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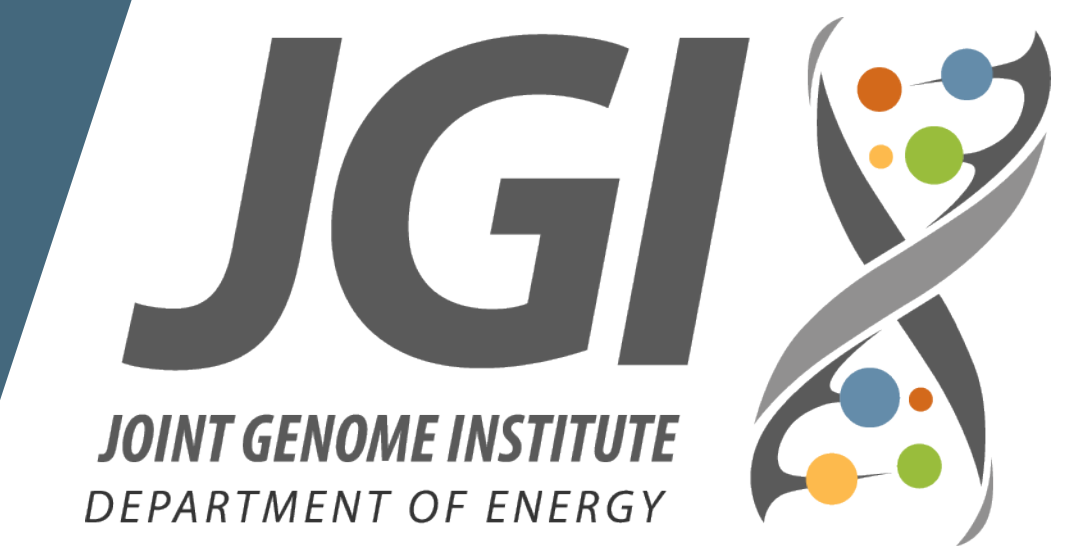
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Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white rot/brown rot paradigm for wood decay fungi

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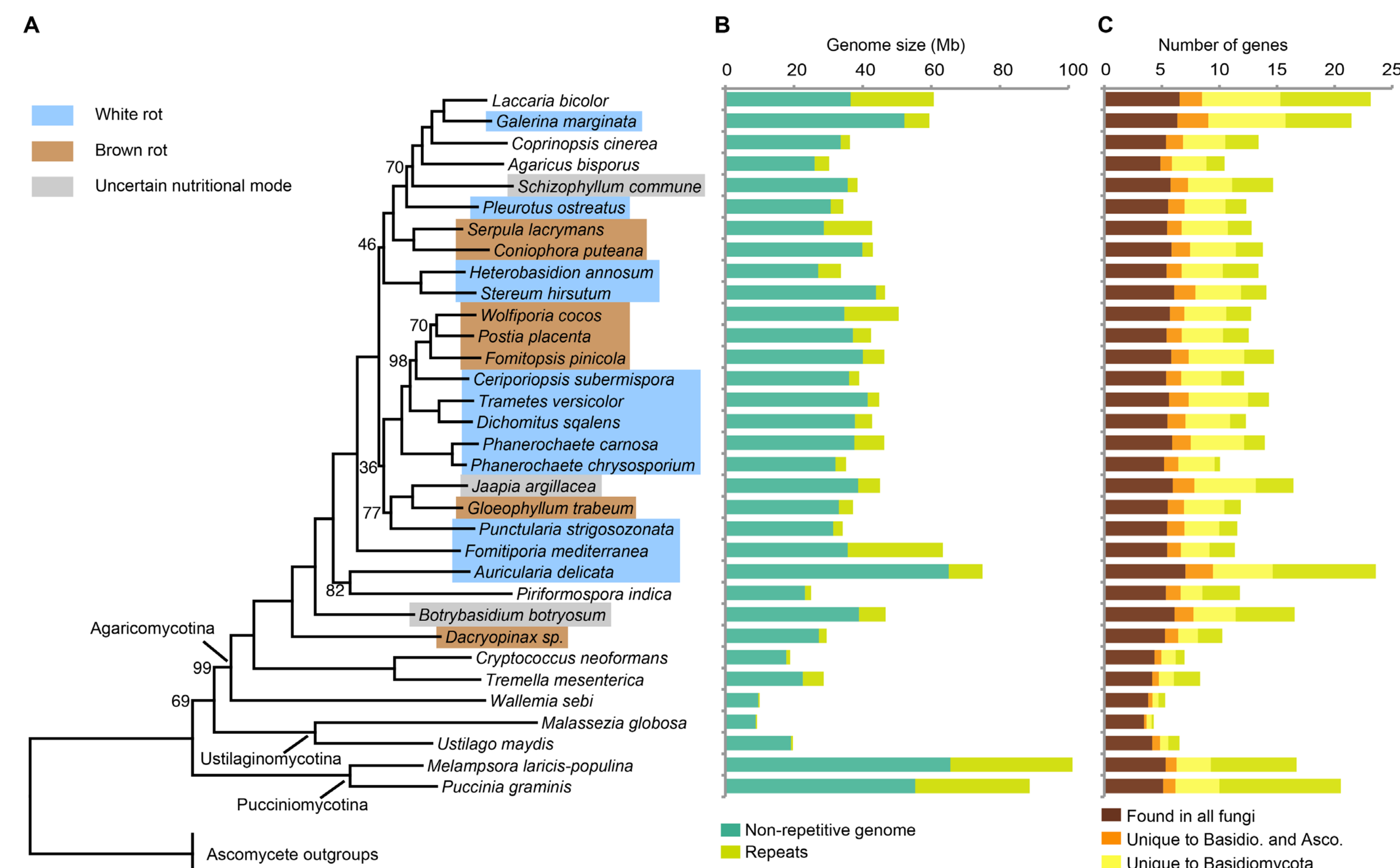
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Abstract

