

# Plant-Microbe Interfaces: The key fungal lineage of the Russulaceae, a new resource for untangling and linking beneficial plant-fungal associations and ecosystem functions

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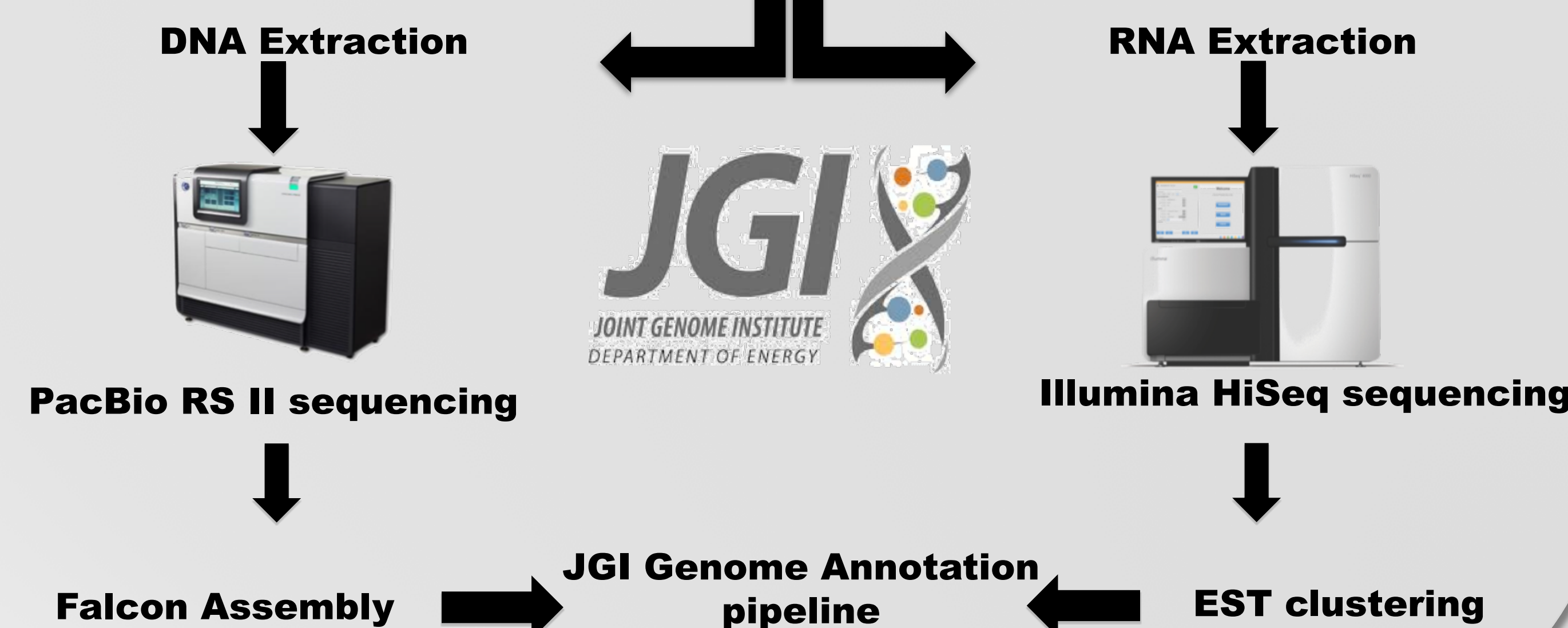
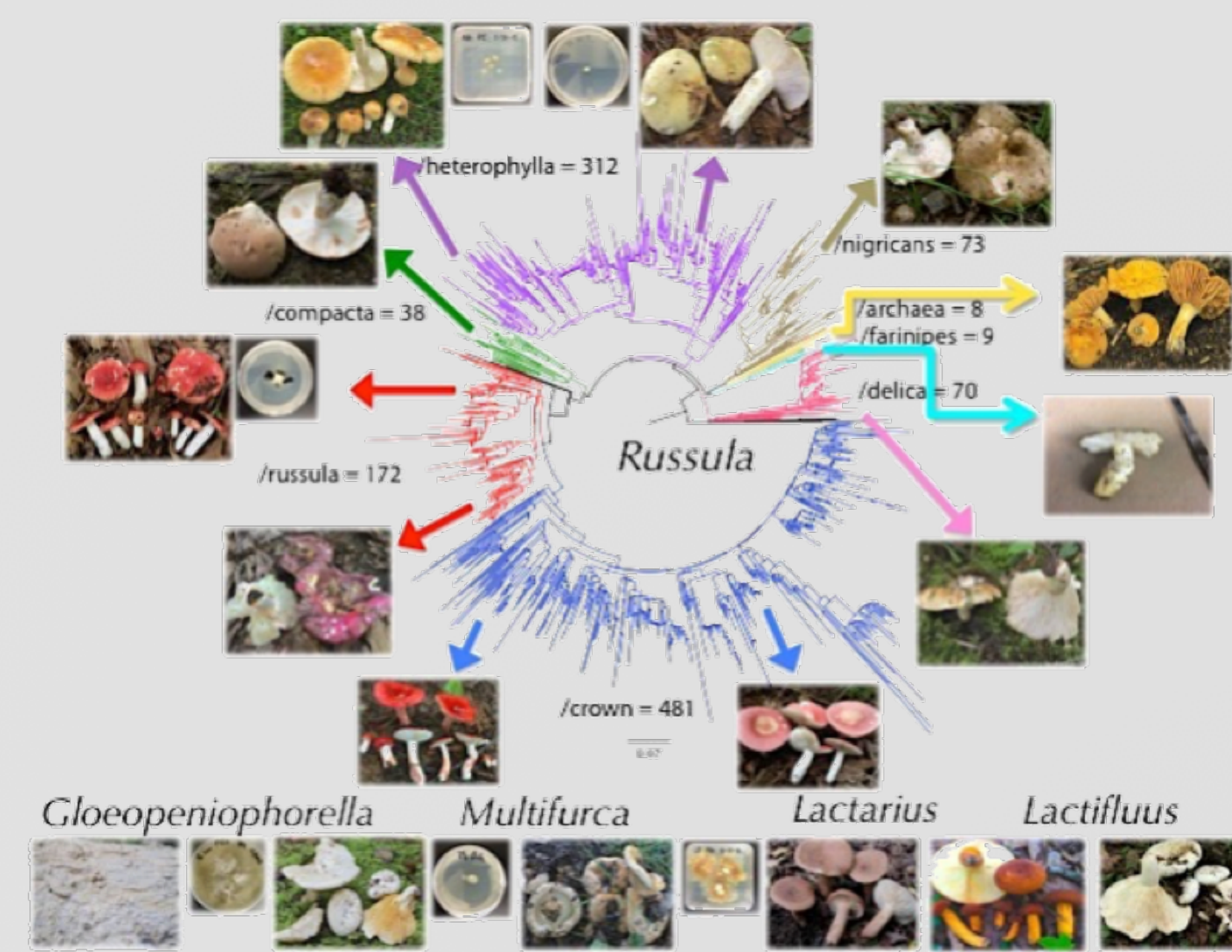
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## Abstract

