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Amanita thiersii is a saprotrophic fungus expanding its range in the United States

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Abstract: Although most species in the genus *Amanita* form ectomycorrhizal associations, a few are reported to be saprotrophs living in grassland habitats. Little is known about the ecology and distribution of these free-living *Amanita* species. We describe the ecology of *Amanita thiersii*, a species commonly collected in lawns throughout the Mississippi River Basin. Stable isotopes of carbon, transcriptomic sequences and patterns of growth on complex carbon sources provide evidence for *A. thiersii* as a saprotrophic species. Sporocarps of *A. thiersii* are less depleted in ^{13}C compared to published data for ectomycorrhizal fungi, supporting a saprotrophic mode of carbon acquisition in the field. Orthologs of cellulase genes known to play key roles in the decomposition of cellulose in other basidiomycetes were identified in a transcriptome of *A. thiersii*, establishing that this species has the genetic potential to degrade cellulose. *Amanita thiersii* also can use artificial cellulose or sterile grass litter as a sole carbon source. DNA sequences of three nuclear gene regions and banding patterns from four inter-simple sequence repeat markers were identical across 31 populations from throughout the known range of the species, which suggests the genetic diversity of *A. thiersii* populations is low. Maps of *A. thiersii* collections made from the 1950s until present suggest this species is experiencing a range expansion. It was reported first in 1952 in Texas and now occurs in nine states north to Illinois. These data provide an ecological context for interpreting the genome of *A. thiersii*, currently being sequenced at the United States Department of Energy's Joint Genome Institute.

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Introduction

Most of the 500-plus species in the genus *Amanita* form ectomycorrhizal associations with woody plants (Yang et al. 1999). However sporocarps of approximately 15 species of *Amanita* are typically collected at some distance from potential woody plant hosts, usually in natural or artificial grasslands. These *Amanita* species have been identified tentatively as saprotrophs (Bas 1969). However the saprotrophic status of these species has never been experimentally confirmed, and almost nothing is known about their natural histories. While most species of Agaricales in grass-dominated ecosystems are saprotrophs that decompose grass litter, some species are pathogens of grasses while other species form ectomycorrhizal associations with sedges (as reviewed in Griffith and Roderick 2008). These latter cases highlight the problems associated with making assumptions about the trophic status of fungi based simply on habitat.

Several approaches, including stable isotopes, the growth of a fungus on different carbon sources and the identification of putative functional genes, can be used to infer the trophic status of poorly understood fungi, including these free-living *Amanita* species. Stable isotopes of carbon are widely used to distinguish between ectomycorrhizal and saprotrophic fungi (e.g. Henn and Chapela 2001, Hobbie et al. 2001, Trudell et al. 2004) and have been used to infer the ecology of other poorly understood species (e.g. Wilson et al. 2007). Because ectomycorrhizal fungi use carbon taken from host plants that is more depleted in ^{13}C, as compared to carbon from dead organic matter, sporocarps of ectomycorrhizal fungi are generally more depleted in ^{13}C than saprotrophic fungi (Mayor et al. 2009). Saprotrophic fungi also generally have a much greater capacity than ectomycorrhizal fungi for growth on complex carbon sources, for example cellulose, and the growth of a fungus on different carbon sources can provide a useful basis for understanding its saprotrophic potential (Maijala et al. 1991). Genome and transcriptome sequencing can identify specific genes considered hallmarks of an ecological niche. For example, to completely decompose cellulose polymers in dead organic matter, saprotrophic fungi typically use three types of cellulases: endo-1,4-β-glucanases, cellobiohydrolaes, and β-glucosidases (Baldrian and Valášková

2008). Saprotrophic fungi tend to have a diversity of the genes encoding these cellulases, while ectomycorrhizal fungi generally have fewer copies of these genes or may lack some cellulases, such as glycoside hydrolase family 7 cellobiohydrolases (Martin et al. 2010, Nagendran et al. 2009). The presence of these genes in a genome or transcriptome provides strong evidence for the genetic potential of a fungus to obtain carbon through the decomposition of organic matter.

Amanita thiersii is one apparently saprotrophic *Amanita*, found in lawns in the central and southeastern United States. This species was originally described as *Amanita alba*, a homonym, from a lawn in Texas (Thiers 1957). Bas (1969) renamed it *Amanita thiersii*. Over the past 10–15 y anecdotal reports have suggested *A. thiersii* as increasing in abundance in regions of the central United States where it was not observed previously (Kuo 2007, McFarland and Mueller 2009, Kuo and Methven 2010), suggesting that like other species of *Amanita* (Pringle and Vellinga 2006, Pringle et al. 2009, Vellinga et al. 2009, Wolfe et al. 2010) the fungus is expanding its range. However several other potentially saprotrophic *Amanita* species with morphological similarities to *A. thiersii*, including *A. manicata* (Pegler 1986), *A. armillariiformis* (Miller et al. 1990) and *A. nauseosa* (Guzmán 1981), occur in the United States. Because specimens in historical collections of macrofungi may be misidentified (Pringle and Vellinga 2006, Pringle et al. 2009) apparent changes in the distribution of *A. thiersii* in the United States might reflect a confused species concept and not an actual range expansion.

In this paper we describe the natural history of *A. thiersii* in the United States, using both field observations and laboratory experiments. We explored these questions:

(i) Does *A. thiersii* possess hallmarks of a saprotrophic niche, based on stable isotope measurements of sporocarps, the presence of genes encoding cellulases in transcriptomic sequences and patterns of growth on complex carbon sources?
(ii) What is the current distribution of *A. thiersii* in the United States? Does mapping the distribution of *A. thiersii* records over time provide support for the perception of *A. thiersii* as a species experiencing a range expansion?
(iii) How genetically diverse are populations of *A. thiersii* across the United States?

We also collected additional natural history data, including descriptions of pure culture morphology, counts of nuclei and data on the effects of *A. thiersii* on the growth of grasses. Our research provides the first detailed study of the ecology of a free-living *Amanita* species, and these data provide an ecological context for interpreting the genome of *A. thiersii*, currently in progress at the United States Department of Energy's Joint Genome Institute (http://www.jgi.doe.gov/sequencing/why/athiersii.html).

MATERIALS AND METHODS

Isolation of axenic cultures of Amanita thiersii *and nuclear counts of spores.*—A mushroom collected by Sherry Kay from a lawn in Baldwin City, Kansas, in Jul 2009 (A thiersii 2009 Baldwin City in SUPPLEMENTARY TABLE I) was used to generate monokaryotic (*Amanita thiersii* Skay4041) and dikaryotic (*Amanita thiersii* Skay4041het) cultures of *A. thiersii*. The monokaryotic strain is being sequenced by the Department of Energy's Joint Genome Institute and has been deposited at the Fungal Genetics Stock Center (FGSC10297).

Monokaryotic cultures were generated by pressing lamallae onto the surface of 0.2% glucose solid MMN medium (Marx 1969). Spores were dispersed across the surface of the medium with 20 μL sterile water. Dikaryotic cultures were generated by excising small pieces of tissue from between the pileus and lamellae of the sporocarp. Tissues were plated onto solid, modified MMN medium (Marx 1969), with 0.2% glucose, no malt extract, and containing 100 μg/mL chloramphenicol. Plates were incubated at 27 C.

To determine the number of nuclei per spore we followed protocols outlined by Horton (2006) for staining of nuclei with DAPI. Spores were observed with a Zeiss Axioscope fluorescence microscope (Carl Zeiss Inc., Jena, Germany) using a DAPI filter at 400× magnification.

