Hypholoma lateritium isolated from coarse woody debris, the forest floor, and mineral soil in a deciduous forest in New Hampshire

Therese A. Thompson, R. Greg Thorn, and Kevin T. Smith

Abstract: Fungi in the Agaricomycetes (Basidiomycota) are the primary decomposers in temperate forests of dead wood on and in the forest soil. Through the use of isolation techniques selective for saprotrophic Agaricomycetes, a variety of wood decay fungi were isolated from a northern hardwood stand in the Bartlett Experimental Forest, New Hampshire, USA. In particular, *Hypholoma lateritium* (Schaeff.: Fr.) P. Kumm. was isolated from basidiocarps, decaying *Acer rubrum* L. logs, the Oe organic soil horizon, and the E and BC mineral soil horizons. Identification was confirmed by sequence analysis of the internal transcribed spacer region of nuclear ribosomal DNA. All isolates had identical sequences in this region to previously published sequences for the species; some were monokaryotic and simple-septate and others were dikaryotic, with clamp connections. Isolates were further characterized by banding patterns (DNA fingerprints) produced with PCR primers based in simple repetitive sequences and the minisatellite M13. Nine dikaryotic isolates from basidiocarps and from soil horizons Oe, E, and BC had identical fingerprint patterns with all primers tested. The confirmed presence of *H. lateritium* suggests that this fungus could form a mycelial translocation network that bridges mineral and organic soil horizons and decaying logs.

Key words: Hypholoma sublateritium, DNA fingerprinting, nutrient recycling, coarse woody debris, DAPI.

Résumé : Les champignons appartenant aux Agaricomycetes (Basidiomycota) venant sur le bois mort et les sols forestiers représentent les décomposeurs primaires des forêts tempérées. À l'aide d'une technique d'isolement sélective pour les Agaricomycetes, les auteurs ont obtenu une variété de champignons décomposeurs du bois, dans un peuplement nordique de bois feuillus de la Forêt expérimentale de Bartlett, dans l'état du New Hampshire aux États-Unis. Ils ont obtenu en particulier l'*Hypholoma lateritium* (Schaeff. : Fr.) P. Kumm, à partir de basidiocarpes, de billes d'*Acer rubrum* L. en décomposition, ainsi que de l'horizon organique Oe et des horizons minéraux E et BC du sol. Ils en ont confirmé l'identification par l'analyse des séquences de la région de l'espaceur interne transcrit de l'ADN ribosomal nucléique. Tous les isolats montrent pour cette région des séquences identiques aux séquences déjà publiées pour cette espèce; on y trouve des isolats à monocaryons avec septations simples et d'autres à dicaryons avec anses d'anastomose. Les auteurs ont poursuivi la caractérisation à partir de patrons de bandes (empreintes ADN) produits avec des amorces et PCR basées sur des séquences répétitives simples et du microsatellite M13. Neuf isolats dicaryotiques provenant de basidiocarpes et des horizons Oe, E, et BC du sol montrent des empreintes identiques, avec toutes les amorces. La présence confirmée du *H. lateritius* suggère que ce champignon pourrait former un réseau mycélien de translocation, faisant le pont entre les horizons minéraux et organiques du sol, avec le bois en décomposition.

Mots-clés : Hypholoma sublateritium, empreinte ADN, recyclage des éléments, débris ligneux grossiers, DAPI.

[Traduit par la Rédaction]

Introduction

Coarse woody debris (CWD), defined as fallen limbs and tree boles having a diameter greater than 10 cm (Harmon et al. 1986), is a major element of the carbon cycling in temperate forests. It is also a reservoir of other nutrients including nitrogen and cations such as calcium and magnesium, and CWD contributes to habitat structure (Huston 1993). CWD provides a nutrient source and habitat for many fungi, bacteria, and invertebrates, as well as some vertebrates such as salamanders. Much of this ecological contribution depends on the wood decay process, the rates of which are influenced by multiple biological and physical factors (van der Wal et al. 2007; Harmon et al. 1986).