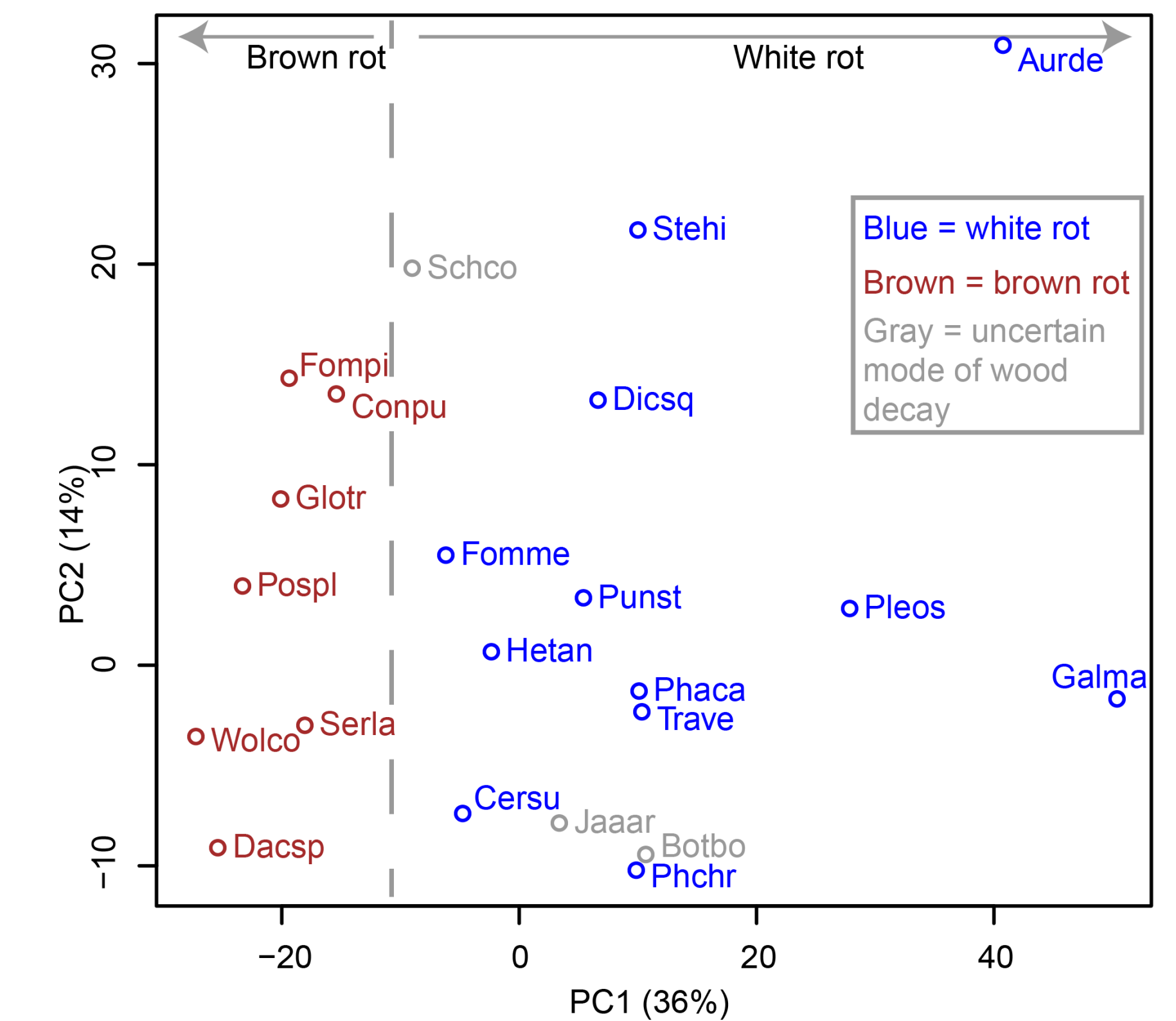
Basidiomycota (basidiomycetes) make up 32% of the described fungi and include most wood decaying species, as well as pathogens and mutualistic symbionts. Wood-decaying basidiomycetes have typically been classified as either white rot or brown rot, based on the ability (in white rot only) to degrade lignin along with cellulose and hemicellulose. Prior genomic comparisons suggested that the two decay modes can be distinguished based on the presence or absence of ligninolytic class II peroxidases (PODs), as well as the abundance of enzymes acting directly on crystalline cellulose (reduced in brown rot). To assess the generality of the white rot/brown rot classification paradigm we compared the genomes of 33 basidiomycetes, including four newly sequenced wood decayers, and performed phylogenetically-informed Principal Components Analysis (PCA) of a broad range of gene families encoding plant biomass-degrading enzymes. The newly sequenced *Botrybasidium botryosum* and *Jaapia argillacea* genomes lack PODs, but possess diverse enzymes acting on crystalline cellulose, and they group close to the model white rot species *Phanerochaete chrysosporium* in the PCA. Furthermore, laboratory assays showed that both *B. botryosum* and *J. argillacea* can degrade all polymeric components of woody plant cell walls, a characteristic of white rot. We also found expansions in reducing polyketide synthase genes specific to the brown rot fungi. Our results suggest a continuum rather than a dichotomy between the white rot and brown rot modes of wood decay. A more nuanced categorization of rot types is needed, based on an improved understanding of the genomics and biochemistry of wood decay.