The *Populus* root microbiome harbors a diverse community of ectomycorrhizal fungi (EMF) that significantly increase nutrient uptake and acquisition by the plant host while also providing protection against antagonistic parasites. Over 30 genera of EMF are known to associate with *Populus*, including many groups of mushroom-producing families including Boletaceae, Russulaceae, Cortinariaceae, Tricholomataceae, and Amanitaceae. A major aim of the PMI project is to extend knowledge acquired or being so, in the *Populus-Laccaria bicolor* system model to representative emerging fungal-*Populus* models. To this end, the isolation and characterization of major EMF fungal associates of *P. trichocarpa* has been initiated and revealed that the most represented EMF taxa collected is the family of the Russulaceae, for which a genome initiative has been started. Comparative genome analyses and experimental *Populus*-Russulaceae model systems have been thereby carried out, opening-up the potential for thorough investigations using multi-omics approaches. Russulaceae being one of the most diverse EMF lineage this new resources becomes tremendous to link plant-fungal associations and ecosystems functions.

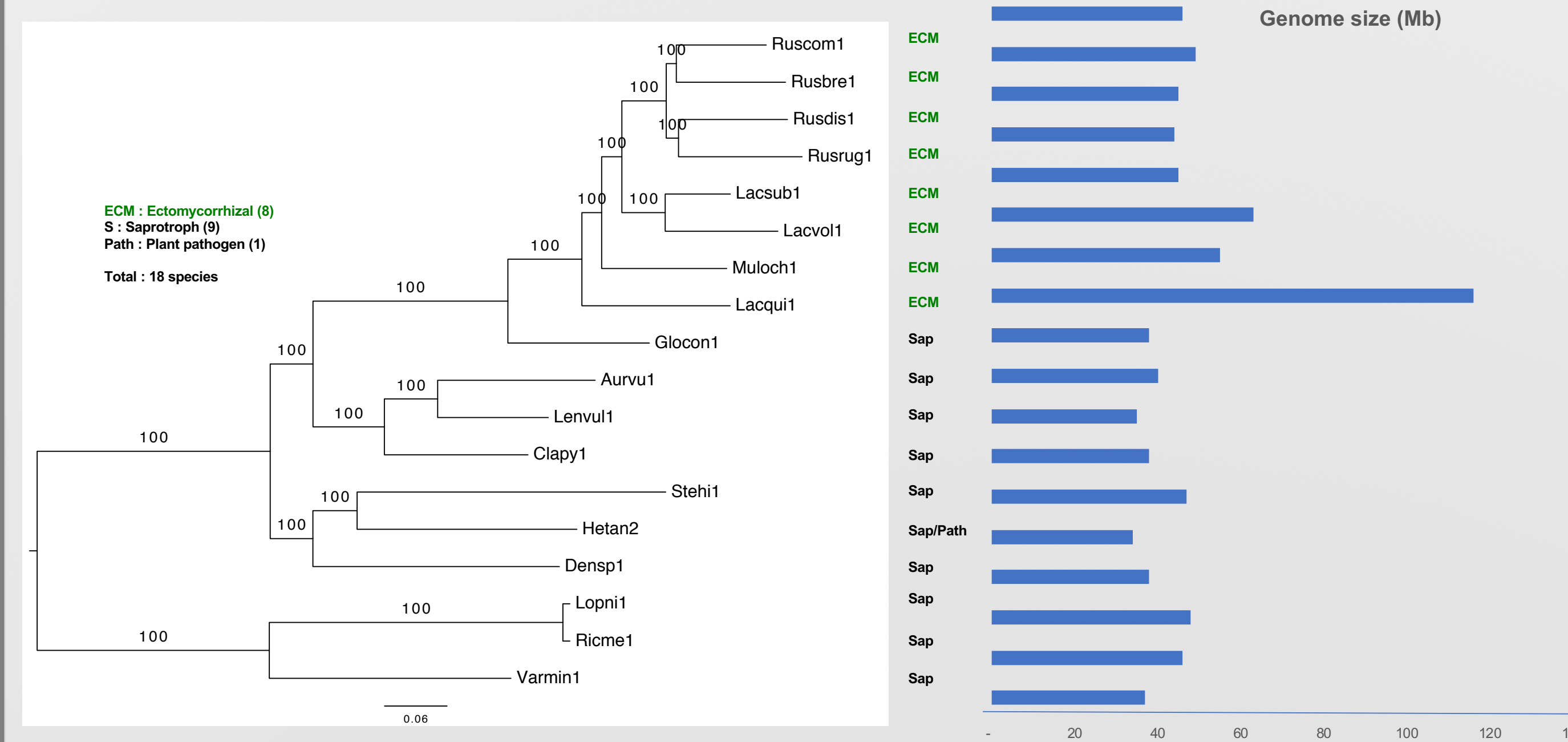
<http://pmi.ornl.gov>

## Dense genome sampling of Russulaceae



## Comparative genomics

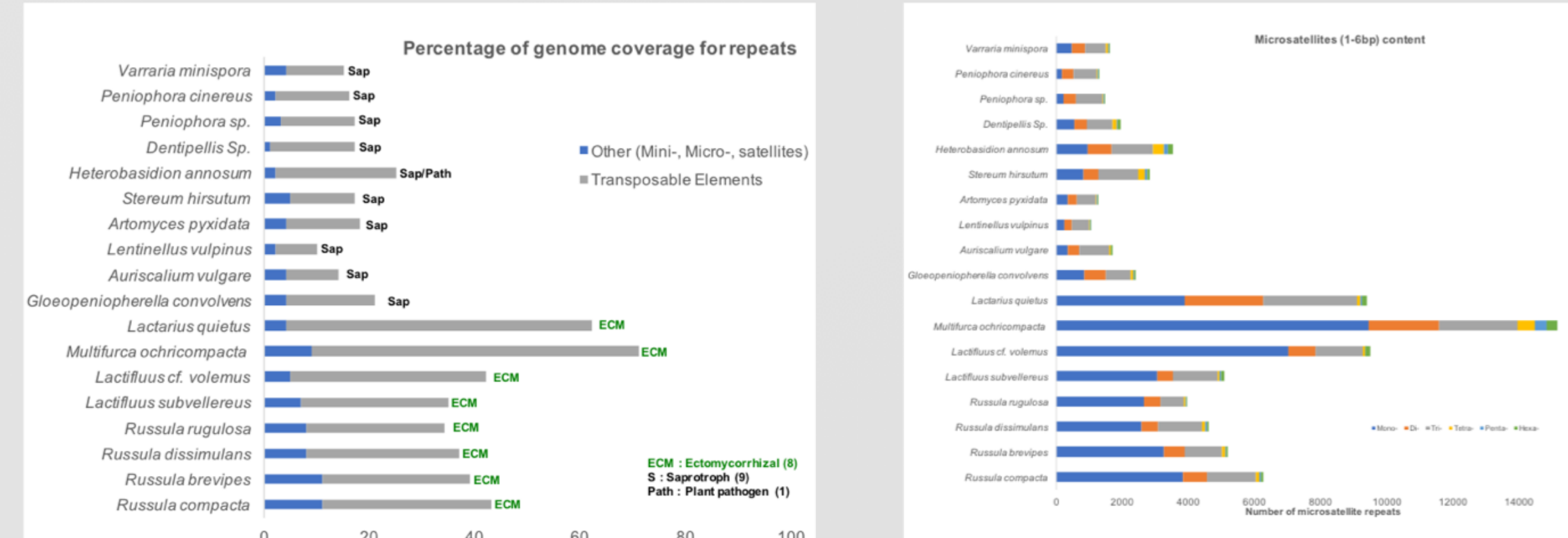
Phylogenomics demonstrates strong relationships between ectomycorrhizal (ECM) and saprotrophs (SAP) in the Russulaceae