Nutrient concentrations of certain elements (primarily cations) increase during wood decomposition, and this effect is not solely due to the loss of wood mass during decay (Foster and Lang 1982; Arthur and Fahey 1990; Ostrofsky et al. 1997; Smith et al. 2007). Presumably these elements are being transported from mineral or organic soil to CWD by de-

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cay fungi, which can lead to the accumulation of nutrients in the CWD (Jennings 1990; Boddy and Watkinson 1995; Connolly and Jellison 1995; Ostrofsky et al. 1997; Smith et al. 2007). Wood decay fungi play a complex role in forest biogeochemistry through the long-term release of C and mineral elements from woody debris, the transient immobilization of essential elements, and humus production that provides storage sites in the soil, particularly for essential base cations (Rayner and Boddy 1988; Paul 2007). These fungi, primarily Agaricomycetes (Basidiomycota, informally referred to as basidiomycetes), form extended hyphal networks that enable translocation and redistribution of elements throughout the forest. This translocation is fueled through the respiration of the organic components of wood as CO_2 (Jennings 1990; Smith et al. 2007). Some basidiomycete mycelia form specialized conducting strands called hyphal cords (fascicles of only slightly differentiated hyphae) or rhizomorphs (in which there are inflated central conducting hyphae surrounded by thick-walled and often melanized protective hyphae). Mycelial cord systems and rhizomorphs may be extensive across the forest floor and can translocate nutrients over long distances (Connolly and Jellison 1995; Cairney 2005). These cords often form extensive, long-lived systems that interconnect discrete nutrient resources, e.g., woody litter components on the floor of boreal, temperate, and tropical forests (Boddy 1999). This foraging habit occurs across taxa of basidiomycetes that decay wood.

The cord-forming habit provides an opportunity to focus research on linkages among spatially discrete ecological compartments. Species of the basidiomycete genus *Hypholoma* are cord-forming saprotrophs that are broadly distributed in temperate forests of the world (Breitenbach and Kränzlin 1995). A field study using radioactive P^{32} showed that *Hypholoma fasciculare* (Huds.: Fr.) P. Kumm. cord systems transported labeled phosphate from a central inoculum placed 13 months earlier to decayed wood (buried and at the surface), fresh leaf litter of beech (*Fagus*) and oak (*Quercus*), and well-decayed leaf litter at distances of 7–25 cm (Wells and Boddy 1995). Soil microcosm studies have demonstrated that *H. fasciculare* cord systems translocate phosphorous through forest soil bidirectionally between colonized wood baits, and that some of this phosphate may be captured by the ectomycorrhizal fungus *Suillus variegatus* (Sw.) Kuntze and then transmitted to its plant host *Pinus sylvestris* L. (Wells and Boddy 1995; Lindahl et al. 1999, 2001*a*, 2001*b*).

In this research, we set out to determine what species of cord-forming basidiomycetes occur simultaneously in organic soil, mineral soil, and CWD in a temperate hardwood forest ecosystem and could thus connect nutrients in these ecosystem compartments. Among a variety of wood-decay Agaricomycetes recovered, the cord-forming *Hypholoma lateritium* (Schaeff.: Fr.) P. Kumm. (syn. *Hypholoma sublateritium* (Fr.) Quél.) was isolated from basidiocarps on fallen boles of *Acer rubrum* L. (red maple), and from bole wood and soil horizons Oe, E, and BC.

Materials and methods

Field site and sample collection

The study site was part of a mixed northern hardwood stand in the Bartlett Experimental Forest of the Northern Research Station, USDA Forest Service. The forest is located in the White Mountains of New Hampshire and is operated for long-term forestry research. The soils in the study site are spodosols developed on glacial till derived from granite and gneiss (Hoyle 1977). Using the Munsell soil color charts and soil classification methods, the soil horizons were differentiated and described. For the work reported here, six canopy-dominant *A. rubrum* trees (20–40 cm in diameter at 1.4 m above ground) were felled in 1990, trimmed of branches, and the stems cut into sections (bolts) 1–2 m in length. Sawn bolts were left in ground contact and undisturbed until sampling. Basidiocarps were collected from the sample logs during August, September, and October 1997, October 1998, and August 1999.

