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**Conference Abstracts**

**Rhizospheric mycobiota associated with *Populus deltoides***

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*Populus deltoides* is a common riparian tree species in southeastern North America. *Populus* forms root associations with arbuscular, ectomycorrhizal, and endophytic fungi. To elucidate the structure of rhizospheric fungal assemblages on *Populus deltoides* we carried out a series of field campaigns in natural *P. deltoides* populations in NC and TN. Field studies were coupled with trap-plant experiments using cuttings of *Populus*. Fungal root communities were characterized through 454 amplicon pyrosequencing. Efforts were also made to culture fungi from *Populus* roots and characterize the arbuscular mycorrhizal community through spore studies. Our results indicate that in addition to hosting mycorrhizal taxa, *P. deltoides* supports a high diversity of fungal root endophytes. In fact, endophytic fungi accounted for the majority of sequences in field and trap-plant samples, and pure culture isolates from roots. There was considerable overlap between datasets. Well represented were ascomycete taxa belonging to the *Hypocreales*, *Chaetothyriales*, *Pleosporales* and *Helotiales*, and basidiomycete taxa belonging to the *Agaricales*, *Polyporales*, and *Atractiellales*. Spore and molecular data indicate that *Glomerales* and *Paraglomales* are the main arbuscular mycorrhizal associates of *P. deltoides*. Although fruitbodies of ectomycorrhizal taxa were uncommon in the field, a new truffle species was collected under *P. deltoides* in TN and NC field sites and has been described (*Tuber mexiusanum*). Additionally, fruitbodies of *Laccaria*, *Cortinarius*, and *Geopora* spp. were produced in our trap-plant experiments. Other ectomycorrhizal taxa recovered in our molecular surveys included *Inocybe*, *Hebeloma*, *Thelephora*, and *Russula* spp. Pure cultures of *Laccaria* and *Thelephora* spp. have been established.

**Diversity in root-associated fungal communities isolated from *Rhododendron maximum* in the southern Appalachian mountains**

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In recent years, *Rhododendron maximum* has become a dominant understory shrub in the southern Appalachian mountain range. This has profound implications for the future structure of the forest canopy, because *R. maximum* reduces understory diversity and tree seedling recruitment by creating dense shade. The chemistry of the soil beneath *R. maximum* is altered by the deposition of litter containing phenolic compounds, some of which are broken down and returned to the shrubs by the ericoid mycorrhizal communities associated with their roots. This community of fungi is essential to the success of *R. maximum*, as the fungi allow the plants to thrive in nutrient-poor soils by acquiring nitrogen from organic sources. We expect that there are differences in the edaphic conditions across the growing season that may affect diversity and community composition of the associated fungi. In this study, I attempt to identify the

fungi that make up these communities and to reveal whether there is a seasonal shift in community structure over the course of a year. I sampled roots during the spring, summer, and fall of 2011 from two transects of ten *R. maximum* plants each located at the Gilley Research Station in North Carolina. I extracted genomic DNA from the fungi isolated from root sections and amplified the ITS region of the samples from the spring and summer. I will analyze and present an initial comparison of the diversity and composition of the fungal communities associated with *R. maximum* across the first two seasons.

### **A seasonal assessment of *in situ* marine fungal biofilm communities on artificial reefs**

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This study was the first characterization of natural mixed-species fungal biofilm communities on artificial reefs in the marine environment. Previous fungal biofilm studies have failed to observe the naturally-occurring biodiversity in coastal areas, which are the most utilized zone of the marine environment. Fungi have evolved to form biofilms for protection, to communicate via quorum sensing, to gain access to nutrients, and to exchange genetic information. Furthermore, these communities fuel many primary consumers that support artificial reef habitats. Fungi, along with bacteria, archaea, protists, algae, and diatoms, quickly form a complex biofilm on immersed surfaces in seawater. This settlement and succession calls for further examination, given the major trophic implications therein, as properties of this biofilm will influence future establishment of other marine organisms. For this study, an optimized extraction technique for fungal DNA from biofilm was developed. The fungal biofilm communities were characterized by morphological identification, ITS T-RFLP analysis, and by ITS gene sequencing. The role of seasonality and other abiotic factors in structuring these communities are also being examined. Fungal biodiversity in these biofilms is high, spanning over 25 genera, including species from the Ascomycetes, Zygomycetes, and potential novel yeast species. This assessment of fungal biofilm communities yields insight into artificial reef microbial succession and functionality.