Phylogenetic reconstruction. Maximum likelihood reconstruction of Russulales genomes in RAxML using 2,518 genes with a total of ~1.68 million AA residues and mid-point rooting at the longest internode. All Megabases (Mb) graphed as bars. Genomes of nodes received maximum bootstrap support following 1,000 bootstrap iterations.

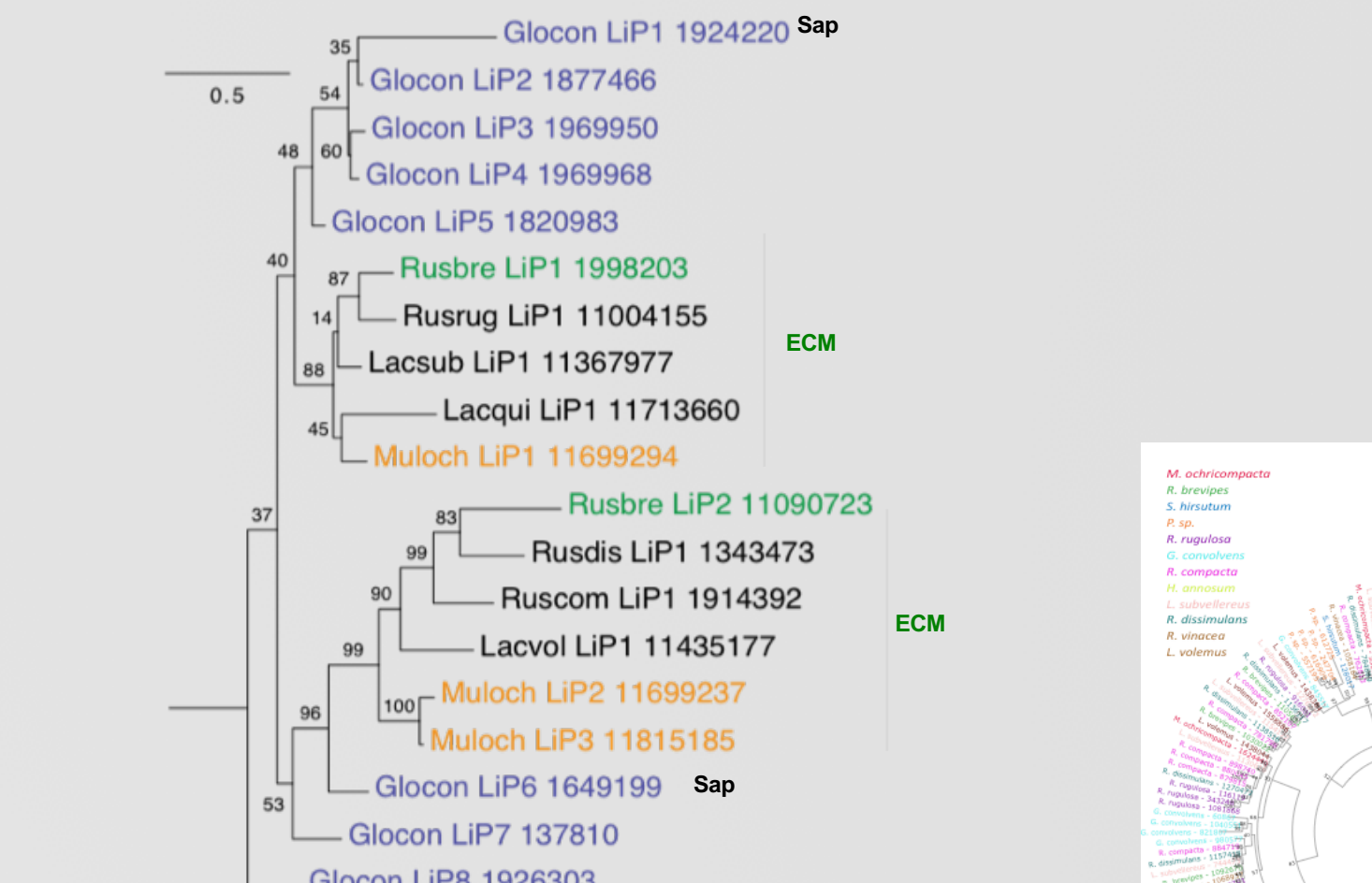
Genome size. Genomes are coded for nutritional mode as Ectomycorrhizal (ECM), Saprotrophic (Sap), and Pathogen (Path) with genome size in and mid-point rooting at the longest internode. All Megabases (Mb) graphed as bars. Genomes of ECM members of Russulales were found to be significantly larger than genomes of Sap members of Russulales ( $p=0.04$ ).