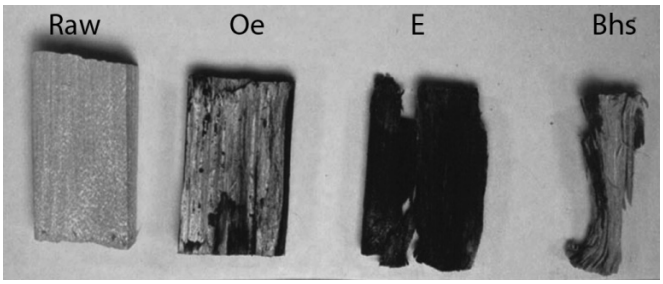


Fig. 1. Soil pit baits from each soil horizon, photographed after incubation in forest soil. A raw (not incubated in soil) wood bait is included to illustrate the change in size and decay of the wood baits.

In June 1997 and in June and October 1999, wood pieces 10–20 cm in length were sawn from the decaying bolts and placed in paper bags for transport to the laboratory. During these site visits, soil pits were dug adjacent to each decaying bolt. With a sterile trowel, the exposed face of the soil profile was cleaned and small samples of soil were aseptically removed from the discernable soil horizons and placed into sterile containers for transport to the laboratory.

In June 1997, small prisms (5 cm × 2.5 cm × 1.5 cm, axial:radial:tangential orientation) of previously-sterilized red maple wood (Raw block of Fig. 1) were aseptically introduced into the Oe, E, and Bhs horizons of the exposed soil profile as bait ("pit bait") for later recovery and isolation of basidiomycetes. After introduction of the bait, the soil pits were backfilled with sterile sand.

Culture media and isolation

Basidiomycetes were isolated from the various wood and soil samples using several semisynthetic agar media (Thompson 2004) based on malt extract and amended with yeast extract, antibacterial antibiotics, and selective fungicides (Table 1).

Wood pieces cut from the sample logs were surface-sterilized in the laboratory and the interior exposed by splitting with a flamed hatchet and rubber mallet. Small wood chips were aseptically transferred from the exposed wood to culture media using a sterile wood gouge and forceps. Chips were plated onto 10 replicate plates of MYBDA and LGBDA in June 1997 and June 1999. Chips from log pieces collected in October 1999 were plated onto five replicate plates of LGBDA, MYBDA, and LGBA (Thorn et al. 1996). The pit bait was aseptically removed from the soil pits in October 2000 and chips from the interior of each bait piece were

Table 1. Composition of isolation and culture media.

	LGBDA	MYBDA	MYA	MEA	LGBA	WA
Basal Medium (g·L^{-1})						
KH_2PO_4	0.5				0.5	
$MSO_4{\cdot}7H_2O$	0.2				0.2	
NH_4NO_3	0.1				0.1	
KCl	0.1				0.1	
$FeSO_4{\cdot}7H_2O$	0.02				0.02	
$Ca(NO_3)_2{\cdot}4H_2O$	0.05				0.05	
Malt extract	2.0	10	10	15	2.0	
Bacto-agar	15	20	20	20	15	20
Yeast extract		2	2			
Distilled water to volume						
Added aseptically (mL·L^{-1}) to sterilized, cooled (55 °C) basal medium						
1 mol·L^{-1} KOH	5				5	
Guaiacol	0.4				0.4	
Indulin AT in dioxane (100 g·L^{-1})	10				10	
Added antibacterial and antifungal compounds (mg·L^{-1})						
Chlortetracycline-HCl	60	60			60	
Streptomycin sulfate	30	30			30	
Penicillin G	30	30			30	
Benomyl	2	2			2	
Dicloran	2	8				

plated onto five replicate plates of LGBDA, MYBDA, and LGBA.

For direct isolation from the soil, a few grains from each soil horizon were streaked across 10 plates each of LGBDA, MYBDA, LGBA, and 2% water agar (WA) (direct plating, Warcup 1951). In addition, 5 g fresh weight of each soil sample were placed in 500 mL of sterile 0.1% (*w/v*) sodium pyrophosphate in a 1 L Erlenmeyer flask and hand shaken for 5 min to disperse the soil clumps. Then, the entire suspension was poured into a stack of sieves (250 and 45 μm). Liquid and grains from the 45 μm sieve were plated onto media listed in Table 1 (sieved method, Thorn et al. 1996).