Stable isotopes of carbon and nitrogen.—To determine whether the biomass of *A. thiersii* sporocarps is composed of carbon derived from the decomposition of litter in the environment or from ectomycorrhizal associations we measured stable isotope ratios of carbon and nitrogen in at least one sporocarp from each of 31 unique populations collected throughout the current range of *A. thiersii* in the United States (SUPPLEMENTARY TABLE I). Homogenized samples of dried gill tissue were analyzed with standard protocols (Hobbie et al. 2001). Measurements were made with a Costech ECS4010 Elemental Analyzer configured with a DeltaPlus XP mass spectrometer at the University of New Hampshire Stable Isotope Lab. Stable isotope data are reported as δ according to this equation:

$$\delta X = \left(R_{sample}/R_{standard} - 1\right) \times 1000(‰)$$

where R_{sample} and $R_{standard}$ are the ratios of heavy to light isotopes of sampled sporocarps and standards, and X is either carbon (C) or nitrogen (N). Vienna Pee Dee Belemnite and air were used as references for C and N respectively. Standards for calibration included NIST 1515 (apple leaves), NIST 1575a (pine needles) and tuna muscle.

Transcriptome sequencing.—To assess the genetic potential for saprotrophic decomposition by *A. thiersii* we sequenced the transcriptome of *Amanita thiersii* Skay4041het growing on sterile grass litter. Using a QIAGEN RNeasy Maxi Kit (QIAGEN, Valencia, California) RNA was extracted from *Amanita thiersii* Skay4041het mycelia that had been grown on litter 2 wk. cDNA was generated from this RNA with the Roche rapid library preparation method (454 Life Sciences, Branford, Connecticut) and sequenced on a Roche GS-FLX with titanium chemistry at the Duke University Genome Sequencing and Analysis Core Facility. The sequencing produced 558 849 reads with an average length of 414 bp. Sequences were assembled into contigs with Newbler 2.5, resulting in 7444 contigs that were placed into a BLAST database. Raw sequence data were deposited in the NCBI Sequence Read Archive under accession number SRA026549.

We searched the *A. thiersii* litter transcriptome for orthologs of glycoside hydrolases previously identified as playing important roles in the decomposition of cellulose in two other basidiomycetes, *Volvariella volvacea* and *Phanerochaete chrysosporium*. These cellulase genes have been shown to be highly upregulated in the presence of artificial cellulose substrates (Jia et al. 1999; Ding et al. 2001, 2002, 2006). Each cellulase sequence was used as a query in a tBLASTx search of the assembled transcriptome. The *A. thiersii* transcript sequence producing the most significant alignment (based on e values) with each queried cellulase sequence used in a tBLASTx search of GenBank to confirm that the *A. thiersii* transcript had significant homology with previously identified cellulases in other basidiomycetes. A future set of studies will explore the diversity of this *Amanita* transcriptome in more detail, but here we briefly describe the results of this targeted probe for cellulases considered hallmarks of saprotrophy in basidiomycetes (Baldrian and Valášková 2008).

Growth of Amanita thiersii *Skay4041 on different sources of carbon.*—To determine whether *A. thiersii* has a carbon utilization profile similar to other saprotrophic basidiomycetes we grew *A. thiersii* Skay4041het on media containing carbon sources varying in complexity from simple sugars to complex artificial cellulose. Media contained the same basal nutrient concentrations as the MMN media described above but with 1% of these sources of carbon: glucose, D-cellobiose, D-galactose, D-mannose, xylan, starch, carboxymethylcellulose (CMC), Sigmacell or Avicel. We also included a treatment where carbon was supplied as 1 g dried, sterile grass litter. The grass litter was a mix of the species *Panicum virgatum*, *Elymus canadensis*, *Andropogon gerardii* and *Koeleria cristata*. These grasses were grown from seeds in a controlled environment growth chamber 3 mo, left to senesce, chopped into ~ 1 cm fragments and steam-sterilized twice 20 min at 121 C. The carbon source was supplied in media solidified with Phytagel (Sigma-Aldrich, St Louis, Missouri).

Each experimental unit consisted of a Petri dish containing one of the various carbon sources and was inoculated with a small agar plug (2 mm × 2 mm) taken from the actively growing hyphal front of *Amanita thiersii* Skay4041het grown on 1% glucose MMN. Each carbon source was replicated five times. A no-carbon control treatment also was included. Experimental units were incubated in the dark at 27 C for 30 d in a randomized design. Mycelial biomass was harvested from each experimental unit by dissolving Phytagel overnight in citrate buffer (Doner and Bécard 1991). The wet weight of each mycelium was determined after patting the harvested mycelium twice with KimWipes™. The mean weight of the no-carbon control treatment was deducted from the weight of each replicate from the carbon treatments to control for carbon stored in the inoculum plug. Statistical significance of differences between treatments was assessed with ANOVA.

Effect of Amanita thiersii *on grass growth.*—To test whether *A. thiersii* can function as a pathogen or mutualist of grasses we measured the effect of *A. thiersii* on the growth of four grass species, *Panicum virgatum*, *Elymus canadensis*, *Andropogon gerardii* and *Koeleria cristata*. These are native grasses that occur throughout the current range of *A. thiersii*. Grasses were germinated from surface-sterilized seeds (Prairie Moon Nursery, Winona, Minnesota) and grown in a sterile growing medium 3 wk. An individual grass seedling was transplanted into a 2.5 mm × 16 mm pot filled with 1 : 1 sterile field soil : sand mix. Field soil was collected from an experimental garden at Harvard University (Cambridge, Massachusetts) composed of a mix of herbaceous grasses and forbs, as well as diverse coniferous and deciduous trees. Field soil was steam-sterilized twice for 30 min each at 121 C before it was mixed with sterile sand. Twenty pots were planted with each grass species. After grasses had established for 3 d half (10) of the pots of each grass species received a 7.5 mm × 7.5 mm piece of agar taken from the actively growing hyphal front of *A. thiersii* Skay4041het on potato dextrose agar, while the other half received an uncolonized 7.5 mm × 7.5 mm piece of potato dextrose agar. The agar plugs were placed 4 cm below the soil surface, in contact with the seedling root system. Each grass-inoculum combination was replicated 10 times, for a total of 80 experimental units. Pots were placed in a randomized design in a controlled-environment growth chamber (20/25 C, 12 h day/night periods, 60% humidity and 500 μmol photon m^{-2} s^{-1} illumination) and were rerandomized every 2 wk. After 4 mo the biomass of each seedling was removed, dried at 40 C for 2 d and weighed. Statistical significance of differences in growth was assessed with ANOVA.

Herbarium material and DNA extractions.—We assembled a set of 31 recently collected specimens of *A. thiersii* from the central and southeastern United States; collections encompassed one sporocarp from each of 31 unique populations (SUPPLEMENTARY TABLE I). We also obtained a portion of gill tissue from the holotype of *A. thiersii* (H.D. Thiers 1713) from MICH. DNA was extracted from all specimens as described in Wolfe et al. (2010).