### **Community composition and diversity of ericoid root associated fungi at different elevations in the southern Appalachian Mountains**

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Mountains are among the most floristically diverse areas on earth. This is due to the mountain's perturbation of weather fronts frequently causing increased precipitation, and the temperature gradient caused by ascending higher into the atmosphere. Due to the changes in temperature and precipitation across elevational gradients, associated differences in edaphic conditions such as C/N are also expected to occur. Differences in moisture, temperature, and edaphic conditions may drive changes in the diversity and composition of root associated fungi. *Rhododendron maximum* is an endemic species found at all elevations in the Southern Appalachians. This study focuses on the dynamics of ericoid mycorrhizal fungal diversity and colonization levels in *R. maximum* roots along elevational gradients in the southern Appalachian Mountains. To address this we sampled roots and soils from three iso-elevational transects from high to low elevations at two locations. Root associated fungi were cultured from the roots. DNA amplified from these cultures was typed by restriction fragment length polymorphism (RFLP) analysis of the ITS regions and representatives from each group were sequenced. Root colonization was quantified by counting

mycorrhizal hyphae at random locations within the root using light microscopy. Preliminary analysis indicates that colonization, diversity, and species composition of fungi within *R. maximum* roots may be correlated with changes in elevation. This research will provide valuable information on how *R. maximum* interacts with fungi that inhabit the range of environments in which this species is found.

### **Firing range soils yield a diverse fungal community capable of Pb-mineral solubilization and organic acid secretion**

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Lead (Pb) contaminated soils represent severe environmental problems as well as a significant hazard to human health. Many species of fungi are capable of transforming insoluble metal compounds into soluble derivatives, an environmentally important process when these fungi constitute a significant portion of the overall soil community. The objective of this study is to understand the potential capacity of the soil fungal community for Pb-mineral solubilization via organic acid secretion at an abandoned small-arms firing range in east Tennessee. Six soils were collected from locations around the abandoned firing range; these soils were analyzed for total Pb content and used to derive fungal isolates via serial dilution plating. Isolates were screened for organic acid production and solubilization of lead carbonate at concentrations ranging from 3mM to 18mM. Soil Pb concentrations at the site ranged up over 2500 mg kg<sup>-1</sup> and the number of cultivable fungi was not significantly different between soil Pb levels. We obtained 220 total isolates, 26% of which were capable of acidification of two different types of growth media. Types and amounts of organic acids secreted varied with phylogenetic affiliation and several of these species were also capable of solubilizing lead carbonate at all tested concentrations. Many of these acid-producing fungi were also capable of solubilizing lead-carbonate, a Pb-mineral frequently found at both active and abandoned firing ranges. This could mean these fungi are capable of not only acidification but also solubilizing Pb-minerals in their native soil habitats.

### **Ectomycorrhizal diversity associated with seedlings and mature trees in the Neotropics**

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The ectomycorrhizal (EM) leguminous canopy tree *Dicymbe corymbosa* forms monodominant stands in the tropical forests of the central Guiana Shield region. Within these stands, *D. corymbosa* maintains a large bank of shade-tolerant seedlings that result from mast fruiting events. It has been proposed that common mycorrhizal networks support *Dicymbe* seedling recruitment and facilitate persistent monodominance, and that an absence of appropriate symbionts may inhibit these seedlings from successfully establishing in nearby mixed forests of AM-associated trees. Little is known, however, about the actual fungi forming mycorrhizae with these seedlings. Using molecular methods we examined EM fungi associated with roots of *D. corymbosa* seedlings within monodominant stands and along transition zones into AM-dominated

mixed forest. Seedlings within monodominant stands hosted significantly higher EM fungal species diversity than those in transition zones, but no effect of distance from parent tree was detected in either location. EM fungi commonly recovered on seedlings included members of the *Clavulinaceae*, *Inocybaceae*, *Russulaceae*, and *Thelephoraceae*, all of which have been consistently found on adult *D. corymbosa* roots. The *Boletaceae*, however, were notably under-represented on seedlings.