Fungi were also isolated from baits incubated with collected field soil ("jar baits"). Soil (10 g) from each collected horizon was placed on a moist paper towel in a sterile French square jar, and baited with small pieces (approximately 5 mm × 5 mm × 5 mm) of autoclaved red maple and white pine wood (Fig. 2). After 16–25 months of incubation at room temperature, chips from the interior of the bait were plated onto MYBDA.

Tramal tissue was excised from the collected basidiocarps and plated onto MYBDA, WA, WA+, MYA+, MEA+, and LGBDA. A plus sign (+) in the abbreviation of a culture medium name indicates the addition of the three antibiotics (chlortetracycline-HCL, streptomycin sulfate, and penicillin G) used in other media at the same concentrations.

Cultural and microscopic characteristics

Isolates grown on MEA were compared on the basis of macroscopic cultural characteristics, growth rates, microscopic anatomy, and reactions on gallic acid and tannic acid agar media (Davidson et al. 1938). To determine nuclear condition, hyphae were stained 1 h with DAPI (4′,6-diamidino-2-phenylindole) (Torralba et al. 2004) and observed using a Zeiss Axioplan fluorescent microscope at 358/461 nm (excitation / emission wavelengths).

DNA sequencing

Genomic DNA was extracted from ground hyphae from cultures and Qiagen DNeasy plant mini kit, with minor modifications. The nrDNA ITS1–5.8S–ITS2 region and approximately 600 bp of the large subunit were amplified using primers ITS1 and LR3 (Vilgalys and Hester 1990; White et al. 1990) and then sequenced with primers ITS1 and ITS4 (White et al. 1990) to obtain the forward and reverse sequences of the ITS region. The SeqMan package of DNASTAR software was used to align and compare the sequences.

DNA fingerprinting using simple sequence repeats and minisatellite M13

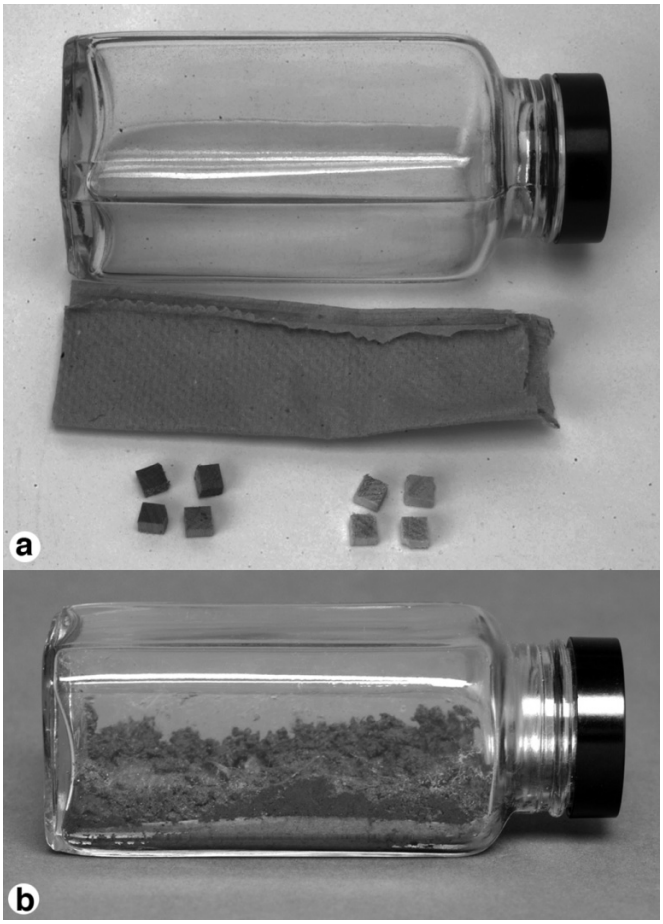
Two primers based on the simple repetitive sequences $(GACA)_4$, $(GTG)_5$, plus the minisatellite M13 primer (5′–GAGGGTGGCGGTTCT–3′) (Meyer et al. 1993) were used to compare all the isolates, including those with different growth habits, using standard PCR conditions. Variation in anonymous regions of DNA between simple repetitive sequences is used in this technique (referred to as ISSR, for inter simple sequence repeat) to recognize and differentiate individuals (Meyer et al. 1993; Franzén et al. 2007).