Significance

Wood decay fungi have historically been characterized as either white rot, which degrade all components of plant cell walls, including lignin, or brown rot, which leave lignin largely intact. Genomic analyses have shown that white rot species possess multiple lignin-degrading peroxidases (PODs) and expanded suites of enzymes attacking crystalline cellulose. To test the adequacy of the white/brown rot categories, we analyzed 33 fungal genomes. Some species lack PODs, and thus resemble brown rot fungi, but possess the cellulose-degrading apparatus typical of white rot fungi. Moreover, they appear to degrade lignin, based on decay analyses on wood wafers. Our results indicate that the prevailing paradigm of white rot vs. brown rot does not capture the diversity of fungal wood decay mechanisms.



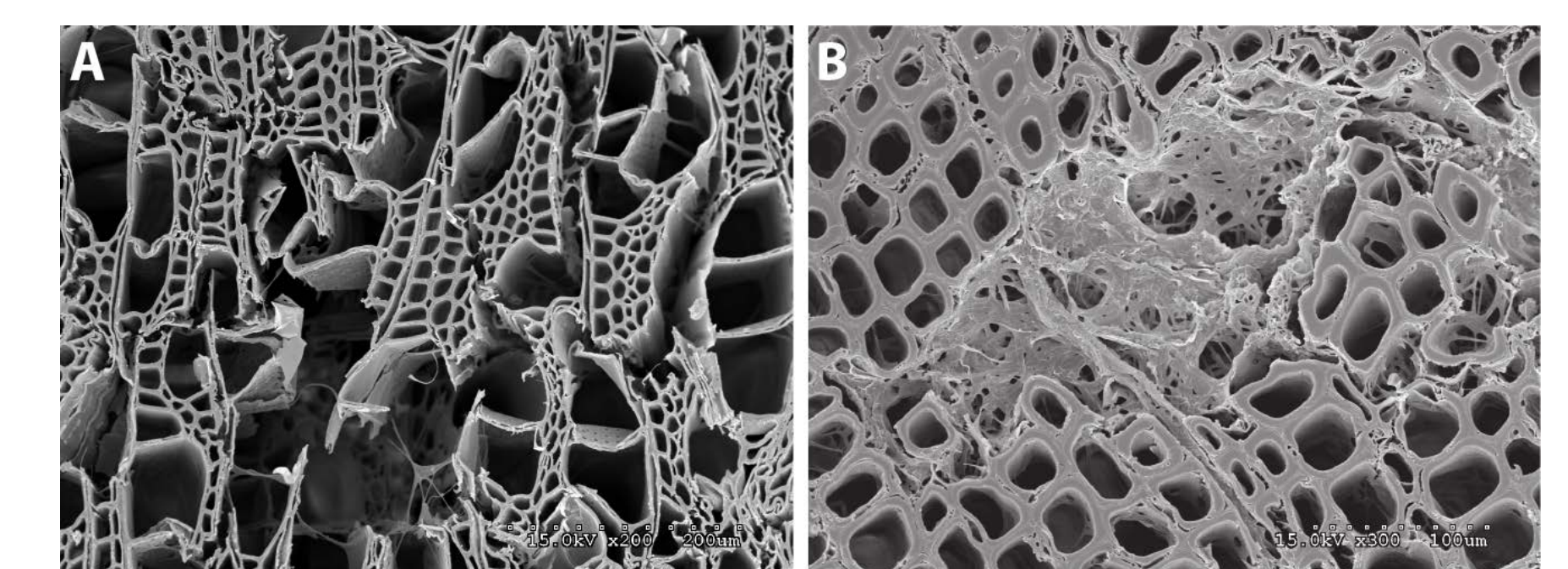
Phylogeny, genome size and repeats, and gene conservation among basidiomycetes. A. Maximum likelihood tree of 33 basidiomycetes based on concatenated alignments of 183 widely conserved genes. Ascomycete outgroups are omitted from the figure. Bootstrap values of branches are 100% except where indicated. Notice that white rot and brown rot phenotypes are polyphyletic. B. Repeat content in basidiomycetes is highly variant, ranging from 1% to 44%. C. Conservation of genes in basidiomycetes



Phylogenetic PCA of wood decaying fungi suggests *B. botryosum* and *J. argillacea* are similar to white rot fungi. Wood-decaying basidiomycetes plotted on the first two principal components from phylogenetic PCA of CAZymes (including lignin-related auxiliary activities) of the organisms. For this analysis we again relied on categorization of fungi as producing either white or brown rot whenever possible. Clear separation of known white and brown rotting fungi is seen along PC1. *B. botryosum* and *J. argillacea* group with the white rot fungi on PC1, and are closest to the model white rot fungus, *P. chrysosporium*. In contrast, *S. commune* is intermediate between white rot and brown rot species along PC1. Both white rot and brown rot species are widely distributed along PC2, which suggests heterogeneity in modes of wood degradation within these functional classes. Despite the lack of PODs (Fig. 2), PCA analysis suggests that *B. botryosum* and *J. argillacea* have wood decay properties that are similar to certain white rot fungi.