### **Testing *Geomyces pannorum* for enzymes that could play a role in the ecology and pathogenesis of White-nose Syndrome agent *G. destructans***

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White-nose Syndrome, an emerging disease of hibernating bats, has killed over 5.5 million bats in the northeastern United States and Canada. Its rapid spread across the country, beginning in New York in 2006 and currently reaching as far south as Alabama, has led to numerous cave closures and potentially dramatic agricultural costs. The fungus *Geomyces destructans*, first isolated from infected bats, was recently shown to be the etiological agent of the disease. Understanding the natural history of *G. destructans*, particularly its ecology in bat hibernacula, is crucial for disease management. *Geomyces*, the anamorph of *Pseudogymnoascus* (Leotiomycetes), consists of 11 saprotrophic species known predominately from cold soils. The best understood species, *G. pannorum*, causes an opportunistic skin infection in immunocompromised patients. Using *G. pannorum* as a model organism, we tested for several enzymes that could play a role in the ecology of *G. destructans*. Already known for its keratinase and chitinase production, *G. pannorum* also tested positive for  $\beta$ -hemolysis. Assays using three cellulose sources found that *G. pannorum* was weakly positive for  $\beta$ -glucosidase, but negative for endoglucanase and cellobiohydrolase activity. We also determined that *G. pannorum* produces iron-scavenging siderophores that permit growth in iron-poor environments and can be key components of pathogenesis. These enzymes, if also present in *G. destructans*, may be important components in host infection and proliferation in the environment. Future research will investigate *G. destructans* enzyme activity and growth in diverse cave sediments.

### **Accelerated fungal evolution with metabolic gene clusters**

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Shifts in fundamental ecological niche and competitive strategies are apparent in many lineages of Ascomycete and Basidiomycete fungi. The recent explosion of fungal genome sequencing projects has enabled investigations of the molecular underpinnings of these shifts. Through comparative investigations of gene phylogenies and genomic clustering, we have documented several cases of metabolic gene clusters facilitating fungal evolutionary leaps, involving horizontal gene transfer, concerted pathway loss and metabolic rewiring. Polymorphic loci encoding alternative pathways as gene clusters may serve as reservoirs for metabolic diversity underlying the exceptional adaptability of fungal genomes.

## Bayesian inference of species diversification rates using DivBayes and SubT

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One of the major tasks of evolutionary biology is to explain the vast diversity of organisms. Although it is controversial on what basis to recognize species, species are a basic unit of diversity. However, different species recognition concepts tend to agree the further the speciation process has progressed. The diversity of species in a specific group depends on the age of the group and the rate at which it has diversified through time. Differences in diversity between groups may therefore depend on either different ages or different rates of net diversification (speciation minus extinction). In this talk I will present DivBayes and SubT, two programs using Bayesian statistics to estimate diversification rates. DivBayes uses species number and clade age for the estimation while SubT uses node depths in an ultrametric tree. SubT is also able to account for missing taxa in the estimation, a characteristic that may otherwise bias the estimate.

## Dispersal of ectomycorrhizal fungi by mammals and its implications for Pinaceae invasion: results from Isla Victoria, Argentina

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Invasive pines in New Zealand and Argentina are not associated with native EM fungi to any great extent, and tend to form symbiosis with non-indigenous fungi. Establishment of Pinaceae in such locations requires the co-introduction of compatible ectomycorrhizal (EM) fungi. Dispersal vectors of compatible ectomycorrhizal fungi from established plantations into native forests include wind and mammals. This project aimed to determine which local mammals can act as vectors of the mycorrhizal fungi on Isla Victoria, Argentina. We used non-indigenous deer and wild boar feces collected on the island to inoculate Pinaceae in a greenhouse experiment. We found that both mammals are dispersing fungi compatible with Pinaceae. Approximately 15 percent of the seedlings growing with deer fecal pellets were colonized and 30 percent of the seedlings growing with wild boar feces were colonized by EM fungi. Rodents are also well known to disperse fungi but observational evidence (by trapping) confirms that rodents are rare in areas adjacent to plantations of non-indigenous trees, so their ecological role seems to be insignificant. Very few fungal species were able to disperse via animal feces, possibly owing to animal preferences and/or the differential ability EM species to germinate after being ingested. The hypogeous genus *Rhizopogon* was the most abundant genus found. Wild boar, a recent addition to the local fauna, seems to be a key agent dispersing the fungi, so they may contribute to promote the invasions of pine trees. These results show that dispersal of ectomycorrhizal fungi can have an important role controlling the invasion of associated plant species.