The amplified DNA was separated by electrophoresis on 2.0% agarose in 1× TBE buffer for ca. 2 h and stained with 0.5 mg·L^{-1} ethidium bromide (EtBr), destained and then visualized and photographed with UV light. The gels were manually analyzed for absence or presence of bands of specific sizes as compared with a 1KB+ ladder (Invitrogen, Carlsbad, Calif., USA). Genetic similarity (Nei 1987) of isolates was determined using the Dice coefficient of $2c/(a + b)$, where a and b are the number of bands in each of the isolates being compared and c is the number of shared bands in both isolates.

Results

Soil classification of organic and mineral soils for the re-

Fig. 2. (*a*) Components of a French square jar bait culture including sterilized red maple (left) and white pine (right) wood baits (5 mm cubes). (*b*) Illustration of the French jar wood bait culture including paper towel moistened with sterile distilled water, red maple wood baits at one end of the jar and white pine wood baits at the opposite end of the jar and, placed on top of the baits, approximately 5 g of a single soil horizon from one research site. Jars were capped, incubated at room temperature, and moistened with sterile distilled water as necessary.



search site are illustrated in Table 2 and Fig. 3. The organic Oe soil horizon had a depth of 4 cm (extending +4–0 cm in depth), the E mineral soil horizon had a depth of 15 cm (extending 0–15 cm in depth), the Bs mineral soil horizon extended 15–18 cm (too irregular to culture from), the Bhs mineral soil horizon extended 18–26 cm, and the BC mineral soil horizon started at a depth of >26 cm. No visible decay was seen on jar wood baits after 16 months, so the soil pit baits were left in place and retrieved after 3 years in October 2000 (Fig. 1). Forty-four fungal isolates of 22 different species including 11 species of Agaricomycetes were recovered from soil horizons by direct plating, the sieved method, pit baits, or jar baits (Supplemental Table 1[1]; Thompson 2004). Although structurally weakened and friable after 3 years of in situ and in vitro soil incubation, wood baits also yielded isolates of basidiomycetes. Sequences of ITS rDNA allowed identification of the isolates (see below). Among these, the common bricktop mushroom, *H. lateritium*, stood out as having been recovered from various soil horizons (Oe, E, and BC) and from the red maple boles. This species was also found fruiting on *A. rubrum* at the site. Pure cultures of *H. lateritium* were directly isolated from basidiocarps in 1997 and 1998, decaying *A. rubrum* boles in 1999, and from soil horizons (Oe, E, and BC) of the forest floor by sieve, jar bait methods in 1999 and by soil pit bait method in 2000.

Morphological characteristics and DAPI staining

The *Hypholoma* isolates recovered were of two distinct morphological types: (1) 11 isolates with clamp connections, binucleate hyphae, a fast growth rate (covering a 100 mm Petri dish of MEA in 2 weeks), and zonate aerial mycelium (Figs. 4*a*–4*c*) were recovered from Oe, E, and BC soil horizons (collected October 1999) using the jar bait method, Oe and E soil horizons using the soil pit bait method (collected October 2000), and basidiocarps (collected October 1997 and 1998); and (2) seven isolates with simple septa, uninucleate hyphae, oidia (arthroconidia with rhexolytic secession, see Walther et al. 2005), a slow growth rate (covering only half of the Petri dish of MEA in 2 weeks), and azonate, feathery mycelium (Figs. 4*d*–4*f*) were recovered from bole wood (collected June 1999), Oe soil horizon by sieve method (collected October 1999), E soil horizon by soil pit method (collected October 2000), and a basidiocarp (collected October 1998). The nuclear state of hyphae was confirmed by DAPI staining.

DNA sequencing

Sequences of the ITS region of all *Hypholoma* isolates recovered, including isolates from basidiocarps, soils, and the bole wood, showed 100% identity to GenBank accession AY818349, *H. sublateritium* (AFTOL-ID 597, Yang et al. 2005). Legon et al. (2005), Index Fungorum (http://indexfungorum.org/), and MycoBank (http://www.mycobank.org/) regard this species as a synonym of *H. lateritium,* and we follow their taxonomy here. All sequences were deposited to GenBank as accession numbers JF692729–JF692746.