## Mycorrhizal Russulaceae genomes richer in repeats



Repeats identification: All genomes were scanned using the REPEATSCOPE Tool with default parameters. Transposable elements were identified by a de novo search combining LTR-Struct, Censor (using REPEATMASKER) and MITE tools.

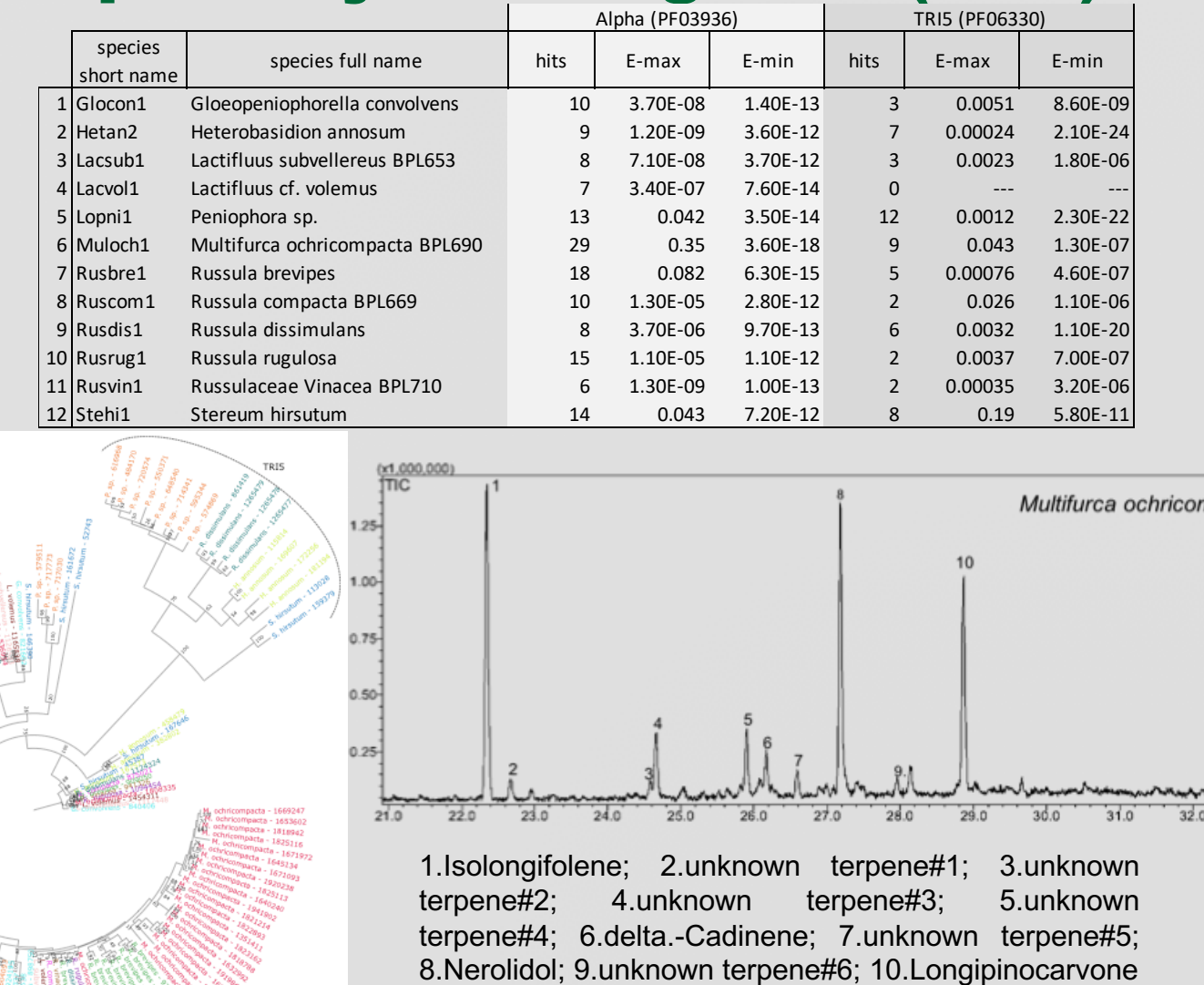
## Lignin peroxidase genes shared between ECM and SAP



