Phylogeny and functional evolution of high affinity nitrate transporters in Fungi

This project investigates the phylogeny and expression of NRT2 in *Hebeloma* and related basidiomycetes. Acquisition of nitrate is mediated by NRT2, a high affinity nitrate transporter found in prokaryotes, viridiplantae and fungi. We have successfully sequenced *nrt2* amplicons from all major clades of *Hebeloma.* Our recent discovery of a putative second locus with a non-random pattern of substitutions suggests that functional diversification of *nrt2* has occurred in these fungi. Multiple analyses of *nrt2* alignments in our lab provide more robust resolution in the backbone of *Hebeloma* phylogeny than has been obtained with rDNA.

Hebeloma is an ectomycorrhizal genus nested within a clade that also includes many decay fungi. Species in this genus inhabit diverse and sometimes extreme nitrogen habitats. Nitrogen is a limiting nutrient in most forest soils, most readily obtained in the form of nitrate. Nitrate concentration in soil and other substrates is constantly shifting so organisms that rely on nitrate possess specific adaptive mechanisms including inducible or repressible regulation and multiple transporters with differential affinities for nitrate. Structural and regulatory evolution in the NRT2 family is crucial to the adaptation of species to diverse carbon and nitrogen niches. *Hebeloma* is an ideal clade in which to study these ecological transitions, in part because a comparative phylogenetic database of ribosomal DNA sequences is available.

This project has two major goals:

1. Characterize the diversity and evolutionary origin of *nrt2* homologs in *Hebeloma* and related taxa.
2. Address evidence of functional diversification between alternate *nrt2* copies and among lineages of different ecologies.

Intellectual merit of the proposed research: This project uses an ecologically significant enzyme to improve phylogenetic resolution in a taxonomically enigmatic clade and to investigate ecological transitions in the fungi.

Broader impacts of the proposed research: This project will engage undergraduate researchers who benefit from the multidisciplinary nature of the work. The results of the work will provide a framework for addressing evolutionary impacts of nitrogen pollution.