DNA fingerprinting using simple sequence repeats and minisatellite M13

The M13 primer yielded 6–7 bands in a range of 275–1900 bp, $(GACA)_4$ primer yielded 4–5 bands of 475–1900 bp, and $(GTG)_5$ primer yielded 4–7 bands of 375–1325 bp. The fingerprint patterns of all strains were very similar. The genetic similarity of the 18 isolates, based on the 18 bands scored for the three PCR primers, ranged from 0.80 to 1.00 (data not shown). Most differences among isolates corresponded to whether they were binucleate (having most or all bands) or uninucleate (lacking some bands). Nine binucleate isolates from basidiocarps and from soil horizons Oe, E, and BC had identical fingerprint patterns with all primers tested (Table 3).

Discussion

The 18 isolates of *H. lateritium* that we recovered had two distinct growth patterns, with differences in growth rate, colony appearance, and presence or absence of clamp connections. These differences were correlated with nuclear state as

[1]Supplementary data are available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/b2012-011)

Table 2. Soil description and ecological properties.

	Horizon				
	Oe	E	Bs	Bhs	BC
Depth (cm)	4–0	0–15	15–18	18–26	>26
Matrix	10 YR 2/1	10 YR 7/1	7.5 YR 5/3	5 YR 4/6	10 YR 6/6
Fe conc.		Few 5 YR 4/6		Few 5 YR 3/4	Few 10 YR 5/8
Texture		Fine sandy loam	Fine sandy loam	Sandy loam	Coarse sandy loam
Structure		Weakly granular	Moderately granular	Moderately granular	Moderately granular
Boundary	Clear, wavy	Clear, irregular	Gradual, broken	Clear, wavy	NA
Stone	0, 0, 0	5, 0, 0	5, 0, 0	5, 0, 0	20, 0, 0
Roots	Many	Many	Few	Few	Few

Note: Matrix is the dominant color or background color on which mottles (redox features) are compared, with color notation from Munsell (1994). Iron concentration (Fe conc.) is the amount of mottles (few, common, or many) and the matrix color of those mottles. Stone is the percentage amount of gravel (0.2–7.5 cm), cobble (7.5–30 cm), and stone (30–60 cm) found in that soil horizon.

Fig. 3. Soil description and ecological properties of the research site. The soil type 143B Monadnock fine sandy loam, with a 3%–8% slope with deep, well drained soils on glaciated uplands. These soils are classified as coarse–loamy over sandy or sandy–skeletal, isotic, frigid, Typic Haplorthods. The organic soil Oe; E – eluvial, grayish color owing to leaching of Fe, Al, and clay into the lower mineral horizons; Bhs – dispersible organic matter (podzolization) with accumulation of Fe and Al; and BC – parent material. Roots and stones found in each soil horizon were illustrated, along with the boundary between soil layers.