Gene family evolution. Reconstruction of a class-II peroxidase gene family that codes for lignin peroxidase. Species with multiple gene copies are highlighted in color, including two ectomycorrhizal species (*M. ochricompacta* & *R. brevipes*) and the saprotroph (*G. convolvans*).

Microsatellites Identification: All genomes were scanned using the Microsatellite Identification Tool (MISA). MISA subsequently identified mono-hexanucleotide motifs of 5, 10, 12 repeats using default parameters.

## 12 genomes enriched in terpene synthase genes (TPS)



Terpene synthase genes. Number of terpene synthase genes found by sequence homology. Two types of Terpene synthases ( $\alpha$  and TRIS) were found in these 12 species. The species *MulochIX* has the largest number of TPS genes, which indicates the species-specific gene expansion. The phylogenetics tree was built using RAxML under the LG+G+I model. The number at the nodes indicate bootstrap values. Metabolite profiling by GC/MS confirms that production of terpenes by most are uncharacterized.

## Culture collection and co-culture systems allow experimental investigations



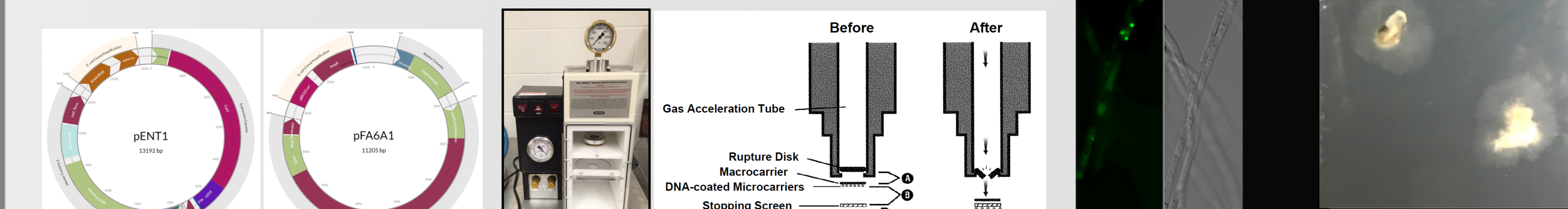
Culture collections: The culture collection, includes 10 different Russulaceae species, mostly isolated from fruiting bodies under native *Populus trichocarpa*

Greenhouse co-culture setting: Co-culture of *Multifurca ochricompacta*, *Lactarius populinus* and *Russula cerolens* in greenhouse with *Populus*. A: co-culture with *P. tremula* X *alba* 717-184; B: *P. trichocarpa* inoculated with *L. populinus* (left) vs. control (right); C: *P. tremula* x *alba* colonized by *L. deliciosus* (left) vs control (right), zoom-in on mycorrhizae in formation; D: *P. trichocarpa* - *L. populinus*; E: Quartz grits setting with *R. cerolens* on *P. trichocarpa* roots (with magnification).