To confirm that sporocarps collected and named *A. thiersii* are a single genetic species and are genetically similar to the material used to describe the species we sequenced the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and part of the nuclear ribosomal

large subunit (nucLSU) from each specimen. These two regions are phylogenetically informative and are useful for delineating species within *Amanita* (e.g. Drehmel et al. 1999, Moncalvo et al. 2000). These regions also have been shown to be variable within morphological species of *Amanita* (Oda et al. 2004; Zhang et al. 2004; Geml et al. 2006, 2008; Zhang et al. 2010). The ITS region was amplified with primers ITS1f and ITS4b (Gardes and Bruns 1993). A portion of the nucLSU was amplified with primers LR0R and LR5 (Vilgalys and Hester 1990, Hopple and Vilgalys 1994). To compare *A. thiersii* to closely related species found in the United States we also sequenced the ITS region of *A. manicata* (RET 387-4), *A. nauseosa* (DPL 6117), A. *armillariiformis* (RET 266-8), *A. prairiicola* (RET 266-1) and *A. silvifuga* (HDT 4630, SFSU). RET specimens are from the personal collection of Dr Rodham E. Tulloss, while DPL 6117 is a specimen held by the Pringle Laboratory at Harvard University.

We were unable to amplify the entire ITS region of the type collected in 1952 with ITS1f-ITS4b or those primers in combination with primers anchored in the 5.8S. To sequence the ITS of this specimen we developed internal primers used in these primer combinations: ITS1f with AthITSi1.1r (GCCTCCCTTCCTATAACCCA), AthITSi1.1f (TGGGTTATAGGAAGGGAGGC) with ITS2, ITS3 with AthITSi2.1r (AATCACGCCATCCATTTAGC) and AthITSi2.1f (GCTAAATGGATGGCGTGATT) with ITS4.

ITS and nucLSU sequences for *Amanita thiersii* SKay4041 have been deposited in GenBank with accession numbers HQ625010 for ITS and HQ619205 for nucLSU. Sequences of ITS and nucLSU from only one specimen were deposited because they were identical across all specimens (see RESULTS).

Survey of genetic diversity among populations.—To assess genetic variability across different populations of *A. thiersii* with markers other than the ITS and nucLSU regions we used restriction fragment length polymorphism (RFLP) of the intergenic spacer 1 (IGS1) region and intersimple sequence repeat (ISSR) banding patterns. The IGS1 is a rapidly evolving region and has been shown to be highly variable among populations, within populations and in some cases even within individual sporocarps of basidiomycetes (e.g. Selosse et al. 1999, Guerin-Laguette et al. 2002, Kauserud and Schumacher 2003, Roy et al. 2008). Initial sequencing of the entire IGS1 region of specimens from several different populations indicated that only one IGS haplotype was present in each sporocarp and that sequences were identical across sporocarps from different populations; so we used restriction fragment length polymorphism (RFLP) patterns of IGS1 amplicons to confirm this pattern across the remaining samples. The IGS1 region was amplified with primers LR20R (James et al. 2001) and 5SA (Guidot et al. 1999) and digested with restriction enzymes *Hinf*I, *Hae*III, *Alu*I and *Msp*I (New England Biolabs, Ipswitch, Massachusetts) according to manufacturer's instructions. Digested PCR amplicons were visualized on 2% agarose gels. The IGS sequence for *Amanita thiersii* SKay4041 was deposited in GenBank with accession number HQ619204.

After an initial screen of a larger set of ISSR primers we chose four ISSR primers with clear patterns of multiple bands that could be scored easily. We used primers $(GTG)_5$, $(ACA)_5S$, $DDB(CCA)_5$ and $(CCA)_5S$ (where D = A, G or T; B = C, G, or T; and S = G or C), with annealing temperatures of 55, 49, 55 and 61 C respectively. Each 25 μL PCR reaction contained 400 nM primer, 200 μM of each of all four dNTPs, 1× Taq buffer, 0.5 U Platinum Taq, 50 mM MgCl2, 5 μL PCR enhancer mix (Ralser et al. 2006) and ~ 100 ng of genomic DNA. PCRs were performed with the same thermo-cycler programs described in Gryta et al. (2006) except that annealing temperatures given above were used. Each PCR was replicated twice to confirm banding patterns.

Mapping historical collections of Amanita thiersii.—To qualitatively assess changes in the distribution of *A. thiersii* since its original identification in 1952 in Texas we used the herbarium specimens above and additional collection records (SUPPLEMENTARY TABLE II) to map the distribution of the fungus over time. We searched for specimens of *A. thiersii* in BPI, CSU, EIU, F, KANU, L, MICH, NY, TENN and UMO. We targeted these herbaria because they house large collections of Agaricales from the central United States, which is the region where most populations of *A. thiersii* have been reported. We also mapped a few additional specimens from the personal herbaria of collectors and our own field collections.

Changes in the distributions of species inferred from herbarium records can be confounded by changes in both historical patterns of collecting and depositions to herbaria (Delisle et al. 2003). To document potential biases we also targeted data on specimens of the basidiomycete *Chlorophyllum molybdites* collected from across the same biogeographic region and period (SUPPLEMENTARY TABLE III). This species has a similar gross morphology to *A. thiersii* and occurs in identical habitats, but *C. molybdites* is clearly distinguishable from *A. thiersii* based on obvious visual differences including its smooth stipe and olive-green spore print. If changes in collecting patterns are driving apparent changes in the distribution of *A. thiersii*, we hypothesized a similar pattern would be found for *C. molybdites*. We used *C. molybdites* specimens from the same herbaria as those used for the *A. thiersii* collections described above. We also included specimens described by Reid and Eicker (1991) that were from the same biogeographic region surveyed for *Amanita thiersii*. We used only specimens that were named *Chlorophyllum molybdites* and intentionally did not include specimens that might be labeled with other synonyms for this species (e.g. *Lepiota molybdites*). The mapping of historical collections of *C. molybdites* was used as a type of control for mapping the distribution of *A. thiersii* and should not be interpreted as an attempt to comprehensively map the distribution of this species in the United States. We are likely to have missed specimens in herbaria outside the biogeographic scope of our study and specimens deposited using one of the many synonyms of this species.

RESULTS

Axenic culture isolation and binucleate spores.—We easily isolated both monokaryotic and dikaryotic

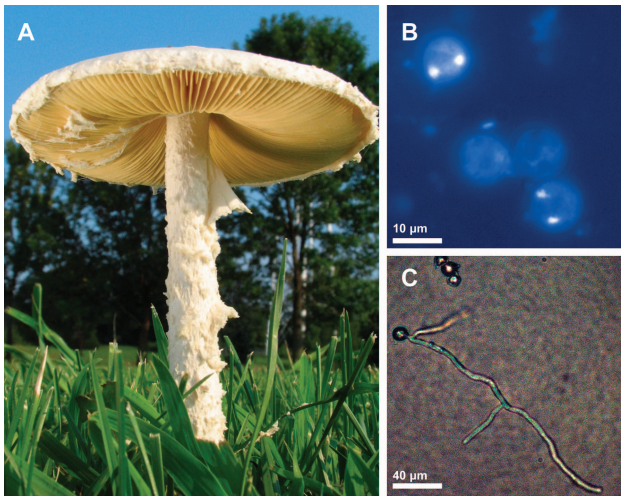

FIG. 1. A. Sporocarp of *Amanita thiersii* growing in a lawn in Illinois (photo used with permission from Joe McFarland). B. Spores of *A. thiersii* stained with DAPI, showing two nuclei per spore. C. A single spore of *A. thiersii* growing on minimal medium. This spore is the source of the monokaryotic strain *A. thiersii* Skay4041, which is being sequenced by the U.S. Department of Energy's Joint Genome Institute.

strains from freshly collected sporocarps. Spores of *A. thiersii* germinated within 2 wk of plating on media (FIG. 1C), and because germlings grew relatively slowly we were able to separate germinated spores onto new Petri dishes without mixing of monokaryotic spore strains. Hyphae grew rapidly from pieces of tissue collected from sporocarps as dikaryotic cultures.