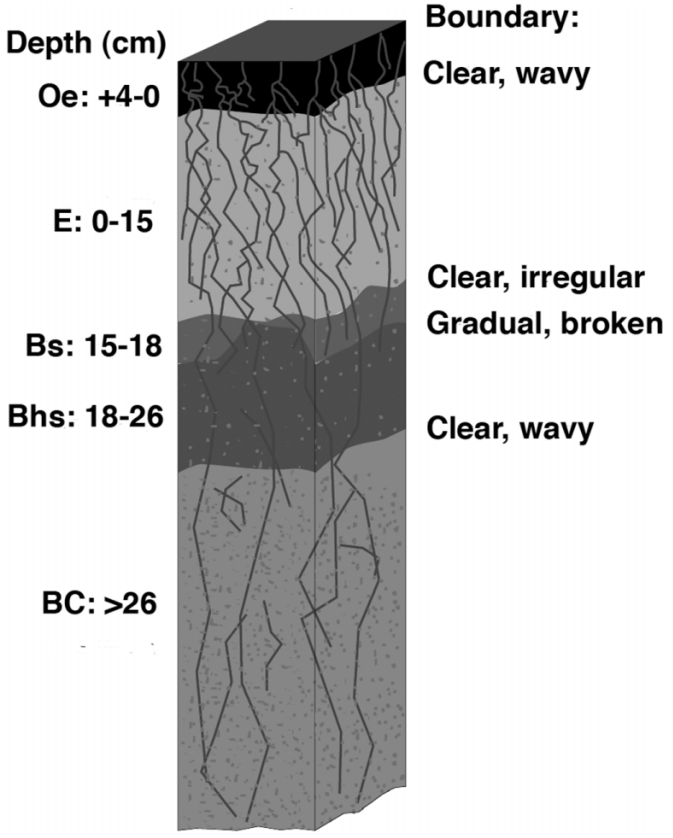
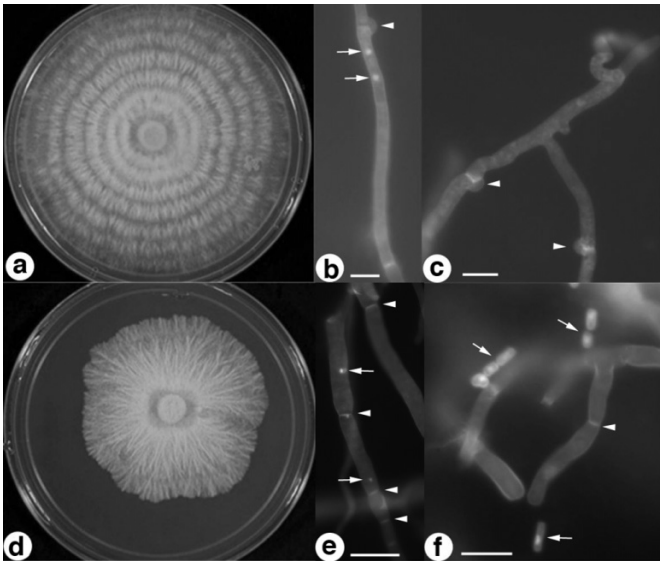


Fig. 4. Morphology and cytology of *Hypholoma lateritium* isolates recovered. (*a*) An isolate with zonate mycelium and fast growth, covering 100 mm plate in 2 weeks; (*b*) using DAPI staining, hyphae are binucleate (arrows) and have clamp connections (arrowheads, *b–c*). (*d*) An isolate with feathery mycelium and slower growth, covering 1/2 plate in 2 weeks; (*e*) using DAPI staining, hyphae are uninucleate (arrows) and simple septate (arrowheads, *e–f*) and produce oidia (arrows, *f*). Scale bars (*b–c*, *e–f*) = 5 μm.



determined by DAPI staining, and all strains were confirmed by sequencing to be *H. lateritium*. Dikaryotic (binucleate) cultures of *H. lateritium* have been shown to produce both uninucleate and binucleate arthroconidia (oidia) (Godio et al. 2004) and to become uninucleate with prolonged culture (Walther and Weiß 2008), so it is possible that our uninucleate strains were binucleate when first isolated. Seven binucleate strains from fruiting bodies and soil had identical fingerprint patterns, suggesting that they may be replicate isolates of the same individual, based on previous studies in other fungi (Meyer et al. 1993; Franzén et al. 2007). However, this identity at the individual level cannot be confirmed without comparison of fingerprint patterns in individuals known to be distinct. This analysis should ideally be done with mating compatible strains from the sampling area as well as isolates from more distant localities.

Translocation of nutrients by wood-decomposing basidiomycete fungi has been shown by a number of other researchers (Jennings 1990; Connolly and Jellison 1995; Cairney 2005; Smith et al. 2007). Wood decaying fungi can take up mineral nutrients as shown by the translocation of ^{32}P in cord systems of *Phanerochaete velutina* (DC.) P. Karst. and *Phallus impudicus* L. (Wells et al. 1990) and the bidirectional

Table 3. Origins, microscopic characters, and DNA fingerprints (using ISSR with $(GACA)_4$, $(GTG)_5$, and minisatellite M13) of *Hypholoma lateritium* isolates recovered.