## Dispersal of fungal and slime mold spores by vertebrate and invertebrate vectors

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Numerous animals, including both vertebrates and invertebrates, serve as vectors for the spores of fungi and slime molds in terrestrial ecosystems. This is particularly important for dictyostelids (cellular slime molds), since the spores in this group of organisms are encased in a mucilaginous matrix that dries out, essentially eliminating the possibility of the spores being dispersed by wind. The latter is thought to be the primary method of dispersal for the spores of myxomycetes (plasmodial slime molds) and most fungi, but the consistent presence of spores in fecal material and on the body surface of animals ranging from insects to salamanders, birds and mammals suggests that many different invertebrates and vertebrates play an important but as yet understudied role in at least short-distance dispersal. Some species of birds travel considerable distances over relatively short time periods, especially during the course of annual migration and it seems likely that they would be able to serve as vectors for long-distance dispersal. Some evidence that this happens for dictyostelids already exists, and circumstantial evidence appears to indicate that birds were responsible for creating advance centers of infection for the chestnut blight fungus when the latter spread rapidly throughout the eastern United States in the early part of the last century. Truffles and other fungi are sought out by mammals yet the link between animal mycophagy and dispersal in North America is still largely unknown.

## Spatiotemporal dynamics of brown rot epidemics and fine-scale structure of *Monilinia fructicola* populations within peach tree canopies

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We examined the spatio-temporal dynamics of brown rot disease (caused by *Monilinia fructicola*) and the structure of the corresponding pathogen population within individual, intensively mapped peach tree canopies. Across 3 years, a total of 12 trees were monitored for disease development throughout the season, tagging each individual symptom (blossom blight, green fruit rot, preharvest fruit rot, and twig canker) and mapping it in three dimensions using a magnetic digitizer. In addition, *M. fructicola* was isolated from each of the mapped symptoms. Trees had between 126 and 739 fruit total and a final preharvest fruit rot incidence of 12.3 to 78.2%. The index of disease aggregation was negatively correlated with disease incidence ( $r = -0.756$ ,  $P < 0.0001$ ), i.e., the lower the disease incidence, the greater the relative degree of aggregation of affected fruit in the canopy. DNA from all 718 single-conidial isolates obtained from a subset of six trees was evaluated with 20 polymorphic SSR markers. For one tree analyzed thus far, haploid diversity for early-season blossom blight isolates was higher than that from mid-season green fruit rot and late-season preharvest fruit rot epidemics ( $h = 0.522, 0.355, 0.393$ , respectively). No spatial genetic structure was detected for either early- or mid-season periods, whereas significant autocorrelation among genetic distances was observed at spatial distances up to 1.5 m during the preharvest interval. The presence of such fine-scale patterns of *M. fructicola* populations within individual trees is likely due to a localized zone of influence in pathogen spore dispersal. Preliminary analysis for 84 of 176 isolates obtained from tree O'Henry 26 in 2009 was conducted. Examining Each period was also analyzed for evidence of population structure, but no structure was observed for either early- or mid-season periods, even when combined (Fig.

2A). Population structure was detected for the late-season fruit rot period, showing significant genetic relatedness of isolates up to 1.25 m in distance.