Spores of *A. thiersii* are binucleate, with two nuclei visible in the DAPI stain (FIG. 1B). DAPI staining of hyphae from the monokaryotic strain showed just one nucleus per cell (data not shown), suggesting the second nucleus of a spore never migrates into the developing germ tube.

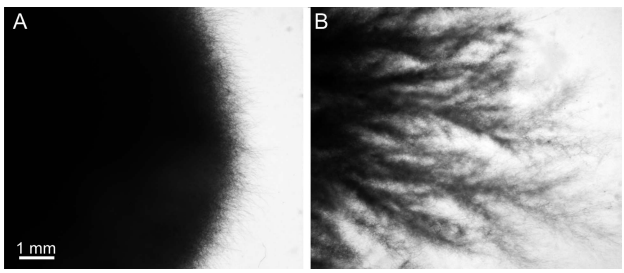

FIG. 2. Actively growing hyphal fronts of (A) the ectomycorrhizal *Amanita muscaria* and (B) saprotrophic *Amanita thiersii*. Both cultures were growing on minimal MMN medium, as described in the text. Color photographs were converted to black and white and then inverted to improve clarity. Scale is identical for A and B.

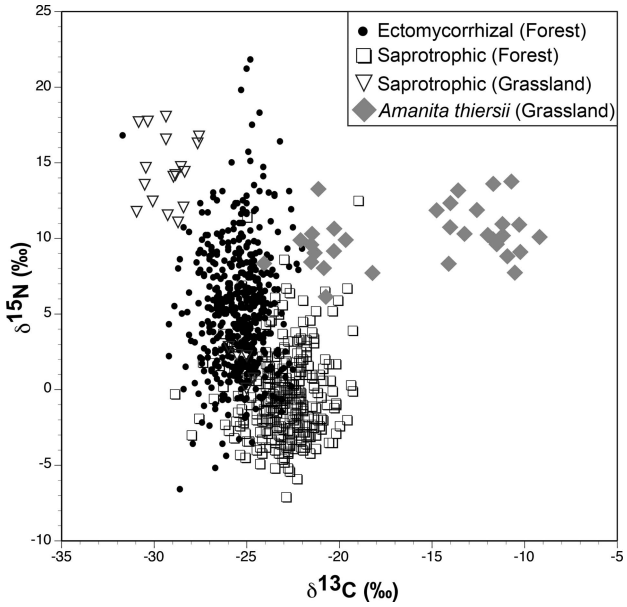

FIG. 3. Stable isotopes of carbon and nitrogen of *Amanita thiersii* sporocarps. Data from ectomycorrhizal and saprotrophic species collected in forests are from Mayor et al. (2009). The only other previously published stable isotope dataset from a grassland (Griffith et al. 2002) is also presented.

Axenic cultures of both monokaryotic and dikaryotic strains of *A. thiersii* have a growth pattern strikingly distinct from the growth of ectomycorrhizal *Amanita* species. The hyphal fronts of ectomycorrhizal *Amanita* are usually smooth (Miller et al. 1983, Iotti et al. 2005), as illustrated by a culture of *A. muscaria* maintained in our lab (FIG. 2A). The hyphal front of *A. thiersii* is diffuse and highly branched (FIG. 2B).

Unusual stable isotope signatures of carbon and nitrogen.—The stable isotope signatures of *A. thiersii* are different from the signatures of any other ectomycorrhizal or saprotrophic species sampled to date. A biplot of the $\delta^{13}C$ and the $\delta^{15}N$ of *A. thiersii* sporocarps, including data from other ectomycorrhizal and saprotrophic fungi (Mayor et al. 2009), demonstrates that most populations of *A. thiersii* have isotopic signatures more enriched in ^{15}N than other recorded saprotrophic fungi (FIG. 3). Two distinct clusters are apparently based on $\delta^{13}C$ values within the *A. thiersii* samples, with one cluster falling within the range of $\delta^{13}C$ reported for other saprotrophic fungi (ranging from −17.86 to −24.05‰) and another cluster being much less depleted in $\delta^{13}C$ and falling well outside values reported for other saprotrophic fungi (ranging from −9.17 to −14.73‰). Only one other dataset, composed mostly of *Hygrocybe* species, contains stable isotope data from

TABLE I. *Amanita thiersii* transcript matches to known basidiomycete cellulases

Gene	Sequenced used for tBLASTx query of *A. thiersii* transcriptome	*A. thiersii* transcript matches		Best match in GenBank (e value) for *A. thiersii* transcripts
		Length (bp)	e value	
Endoglucanase I	AF329732 *Volvariella volvacea* endoglucanase	1272	0	EU716328 *Volvariella volvacea* endoglucanase (egI) (2e-172)
Endoglucanase II	AY559101 *Volvariella volvacea* endoglucanase II	1180	2e-39	M86356 *Agaricus bisporus* cellulose-growth-specific protein (cel1) (1e-106)
Cellobiohydrolase I	AY559102 *Volvariella volvacea* cellobiohydrolase I-I	1710	0	AM262993 *Pleurotus ostreatus* cbhI-1 gene (0.00)
Cellobiohydrolase II	AY559104 *Volvariella volvacea* cellobiohydrolase II-I	1453	0	AY559104 *Volvariella volvacea* cellobiohydrolase II-I (0.00)
Extracellular beta-glucosidase	AF036873 *Phanerochaete chrysosporium* beta-glucosidase	2441	0	XM_001879644 *Laccaria bicolor* glycoside hydrolase family 3 (0.00)
Intracellular beta-glucosidase	AF329731 *Volvariella volvacea* beta-glucosidase	2793	0	XM_002474673 *Postia placenta* Mad-698-R beta-glucosidase (0.00)
Cellobiose dehydrogenase	U46081 *Phanerochaete chrysosporium* cellobiose dehydrogenase	2161	0	XM_001834980 *Coprinopsis cinerea* cellobiose dehydrogenase (0.00)

sporocarps of basidiomycete species collected from grasslands (Griffith et al. 2002). *Amanita thiersii* sporocarps are much less depleted in ^{13}C and slightly less enriched in ^{15}N, when compared to these other grassland species.

A complete set of cellulases is found in the litter transcriptome.—The presence of transcripts with high similarity to cellulose-degrading glycoside hydrolases from other saprotrophic basidiomycetes confirms that *A. thiersii* has the genetic potential to decompose cellulose. In tBLASTx queries of the *A. thiersii* litter transcriptome we found clear orthologs to all classes of cellulases examined (TABLE I).

Significant growth on complex carbon substrates, including grass litter.—*Amanita thiersii* is similar to other saprotrophic basidiomycetes in its patterns of growth on single carbon sources, and grew well on complex carbon compounds such as xylan and artificial forms of cellulose (FIG. 4). *Amanita thiersii* produced the greatest biomass when it was grown on sterile grass litter (FIG. 4, SUPPLEMENTARY FIG. 1).

Amanita thiersii *improves growth of grasses.*—Inoculation with *A. thiersii* caused an increase in the total plant biomass of each of the four grass species, with biomass increases of 22–59% in the presence of *A. thiersii*, as compared to plants grown without *A. thiersii* (TABLE II).