	White rot													Uncertain			Brown rot				Correlation with phenotype		Enzyme		
	Trave	Galma	Aurde	Fomme	Phchr	Cersu	Dicsq	Punst	Phaca	Pleos	Hetan	Schco	Stehi	Schco	Jaaar	Botbo	Fompi	Pospl	Wolco	Dacsp	Glotr	Conpu		Serla	W
Crystalline cellulose	23	54	43	6	30	17	17	21	27	33	17	17	5	24	28	0	0	0	1	1	2	8	CBM1	Carbohydrate-binding module family 1	
	1	3	2	2	1	1	1	1	1	1	1	1	1	1	3	0	0	0	0	0	2	1	GH6	Glycoside hydrolase family 6	
	4	8	8	2	9	3	4	5	5	16	1	3	2	5	7	0	0	0	0	0	2	0	GH7	Glycoside hydrolase family 7	
	18	19	20	13	15	9	15	14	11	29	10	16	22	15	32	4	2	2	0	4	10	5	AA9	Lytic polysaccharide monooxygenase	
	25	10	5	16	15	15	12	10	8	9	8	5	0	0	0	0	0	0	0	0	0	0	0	AA2	Class II peroxidase
Lignin	17	32	30	24	27	18	30	19	37	36	29	40	18	16	21	15	21	9	8	20	14	8	AA3_2	GMC oxidoreductase	
	9	15	8	4	7	3	9	9	6	16	5	8	2	4	5	4	3	4	3	2	6	3	AA5_1	Copper radical oxidase	
	7	8	0	10	0	7	11	12	0	11	14	15	2	1	0	5	2	3	0	4	6	4	AA1_1	Laccase	
	4	6	6	3	3	3	4	4	4	4	3	7	4	2	3	5	4	5	1	2	5	5	AA3_3	Alcohol oxidase	
	0	9	2	0	0	0	4	1	0	3	3	4	1	4	5	0	0	3	0	0	0	0	AA7	Glucoglucosaccharide oxidase	
	2	1	1	1	1	1	1	1	1	1	1	2	0	1	1	1	1	1	3	1	1	1	AA1_2	Ferroxidase	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	4	AA3_1	Cellobiose dehydrogenase
	1	0	3	1	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	1	0	AA3_4	Pyranose oxidase
	1	0	7	1	4	1	1	0	8	0	2	3	4	1	4	1	1	1	2	0	1	1	1	AA1_dist	Multicopper oxidase
	1	3	4	3	4	0	1	2	3	2	2	1	4	3	1	1	1	1	1	1	3	2	2	AA6	Benzoquinone reductase
Secondary metabolism	2	1	2	1	2	2	1	2	1	2	2	3	2	2	0	0	0	0	0	4	4	4	AA8	Iron reductase domain	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	3	AA4	Vanillyl alcohol oxidase
	1	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	FAS	Fatty acid synthase
	2	1	1	1	1	2	1	9	1	0	1	7	0	3	0	0	0	0	0	1	8	1	NRPS	Non-ribosomal peptide synthase	
	1	1	0	1	0	1	1	1	1	1	1	2	1	5	1	1	1	0	1	1	1	1	1	NR-PKS	Non-reducing polyketide synthase
	1	4	1	4	0	2	2	4	0	2	5	0	2	1	6	5	6	1	10	4	9	1	1	R-PKS	Reducing polyketide synthase
	5	4	3	13	1	6	9	3	1	7	6	6	2	11	5	8	6	6	2	6	7	5	1	TS	Terpene synthase