**Like a rock? Lack of sequence variation in clinical isolates of the dermatophyte *Trichophyton rubrum***

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*Trichophyton rubrum* (Ascomycota) – the cause of athlete’s foot, ringworm and nail infections – is the most common dermatophyte affecting humans. Despite availability of an annotated genome, this microbe’s pathogenicity, epidemiology, and genetics remain poorly understood due to an inability to conduct genetic crosses, lack of adequate sampling, and apparent clonality. The only evidence of sexual mating in *T. rubrum* is indirect - a single genetic hybrid was formed between *T. rubrum* and *Arthroderma simii*, the teleomorph of a related species (*T. mentagrophytes*). *Trichophyton rubrum* lacks a known teleomorph, and although its genome contains mating type genes, only a single *MAT* allele (*MATI-1*) is known. This study has three aims: (1) survey sequence variation in our 194 clinical samples using sequence-based and previously-developed variable number tandem repeat (VNTR) markers; (2) use paired-end Illumina sequencing to identify additional polymorphism within and among strains belonging to different VNTR types, and (3) search for evidence of (same-sex) mating using genetic and culture-based methods. First, we sequenced 7.5 kb of mainly non-coding regions located within 20 kb of the *ABC1* and *CAP59* genes in ten *T. rubrum* isolates collected over a span of ten years. These sequences revealed six singleton polymorphisms, which distinguished only half of the strains. We are now transitioning to VNTR genotyping and whole genome Illumina sequencing to characterize the diversity more completely. Lastly, in order to test for formation of ascomata (sexual structures), we will co-culture ten of our *T. rubrum* isolates and a *T. tonsurans* isolate (*MATI-2*) in single, pair-wise and triple combinations on dermatophyte mating medium. Cultures with extensive hyphal coiling (indicative of early sexual development) will be dissected and examined for presence of ascomata or ascospores. Thus far, our results provide no evidence of sexual reproduction and re-confirm low sequence variation in *T. rubrum* clinical isolates.

**A systematic revision of species of Clavariaceae (Agaricales) from the Pacific Northwest**

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The diversity of Clavariaceae has been underestimated in the Pacific Northwest of North America and thorough taxonomic revisions are much needed. Molecular methods of species recognition have not been extensively used in the Clavariaceae and are found to elucidate species level diversity, which had been previously unidentified. Several species complexes have been observed that feature high morphological and nucleotide sequence diversity. Morphological differentiation of some molecularly distinctive species is not yet possible, and cryptic species may be present. A monographic revision of the Clavariaceae in the Pacific Northwestern North America with taxonomic keys, illustrations and descriptions is being compiled and will be published pending further investigation. At present, thirty-four species in the family have been identified based on herbarium and recent field collections. Eleven of these are tentatively considered undescribed. Overall, the Pacific Northwest is represented by two species of *Camarophylloopsis* (one new), ten species of

*Clavaria* (one new), one species of *Clavicornia*, seven species of *Clavulinopsis* (three new), four species of *Mucronella*, and ten species of *Ramariopsis* (six new).

#### **A preliminary account of a new genus to accommodate *Cosmospora vilior* and related species**

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*Cosmospora* sensu Rossman (Ascomycota, Hypocreales, Nectriaceae) was erected to accommodate nectroid fungi with small, reddish, KOH+, smooth, thin-walled, laterally collapsing when dry, non- or weakly stromatic perithecia. Recently, the group was found to be polyphyletic based on molecular data, and has been segregated into multiple genera. However, not all *Cosmospora*-like fungi have been treated systematically. Some of these species include *C. vilior* (Starbäck) Rossman & Samuels and many species often labeled as “*Cosmospora* sp.” The objectives of this research were to designate an epitype for *C. vilior*, which has been misapplied based on examination of the type specimen, and determine its phylogenetic position within *Cosmospora* sensu lato and the *Nectriaceae*. A multilocus phylogeny was constructed based on six loci (ITS, LSU, RPB1, MCM7, TEF1, and TUB) to estimate the species tree. Results from the phylogenetic analyses indicated that *C. vilior* forms a monophyletic group with other *Cosmospora*-like fungi that have an acremonium-like anamorph and that parasitize *Eutypa* and *Eutypella* (Diatrypaceae). The group is phylogenetically distinct from other previously segregated genera, and for that reason, a new is described to accommodate these species.