Genetic diversity of A. thiersii is low.—The ITS and nucLSU sequences of specimens taken from 31 populations of *A. thiersii* are identical, and the ITS sequences matched the holotype exactly. While there are many nucleotide positions throughout the ITS

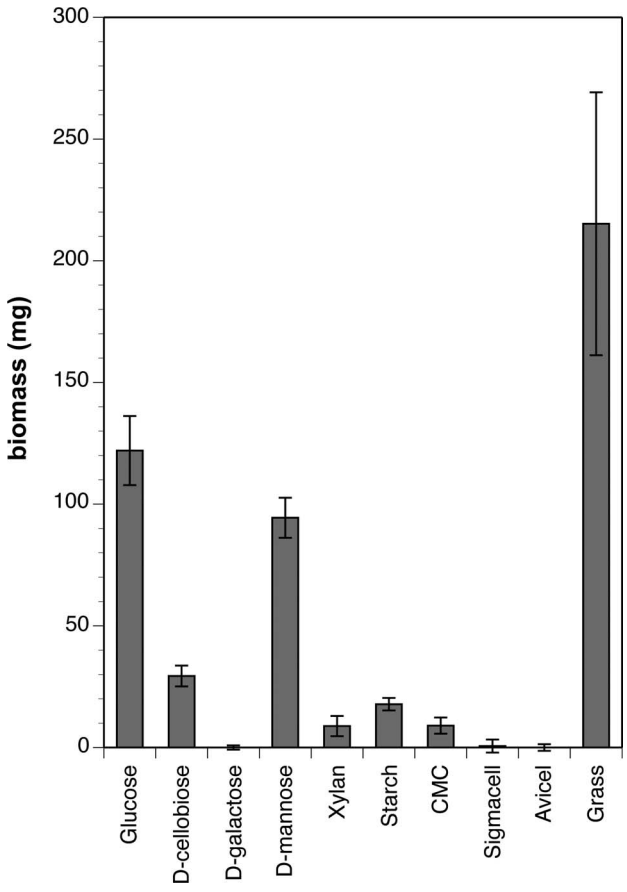

FIG. 4. Growth of *A. thiersii* Skay4041 on different carbon sources. Values are mean (± standard error). Substrates on the X axis are arranged in increasing order of structural complexity.

TABLE II. Effect of *Amanita thiersii* on biomass of four grass species. Data are mean mg dry weight (± SE). n = 10

	− *A. thiersii*	+ *A. thiersii*	
Panicum virgatum	68.7 (9.3)	99.7 (11.8)	$P = 0.053$
Elymus canadensis	82.8 (2.9)	100.6 (5.4)	$P = 0.010$
Andropogon gerardii	97.7 (8.4)	131.2 (17.5)	$P = 0.102$
Koeleria cristata	46.7 (7.4)	74.3 (10.8)	$P = 0.049$

and 5.8s regions that differ between *A. thiersii* and closely related species, no nucleotide polymorphisms are in these regions within *A. thiersii* itself. We report two diagnostic, variable positions located in the 5.8S region that delineate *A. thiersii* from other species (SUPPLEMENTARY TABLE IV).

Identical IGS1 RFLP patterns were also observed across populations, confirming preliminary sequencing of the IGS1 locus (SUPPLEMENTARY FIG. 2A). Moreover, ISSR banding patterns were also identical, with no clearly distinguishable bands unique to sporocarps from different populations (FIG. 5).

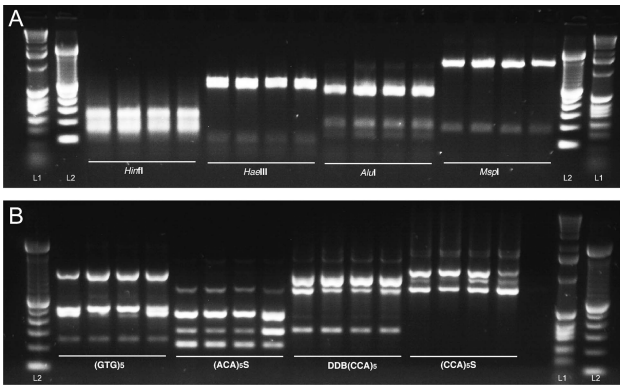

FIG. 5. A. Restriction fragment length polymorphisms of the IGS1 region of *Amanita thiersii*. Data from four specimens are shown, arranged in identical order within the bar marking each enzyme: CSU Ovrebo 4418, DPL 9229, Kuo 07220704 and *A. thiersii* 2009 Baldwin City (see SUPPLEMENTARY TABLE I). L1 = 100 bp ladder (Invitrogen). L2 = 1 kb plus ladder (Invitrogen). B. Intersimple sequence repeat (ISSR) banding patterns of *A. thiersii*. The same four specimens described above are shown for each ISSR primer.

Amanita thiersii *is expanding its range.*—Since it was originally collected in 1952 in College Station, Texas, *A. thiersii* appears to have moved north up the Mississippi River Basin to Illinois (FIG. 6). From 1952 until 1979 the species was limited to eastern Texas and Missouri. In the 1980s populations were reported from Oklahoma and Kansas. By the late 1990s the northern limit of *A. thiersii* was southern Illinois and northern Kentucky. By 2009 the species had been reported in nine states.

In contrast to *A. thiersii*, *C. molybdites* was collected throughout much of North America, from Quebec to Florida and west to Texas, before the 1950s (FIG. 6). The extent of its range in the central United States has not changed since the 1950s, although additional populations have been reported within this range.

DISCUSSION

We provide the first comprehensive evidence for an *Amanita* species as a saprotrophic fungus. We used field and laboratory data, including stable isotope signatures, patterns of growth on complex carbon sources and the presence of cellulase genes, as tests of saprotrophy; in each case there was strong support for a saprotrophic niche. Although we did not see obvious signs of typical mycorrhizal structures in the field or a growth chamber experiment, we did observe an increase in growth when grasses were inoculated with *A. thiersii*, and it is possible *A. thiersii* provides an indirect benefit to plants.

Stable isotopes of carbon and nitrogen taken from *A. thiersii* sporocarps suggest the fungus obtains carbon through the decomposition of organic matter in grasslands. Isotope ratios for carbon were well outside the range previously reported for ectomycorrhizal fungi, with ^{13}C values of *A. thiersii* much less depleted than typical ectomycorrhizal sporocarps. One cluster of ^{13}C values from *A. thiersii* falls outside the range previously observed for saprotrophic fungi (FIG. 3). This pattern may be driven in part by a collection bias in the currently available isotope data. Most stable isotope data of fungal sporocarps are from fungi collected in forest ecosystems, where the majority of live biomass and litter is produced by plants with the C_3 photosynthetic pathway (see Mayor et al. 2009). Plants with the C_4 pathway have much less depleted ^{13}C values compared to C_3 plants because of differences in carboxylation reactions and the fractionation of isotopes between the two photosynthetic pathways (Farquhar et al. 1989). In the only other study where multiple specimens of grassland sporocarps were collected the ^{13}C signatures of *Hygrocybe* spp. were more depleted compared to saprotrophic and ectomycorrhizal basidiomycetes from forests (Griffith et al. 2002), but this grassland also was dominated by a C_3 species (an *Agrostis*). The two distinct clusters formed by differences in ^{13}C signatures across the range of *A. thiersii* may be driven by variation in the abundance of C_3 vs. C_4 grasses, with the more typical saprotrophic signatures measured in sporocarps from C_3-dominated habitats and the less depleted signatures coming from C_4-dominated