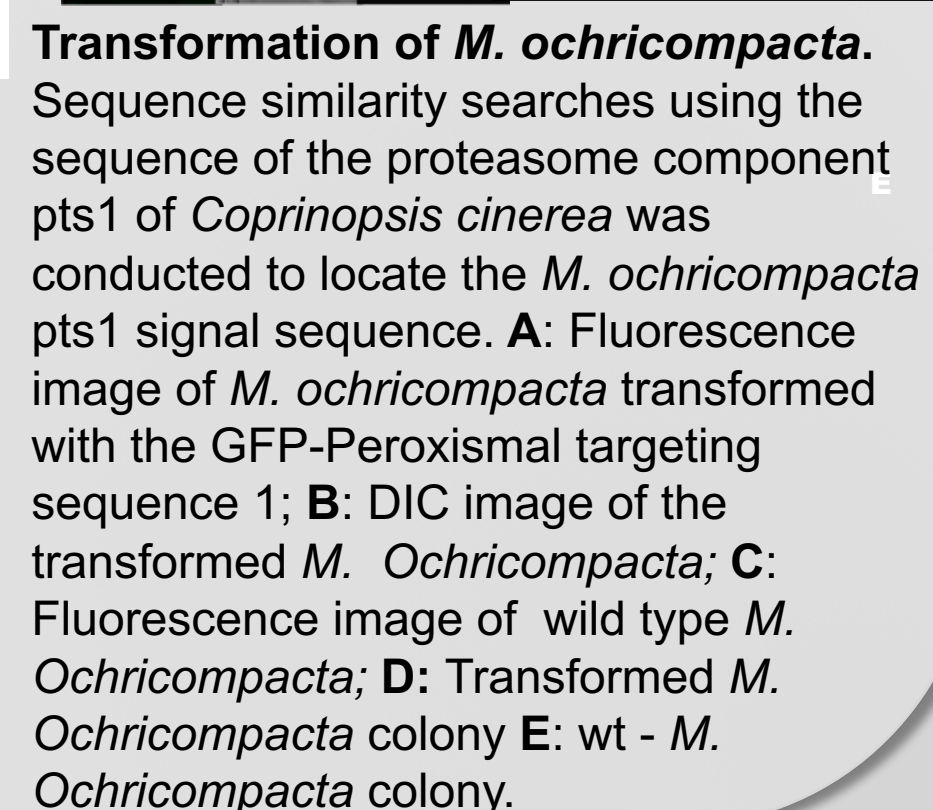
In vitro co-culture setting: Co-culture of Russulaceae species in vitro with *Populus*. A: co-culture with *P. tremula* X *alba* 717-184; B: *P. trichocarpa* inoculated with *L. populinus* (left) vs. control (right); C: *P. tremula* x *alba* colonized by *L. deliciosus* (left) vs control (right), zoom-in on mycorrhizae in formation; D: *P. trichocarpa* - *L. populinus*; E: Quartz grits setting with *R. cerolens* on *P. trichocarpa* roots (with magnification).