#### **Distribution and phylogenetic analyses of the genus *Auricularia* in the southeast United States**

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A recent discovery of *Auricularia fuscossuccinea* in east Tennessee has led to a study of the diversity within the genus *Auricularia* in the southeast United States. *Auricularia* currently comprises 10-15 well-established species, and most are considered to have intercontinental to cosmopolitan distributions. Though most regional field guides treat only one or two species for the southeast USA, five species, *A. fuscossuccinea*, *A. auricula-judae*, *A. mesenterica*, *A. polytricha*, and *A. delicata* have been reported from the region. This study seeks to evaluate current species concepts using phylogenetic and morphological analysis in order to understand regional diversity as well as provide new reports of geographical distributions. Historical collections from the University of Tennessee Herbarium, as well as fresh material observed in the field, have been examined and sequenced at two loci, ITS and *rpb2*. Phylogenetic trees have been constructed using maximum likelihood analysis, and preliminary results show diverse clades in need of taxonomic revision. Variation within traditional taxonomic characters has made it evident that additional characters are required to reflect the genetic diversity in the group. To this end, a new taxonomic character, the schizomedullum, is discussed as an important character to distinguish a new species from the morphologically similar species *A. delicata*. In addition, a reticulate, meruloid hymenial surface can no longer be considered a character unique to *A. delicata*.



## **Hygrophoraceae of the Great Smoky Mountain National Park - how many have misapplied European names?**

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The family Hygrophoraceae includes the brightly colored mushrooms commonly known as the wax-caps. From 2004-2008, we documented 40 species and varieties in the Hygrophoraceae. Of these, half bear European names, but 16 of them differ from the European species based on morphology and/or DNA sequences. Thus, these Smokies taxa have misapplied European names and most will need new names. Three European species also bear misapplied American names. Among the correctly named European species found in the Smokies, *Neohygrocybe ingrata* has not previously been reported in North America, but previous collections of this species have probably been misidentified as *Hygrocybe nitrata*. *Hygrocybe reidii* collected in the Smokies represents the first record for the USA, but this species has previously been reported from North America in Québec, Canada. Previous American collections of *H. reidii* have been misidentified as *H. cantharellus*, *H. coccinea* or *H. punicea*. DNA sequences of collections from the Great Smoky Mountain National Park have played a critical role in delineating species as well as in revision of the family Hygrophoraceae.

## **A phylogenetic evaluation of the tribe Leucopaxilleae: polyphyly, the LPD grade, and novel taxa from the Southern Appalachians**

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The tribe Leucopaxilleae Singer comprises eight genera that have been placed at various taxonomic ranks. Phylogenetic analysis of a four-gene region supermatrix (nuclear rRNA regions, *rpb2*) shows the tribe to be highly polyphyletic, with genera distributed in the Tricholomatoid, Marasmioid, Hygrophoroid and Pluteoid clades. *Leucopaxillus*, *Porpoloma* and *Dennisiomyces*, occur in the Tricholomatoid clade. *Dennisiomyces*, primarily a neotropical genus, has not been previously considered part of the Leucopaxilleae. We present preliminary data that suggest *Dennisiomyces* is closely related to *Leucopaxillus* and *Porpoloma* forming the 'LPD' grade (or clade) from which the ectomycorrhizal (ECM) genus *Tricholoma* appears to be derived. These results also suggest that *Leucopaxillus* and *Porpoloma* are polyphyletic. Additionally, three unknown species that form a clade nested in the 'LPD' grade have been collected recently from the Southern Appalachians of the southeast United States. They are distinguished from other species in the grade by possession of distinct cheilocystidia and pleurocystidia, presence of clamp connections, and inamyloid smooth spores. At present, we are unable to ascribe any known genus to this apparent endemic group.

**The “Big Ditch” project: Trans-Atlantic mushroom disjunction tested using multiple taxonomic tools**

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Written descriptions and illustrations by historical Euro-Scandinavian mycologists crossed the Atlantic to America, where they were matched to fungi of the New World. Doubts about the accuracy of name applications across the ocean have persisted, but molecular phylogenies now provide a new level of resolution. Our research targets fleshy fungi whose names originated in Europe, but which bear the same name and superficial form in eastern North America. Several examples can be identified in which DNA sequences reveal differences across the ocean, including saprophytic (*Marasmius rotula*; *Baeospora myosura*; *Sparassis crispa*) and ectomycorrhizal (*Strobilomyces strobilaceus*; *Tricholoma populinum*) fungi. In addition, it appears that genes for sexual compatibility and recognition are more highly conserved than the internally transcribed spacer (ITS), but morphological differences vary with the individual taxon. Implications of this research include the necessity for new names for heretofore cryptic taxa on both continents and resultant adjustment of biodiversity inventory reports.