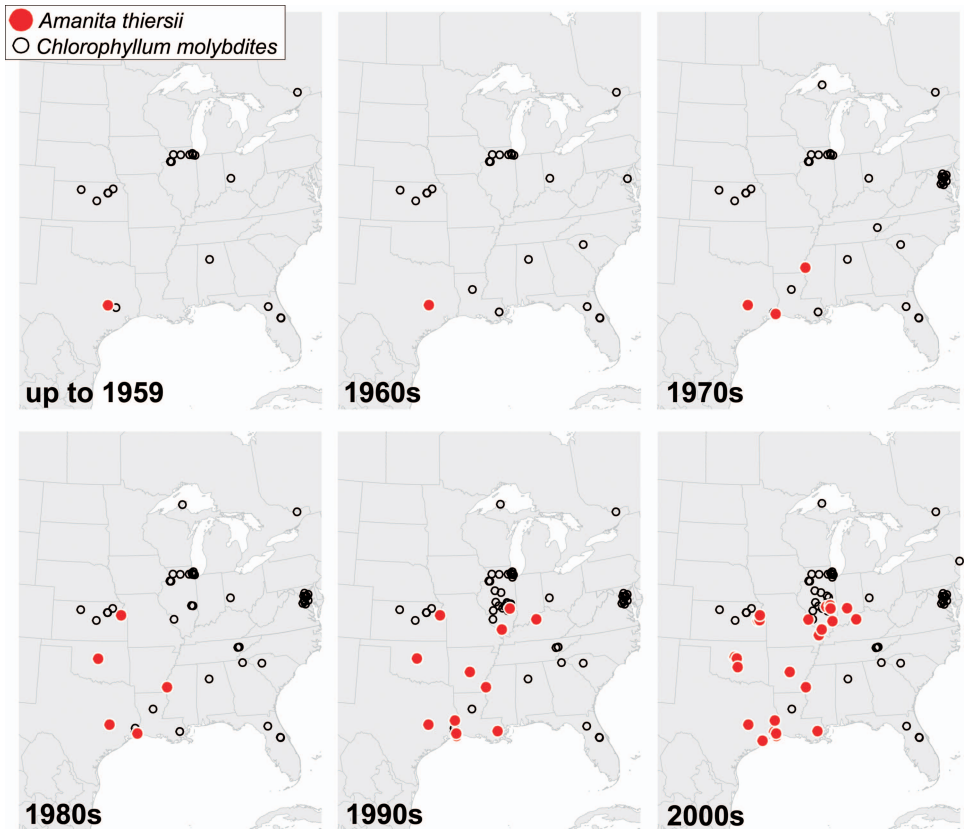

FIG. 6. Historical distributions of *Amanita thiersii* and *Chlorophyllum molybdites* in North America. The top left panel shows the distribution of both species based on records collected through 1959. Following panels track the distribution of both species during subsequent 10 y periods.

habitats. More stable isotope measurements from sporocarps collected in C_4 grasslands are needed to understand whether signatures like those observed for *A. thiersii* are characteristic for fungi growing in these ecosystems or if there is something unique about the carbon dynamics of *A. thiersii*.

While the less depleted ^{13}C signature is consistent with *A. thiersii* functioning as a saprotroph and not a symbiont, the highly enriched ^{15}N signature of *A. thiersii* sporocarps is striking because saprotrophic fungi are typically less enriched in ^{15}N than ectomycorrhizal fungi (Mayor et al. 2009). Ectomycorrhizal fungi are thought to preferentially transfer ^{15}N-depleted nitrogen to host plants (Högberg et al. 1999). However the overall N availability and different N types in soils also may influence sporocarp ^{15}N values (Hobbie and Colpaert 2003, Hobbie and Hobbie 2006). The limited number of samples of ^{15}N from other grassland fungi makes it difficult to know whether the *A. thiersii* ^{15}N signatures are species specific or if they are common among other grassland fungi. The saprotrophic taxa surveyed by Griffith et al. (2002) also were highly enriched in ^{15}N, providing preliminary support for a pattern of ^{15}N enrichment in grassland fungi.

Decomposition of dead organic matter in the environment by saprotrophic basidiomycetes involves the synergistic action of a suite of glycoside hydrolases that degrade plant cell walls (Baldrian and Valášková 2008). *Amanita thiersii* grew on several complex carbon sources that require these enzymes. Moreover we identified a complete set of cellulase genes in the transcriptome sequence of *A. thiersii*. If the transcription of these genes results in the production of cellulases and if the function assigned to orthologs in other fungi is similar in *A. thiersii* then this fungus has the genetic potential to fully degrade cellulose into glucose. Its limited growth on crystalline cellulose (Sigmacell and Avicel) but considerable growth on amorphous cellulose (carboxymethylcellulose) suggest that cellulases in *A. thiersii* might have higher affinities for amorphous regions of cellulose. Ongoing genome sequencing will provide a more complete view of the genetic basis of decomposition by this species.

Saprotrophic basidiomycetes found in natural and artificial grasslands stimulate the growth of grasses through the mobilization of nitrogen in grassland soils (Shantz et al. 1917, Fisher et al. 1977). The increase in the growth of grasses inoculated with *A.*

thiersii is likely caused by these indirect effects on plant growth and not by a direct symbiotic association between the fungus and plants. We found no visual signs of symbiotic structures formed between the plant roots and *A. thiersii* mycelium in both the grass-response experiment and from under *A. thiersii* sporocarps collected in the field. Mycorrhizal associations between grasses and grassland fungi have been reported in the literature (e.g. Harrington and Mitchell 2002), but hosts are always sedge species in the Cyperaceae, which are rarely found in the residential lawns where *A. thiersii* commonly grows.

Placing *A. thiersii* within a phylogenetic context would clarify its ecological role and evolutionary origin. Large-scale phylogenies aiming to resolve family relationships within the Agaricales suggest ectomycorrhizal *Amanita* species evolved from saprotrophs (Matheny et al. 2006), but more detailed phylogenies encompassing the approximately 15 species of putatively saprotrophic *Amanita* species are lacking and remain a focus of research in our laboratory. A recent single-gene phylogeny demonstrates that two other morphologically similar and putatively saprotrophic *Amanita* species, *A. armillariiformis* and *A. nauseosa* (Bas 1969), form a well supported clade apart from an ectomycorrhizal *Amanita* clade (Justo et al. 2010).

The lack of genetic diversity across the current range of *Amanita thiersii*, which spans up to 1200 km, is surprising because both IGS1-RFLP and ISSR consistently detect considerable genetic variation within other species of basidiomycetes (Carriconde et al. 2008, Gryta et al. 2006, Liang et al. 2005, Sawyer et al. 2003). Research with similar genetic markers and closely related ectomycorrhizal *Amanita* species have repeatedly found multiple genotypes within and between populations. For example the IGS2 region of the ectomycorrhizal *Amanita phalloides* is variable within its introduced range in North America (Pringle et al. 2009) and other *Amanita* species possess highly polymorphic ISSR patterns at much smaller spatial scales than those considered here (Sawyer et al. 2003, Liang et al. 2005). The sequencing of more loci and the use of other approaches, such as AFLP, may uncover genetic variation not detected by our methods. However our current data suggest incredibly low genetic diversity across populations of *A. thiersii* in the United States.