Lignocellulose-degrading and secondary metabolism in wood-decaying fungi. Notice a strict white/brown rot dichotomy with respect to the lignin-attacking PODs (AA2) and the CAZymes that target crystalline cellulose (CBM1, GH6, and GH7), and a continuum with other lignin-targeting enzymes. Organisms use the following abbreviations: Aurde = *Auricularia delicata*, Botbo = *Botrybasidium botryosum*, Cersu = *Ceriporiopsis subvermisporea*, Conpu = *Coniophora puteana*, Dacsp = *Dacryopinax* sp., Dicsq = *Dichomitus squalens*, Fomme = *Fomitiporia mediterranea*, Fompi = *Fomitopsis pinicola*, Galma = *Galerina marginata*, Glotr = *Gloeophyllum trabeum*, Hetan = *Heterobasidion annosum*, Jaaar = *Jaapia argillacea*, Phaca = *Phanerochaete carnosia*, Phchr = *Phanerochaete chrysosporium*, Pleos = *Pleurotus ostreatus*, Pospl = *Postia placenta*, Punst = *Punctularia strigosozonata*, Schco = *Schizophyllum commune*, Serla = *Serpula lacrymans*, Stehi = *Stereum hirsutum*, Trave = *Trametes versicolor*, Wolco = *Wolfiporia cocos*. Gene number is shaded red/white and independent contrasts correlation of enzyme with rot type shaded orange/blue.



Wood decay experiments indicating that mode of decay by *B. botryosum* and *J. argillacea* resembles white rot. A Micrograph of *B. botryosum* on aspen wood with vessel, fiber and parenchyma cell walls degraded. Mycelia are visible growing through the voids. B Micrograph of *J. argillacea* on pine showing an area within the wood where the fungus has caused a localized simultaneous decay of the cells. Residual cell wall material and mycelia fill the degraded zone. The removal of cellulose, hemicellulose, and lignin in these areas is consistent with white rot, whereas in brown rot we would observe residual lignin remaining after a diffuse depolymerization of the cellulose.

Conclusion

Our results indicate that the simple dichotomy of white rot vs. brown rot does not adequately reflect the diversity of mechanisms by which wood-rotting fungi obtain nutrition. Specifically, *B. botryosum* and *J. argillacea* show similarities to white-rotting fungi in PCA analysis of all predicted carbohydrate- and lignin-active enzymes and can degrade all components of wood, but they do not have the PODs that are a hallmark of white rot. *S. commune* is another putative white rot fungus (57) that lacks PODs, but it is quite distinct from *B. botryosum* and *J. argillacea* in the PCA analysis (and presumably also in its mode of wood decay). Therefore, we suggest that a more nuanced categorization scheme is needed to describe wood decay by species that degrade all cell wall polymers, including lignin, but lack PODs.