## A functional genetic tool-based on the CRISPR-Cas9 gene-editing system

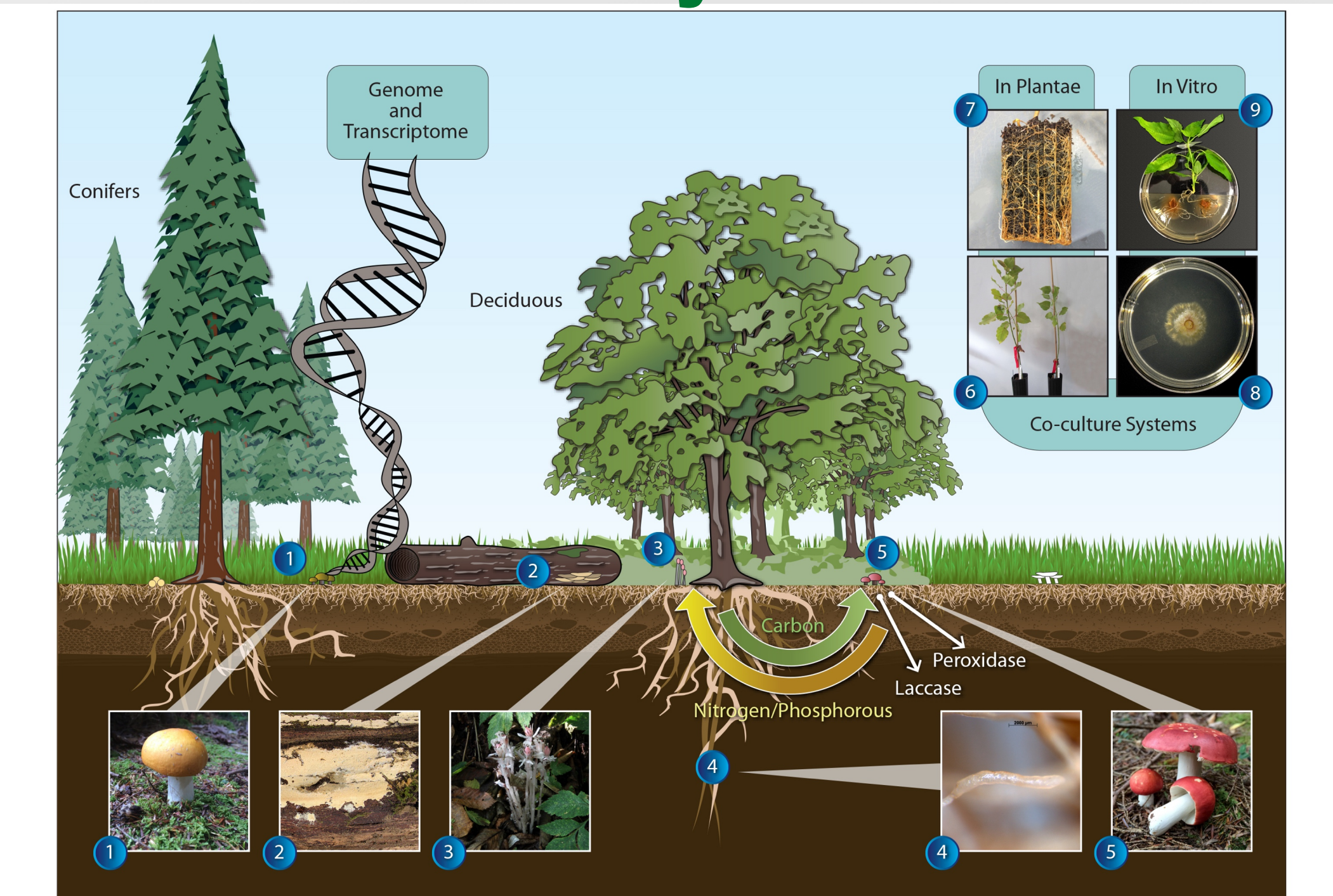
Recently ORNL developed a versatile CRISPR-Cas9 platform to genetically edit and engineer non-model filamentous fungi. This new genetic capabilities has been successfully tested on a couple of the Russulaceae species to date; further will be tested then.



Plasmids designed to be versatile in Biolistic Particle Delivery. A biolistic the three primary fungal phyla – system delivery system was employed due to Ascomycota, Basidiomycota and to the high-efficacy of transformation Zygomycota. Several modules obtained across the diverse fungal phyla. (promoter, reporter genes...) specific of each phyla have been synthesized and assembled using concomitant assembly strategies into the plasmids shown above.



## A package of new, complementary fungal model systems



Schematic representation of key ecological roles played by Russulaceae in forested ecosystems and a characterization of the experimental co culture system along with dense genomic and transcriptomic dataset. (1) *Russula paludosa* basidiocarp in association with conifers (sporocarp source material for genome and transcriptome). (2) The closest extant saprotrophic relative of Russulaceae, *Gloeopneophora convolvans*, as a putative lignin-degrading saprotroph fruiting on dead wood. (3) The achlorophyllous *Ericaceae* plant *Monotropa uniflora* parasitizes the mycorrhizal network of Russulaceae. (4) Ectomycorrhizal mantle of *Lactarius cf. deliciosus* colonizing *Populus trichocarpa*. (5) *Russula rugulosa* in association with hardwood trees secreting laccases and lignin peroxidases to scavenge nitrogen and phosphorus from soil organic matter for its plant host. (6) *Populus trichocarpa* colonized by Russulaceae exhibiting positive growth effects. (7) Root system of *P. trichocarpa* colonized by Russulaceae. (8) *Lactarius psammicola* isolated in culture from sporocarp tissue. (9) *Russula redolens* in association with *P. trichocarpa* in an axenic in vitro system.

## References:

Looney BP, Meidl P, Piatek MJ, Miettinen O, Martin FM, Matheny PB, Labbé JL. Russulaceae: a new genomic dataset to study ecosystem function and evolutionary diversification of ectomycorrhizal fungi with their tree associates. *New Phytologist*. 2018 Jan 30.

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