The increase in range over time suggests an expansion of *A. thiersii* in the central and southeastern United States. It is unclear whether *A. thiersii* is native to the United States and is expanding from a previously restricted southern range or whether *A. thiersii* was introduced to the United States and is currently spreading in a novel range. Low genetic diversity across populations is commonly detected in species that have experienced a founder event caused by a recent introduction to a novel range (Dlugosch and Parker 2007), but natural range expansions also can result in low genetic diversity across populations (Hewitt 2000). As far as we are aware *A. thiersii* has no other identified range throughout the world, so if it has been introduced to the United States the native range of this fungus is currently unknown. However the potential for the successful introduction and spread of *Amanita* species has been demonstrated with the numerous examples of both ectomycorrhizal and saprotrophic *Amanita* species currently spreading in novel ranges (Vellinga et al. 2009, Pringle et al. 2009). Unlike other ectomycorrhizal *Amanita* species that can spread only within the ranges of suitable hosts the saprotrophic *Amanita thiersii* will be unconstrained by host availability. The species thrives in suburban lawns, and because these are a ubiquitous feature of the North American landscape the spread of *A. thiersii* may continue at a rapid pace.

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LITERATURE CITED

Baldrian P, Valášková V. 2008. Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32:501–521, doi:10.1111/j.1574-6976.2008.00106.x

Bas C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section Lepidella. Persoonia 5:285–579.

Carriconde F, Gardes M, Jargeat P, Heilmann-Clausen J, Mouhamadou B, Gryta H. 2008. Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, *Tricholoma scalpturatum*. Microb Ecol 56:513–524, doi:10.1007/s00248-008-9370-2

Delisle F, Lavoie C, Martin J, Lachance D. 2003. Reconstructing the spread of invasive plants: taking into consideration biases associated with herbarium specimens. J Biogeogr 30:1033–1042, doi:10.1046/j.1365-2699.2003.00897.x

Ding S. 2006. Cloning of multiple cellulase cDNAs from *Volvariella volvacea* and their differential expression during substrate colonization and fruiting. FEMS Microbiol Lett 263:207–213, doi:10.1111/j.1574-6968.2006.00433.x

———, Ge W, Buswell JA. 2007. Molecular cloning and transcriptional expression analysis of an intracellular beta-glucosidase, a family 3 glycosyl hydrolase, from the edible straw mushroom, *Volvariella volvacea*. FEMS Microbiol Lett 267:221–229, doi:10.1111/j.1574-6968.2006.00550.x

Ding SJ, Ge W, Buswell JA. 2001. Endoglucanase I from the edible straw mushroom, *Volvariella volvacea*—purification, characterization, cloning and expression. Eur J Biochem 268:5687–5695, doi:10.1046/j.0014-2956.2001.02503.x

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution and the role of multiple introductions. Mol Ecol 17:431–449, doi:10.1111/j.1365-294X.2007.03538.x

Doner LW, Becard G. 1991. Solubilization of gellan gels by chelation of cations. Biotechnol Tech 5:25–28, doi:10.1007/BF00152749

Drehmel D, Moncalvo JM, Vilgalys R. 1999. Molecular phylogeny of amanita based on large-subunit ribosomal DNA sequences: implications for taxonomy and character evolution. Mycologia 91:610–618, doi:10.2307/3761246

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537, doi:10.1146/annurev.pp.40.060189.002443

Fisher RF. 1977. Nitrogen and phosphorus mobilization by fairy ring fungus, *Marasmius oreades*. Soil Biol Biochem 9:239–241, doi:10.1016/0038-0717(77)90028-1

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118, doi:10.1111/j.1365-294X.1993.tb00005.x

Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL. 2006. Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). Mol Ecol 15:225–239, doi:10.1111/j.1365-294X.2005.02799.x

———, Tulloss RE, Laursen GA, Sazanova NA, Taylor DL. 2008. Evidence for strong inter- and intracontinental phylogeographic structure in *Amanita muscaria*, a wind-dispersed ectomycorrhizal basidiomycete. Mol Phylogenet Evol 48:694–701, doi:10.1016/j.ympev.2008.04.029

Griffith GW, Easton GL, Jones AW. 2002. Ecology and diversity of waxcap (Hygrocybe spp.) fungi. Bot J Scotland 54:7–22, doi:10.1080/03746600208685025

———, Roderick K. 2008. Saprotrophic Basidiomycetes in grasslands: distribution and function. In: Boddy L, Frankland JC, van West P, eds. Ecology of saprotrophic Basidiomycetes. London: Academic Press. p 275–297.

Gryta H, Carriconde F, Charcosset JY, Jargeat P, Gardes M. 2006. Population dynamics of the ectomycorrhizal fungal species *Tricholoma populinum* and *Tricholoma scalpturatum* associated with black poplar under differing environmental conditions. Environ Microbiol 8:773–786, doi:10.1111/j.1462-2920.2005.00957.x

Guerin-Laguette A, Matsushita N, Kikuchi K, Iwase K, Lapeyrie F, Suzuki K. 2002. Identification of a prevalent *Tricholoma matsutake* ribotype in japan by rDNA IGS1 spacer characterization. Mycol Res 106:435–443, doi:10.1017/S0953756202005725

Guidot A, Lumini E, Debaud JC, Marmeisse R. 1999. The nuclear ribosomal DNA intergenic spacer as a target sequence to study intraspecific diversity of the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum* directly on *Pinus* root systems. Appl Environ Microbiol 65:903–909.

Guzmán G. 1981. Distribution of *Amanita nauseosa*. Mycotaxon 12:522–524.

Harrington TJ, Mitchell DT. 2002. Colonization of root systems of *Carex flacca* and *C. pilulifera* by *Cortinarius* (*Dermocybe*) *cinnamomeus*. Mycol Res 106:452–459, doi:10.1017/S0953756202005713

Henn MR, Chapela IH. 2001. Ecophysiology of ^{13}C and ^{15}N isotopic fractionation in forest fungi and the roots of the saprotrophic-mycorrhizal divide. Oecologia 128:480–487, doi:10.1007/s004420100680

Hewitt G. 2000. The genetic legacy of the quaternary ice ages. Nature 405:907–913, doi:10.1038/35016000

Hobbie EA, Colpaert JV. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol 157:115–126, doi:10.1046/j.1469-8137.2003.00657.x

———, Weber NS, Trappe JM. 2001. Mycorrhizal vs. saprotrophic status of fungi: the isotopic evidence. New Phytol 150:601–610, doi:10.1046/j.1469-8137.2001.00134.x

Hobbie JE, Hobbie EA. 2006. ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87:816–822, doi:10.1890/0012-9658(2006)87[816:NISFAP]2.0.CO;2

Högberg P, Hogberg MN, Quist ME, Ekblad A, Nasholm T. 1999. Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. New Phytol 142:569–576, doi:10.1046/j.1469-8137.1999.00404.x

Hopple JS, Vilgalys R. 1994. Phylogenetic-relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86:96–107, doi:10.2307/3760723

Horton TR. 2006. The number of nuclei in basidiospores of 63 species of ectomycorrhizal homobasidiomycetes. Mycologia 98:233–238, doi:10.3852/mycologia.98.2.233

Iotti M, Barbieri E, Stocchi V, Zambonelli A. 2005. Morphological and molecular characterization of mycelia of ectomycorrhizal fungi in pure culture. Fungal Divers 19:51–68.

James TY, Moncalvo JM, Li S, Vilgalys R. 2001. Polymorphism at the ribosomal DNA spacers and its relation to breeding structure of the widespread mushroom *Schizophyllum commune*. Genetics 157:149–161.

