy metals (Pb, Cd, Zn, and Mn), nutrients (carbon and sulfur), and spatial factors predicted the occurrence of species in this region. This work has provided valuable information regarding the *Russula* species present in the spruce-fir ecosystem, their ecological preferences, and how aboveground and belowground diversity differ for large ectomycorrhizal genera.