Jia J, Dyer PS, Buswell JA, Peberdy JF. 1999. Cloning of the cbhI and cbhII genes involved in cellulose utilization by the straw mushroom *Volvariella volvacea*. Mol Gen Genet 261:985–993, doi:10.1007/s004380051047

Justo A, Morgenstern I, Hallen-Adams HE, Hibbett DS. 2010. Convergent evolution of sequestrate forms in *Amanita* under Mediterranean climate conditions. Mycologia 102:675–688, doi:10.3852/09-191

Kauserud H, Schumacher T. 2003. Genetic structure of fennoscandian populations of the threatened wood-decay fungus *Fomitopsis rosea* (Basidiomycota). Mycol Res 107:155–163, doi:10.1017/S0953756203007214

Kuo M. 2007. Mushrooming in the age of DNA: now comes the fun part. McIllvainea 17:43–49.

———, Methven A. 2010. 100 cool mushrooms. Ann Arbor: Univ Michigan Press. 393 p.

Liang Y, Guo LD, Ma KP. 2005. Population genetic structure of an ectomycorrhizal fungus *Amanita manginiana* in a subtropical forest over two years. Mycorrhiza 15:137–142, doi:10.1007/s00572-004-0311-8

Maijala P, Fagerstedt KV, Raudaskoski M. 1991. Detection of extracellular cellulolytic and proteolytic activity in ectomycorrhizal fungi and *Heterobasidion annosum* (Fr) Bref. New Phytol 117:643–648, doi:10.1111/j.1469-8137.1991.tb00968.x

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R, others. 2010. Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033–1038, doi:10.1038/nature08867

Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. Phytopathology 59:153–163.

Matheny P, Curtis J, Hofstetter V, Aime M, Moncalvo J, Ge Z, Yang Z, Slot J, Ammirati J, Baroni T, others. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995, doi:10.3852/mycologia.98.6.982

Mayor JR, Schuur EAG, Henkel TW. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol Lett 12:171–183, doi:10.1111/j.1461-0248.2008.01265.x

McFarland J, Mueller GM. 2009. Edible wild mushrooms of Illinois and surrounding states. Champaign: Univ Illinois Press. 232 p.

Miller OK, Miller SL, Palmer JG. 1983. Description and identification of selected mycorrhizal fungi in pure culture. Mycotaxon 18:457–481.

———, Trueblood E, Jenkins DT. 1990. 3 new species of *Amanita* from southwestern Idaho and southeastern Oregon. Mycologia 82:120–128, doi:10.2307/3759971

Moncalvo JM, Drehmel D, Vilgalys R. 2000. Variation in modes and rates of evolution in nuclear and mitochondrial ribosomal DNA in the mushroom genus *Amanita* (Agaricales, Basidiomycota): phylogenetic implications. Mol Phylogenet Evol 16:48–63, doi:10.1006/mpev.2000.0782

Nagendran S, Hallen-Adams HE, Paper JM, Aslam N, Walton JD. 2009. Reduced genomic potential for secreted plant cell wall-degrading enzymes in the ectomycorrhizal fungus *Amanita bisporigera*, based on the secretome of *Trichoderma reesei*. Fungal Genet Biol 46:427–435, doi:10.1016/j.fgb.2009.02.001

Oda T, Tanaka C, Tsuda M. 2004. Molecular phylogeny and biogeography of the widely distributed *Amanita* species, *A. muscaria* and *A. pantherina*. Mycol Res 108:885–896, doi:10.1017/S0953756204000620

Pegler DN. 1986. Agaric flora of Sri Lanka. Kew Bull Addit Ser 12:1–519.

Pringle A, Adams RI, Cross HB, Bruns TD. 2009. The ectomycorrhizal fungus *Amanita phalloides* was introduced and is expanding its range on the west coast of North America. Mol Ecol 18:817–833, doi:10.1111/j.1365-294X.2008.04030.x

———, Vellinga EC. 2006. Last chance to know? Using literature to explore the biogeography and invasion biology of the death cap mushroom *Amanita phalloides* (Vaill. ex Fr.:Fr.) Link. Biol Invasions 8:1131–1144, doi:10.1007/s10530-005-3804-2

Ralser M, Querfurth R, Warnatz HJ, Lehrach H, Yaspo ML, Krobitsch S. 2006. An efficient and economic enhancer mix for PCR. Biochem Biophys Res Commun 347:747–751, doi:10.1016/j.bbrc.2006.06.151

Reid DA, Eicker A. 1991. A comprehensive account of *Chlorophyllum molybdites*. Bot Bull Acad Sin 32:317–333.

Roy M, Dubois M, Proffit M, Vincenot L, Desmarais E, Selosse M. 2008. Evidence from population genetics that the ectomycorrhizal basidiomycete *Laccaria amethystina* is an actual multihost symbiont. Mol Ecol 17:2825–2838, doi:10.1111/j.1365-294X.2008.03790.x

Sawyer NA, Chambers SM, Cairney JWG. 2003. Distribution of *Amanita* spp. genotypes under eastern Australian sclerophyll vegetation. Mycol Res 107:1157–1162, doi:10.1017/S0953756203008426

Selosse MA, Martin F, Bouchard D, Le Tacon F. 1999. Structure and dynamics of experimentally introduced and naturally occurring *Laccaria* sp. discrete genotypes in a Douglas-fir plantation. Appl Environ Microbiol 65:2006–2014.

Shantz HL, Piemeisel RL. 1917. Fungus fairy rings in eastern Colorado and their effect on vegetation. J Ag Res 11:0191–0245.

Thiers HD. 1957. The agaric flora of Texas 1. New species of agarics and boletes. Mycologia 49:707–722, doi:10.2307/3755988

Trudell SA, Rygiewicz PT, Edmonds RL. 2004. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth conifer forests. New Phytol 164:317–335, doi:10.1111/j.1469-8137.2004.01162.x

Vellinga EC, Wolfe BE, Pringle A. 2009. Global patterns of ectomycorrhizal introductions. New Phytol 181:960–973, doi:10.1111/j.1469-8137.2008.02728.x

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246.

Wilson AW, Hobbie EA, Hibbett DS. 2007. The ectomycorrhizal status of *Calostoma cinnabarinum* determined using isotopic, molecular and morphological methods. Can J Bot 85:385–393, doi:10.1139/B07-026

Wolfe BE, Richard F, Cross HB, Pringle A. 2010. Distribution and abundance of the introduced ectomycorrhizal fungus *Amanita phalloides* in North America. New Phytol 185:803–816, doi:10.1111/j.1469-8137.2009.03097.x

Yang Z-L, Weiss M, Kottke I, Oberwinkler F, Nehls U, Guttenberger M, Hampp R. 1999. Amanita. In: Cairney J, Chambers S, eds. Ectomycorrhizal fungi: key genera in profile. Berlin: Springer-Verlag. p 201–230.

Zhang LF, Yang JB, Yang ZL. 2004. Molecular phylogeny of eastern Asian species of *Amanita* (Agaricales, Basidiomycota): taxonomic and biogeographic implications. Fungal Divers 17:219–238.

Zhang P, Chen ZH, Xiao B, Tolgor B, Bao HY, Yang ZL. 2010. Lethal amanitas of east Asia characterized by morphological and molecular data. Fungal Divers 42: 119–133, doi:10.1007/s13225-010-0018-4