		Microscopic hyphal characters		Fingerprint marker[a]		
Isolate / source codes[b]	Collection	Binucleate, clamps	Uninucleate, simple septa	$(GACA)_4$	$(GTG)_5$	M13
Mu 3 28 WA / Mu	Oct. 1997	X		A	A	A
Mu 4 28–4 MYA+ / Mu	Oct. 1998	X		A	A	A
10 JB 28–1 WP 2 2 / Oe	Oct. 1999	X		A	A	A
10 JB 28–2 WP 2 1 / E	Oct. 1999	X		A	A	A
10 JB 28–2 WP 1 6 / E	Oct. 1999	X		A	A	A
10 JB 28–2 RM 1 1 / E	Oct. 1999	X		A	A	A
10 JB 28–3 WP 2 2 / BC	Oct. 1999	X		A	A	A
SPB 28–1 mol/L 1A / Oe	Oct. 2000	X		A	A	A
SPB 28–2 mol/L 1A / E	Oct. 2000	X		A	A	A
SPB 28–2 T 1A / E	Oct. 2000	X		A	A	A
10 JB 28–2 WP 8 / E	Oct. 1999	X		A	A-750, 650	A
Mu 4 28–4 MYBDA / Mu	Oct. 1998		X	A	A	A-1000
BC 28–2-1 L / BC	June 1999		X	A	A-375	A-1900
Soil 28–1 OSL L2 2B / Oe	Oct. 1999		X	A	A-750, 650, 475, 375	A-1900
Soil 28–1 OSL L2 2 / Oe	Oct. 1999		X	A	A-650, 375	A-1900
Soil 28–1 OSL L2 2A / Oe	Oct. 1999		X	A	A-650, 375	A-1900
Soil 28–1 OSL T1 1 / Oe	Oct. 1999		X	A-475	A-375	A-1900
SPB 28–2 T 2A / E	Oct. 2000		X	A-475	A-475	A-1900

Note: A, All bands; A-, missing band(s).

[a]Finger print marker bands for $(GACA)_4$ at 1900, 1300, 800, 750, 475 bp; $(GTG)_5$ at 1325, 1000, 750, 650, 475, 375 bp; and M13 at 1900, 1650, 1000, 800, 500, 325, 275 bp.

[b]Isolate codes are Mu, mushroom; BC, bole chip; OSL, organic soil liquid; 10JB, Oct. jar bait; WP, white pine bait; RM, red maple bait; SPB, soil pit bait; L, LGBDA; T, LGBA; and M, MYBDA. Source codes are Mu and soil horizons Oe, E, and BC.

translocation between ectomycorrhizal and saprotrophic rhizomorphs (Cairney 1992). Lindahl et al. (1999, 2001*b*) demonstrated translocation of ^{32}P from a wood-decomposing saprotrophic fungus *H. fasciculare* to an ectomycorrhizal fungus *S. variegatus* and its plant host *Pinus sylvestris*. Lindahl et al. (2001*a*) demonstrated bi-directional translocation of ^{32}P and ^{33}P between wood blocks by *H. fasciculare*. *Hypholoma fasciculare* was also studied by Wells and Boddy (1995, 2002), who showed translocation of soil-derived phosphorus and carbon exchange between soil and wood by mycelia. Mycelial cord systems can translocate nutrients distances of significance in forest ecology, measured in meters or tens of meters (Cairney 2005), and individuals of these fungi may occupy large areas, from tens of m^2 to several ha (Smith et al. 1992).

The impacts of nutrients on wood decomposition, and the impacts of decomposition on nutrient concentrations in wood have been the subjects of recent studies. Clinton et al. (2009) studied mass loss during decomposition by 12 different wood decay fungi of three species of southern beech (*Nothofagus*) wood in soil microcosms and recorded an increase in concentrations of nitrogen, phosphorus, and calcium. Nutrient mobilization from soil to decaying wood was particularly pronounced in *Ganoderma* cf. *applanatum* (Pers.) Pat. and *Pleurotus purureoolivaceus* (G. Stev.) Segedin, P.K. Buchanan & J.P. Wilkie (Clinton et al. 2009). In a seminatural microcosm study in which *H. fasciculare* was grown together with the ectomycorrhizal fungus *Tomentellopsis submollis* (Svrček) Hjortstam with living roots of the host beech tree, the decomposer fungus released inorganic nitrogen that was taken up by mycorrhizal roots (Wallander et al. 2006). The authors concluded that this release of $^{15}NH_4$ was not the result of combative interactions between the two mycelia, but possibly the result of losses from the *Hypholoma* mycelium during freezing and thawing in winter or during grazing by invertebrates (Wallander et al. 2006). In another soil microcosm study, grazing of *Phanerochaete velutina* mycelium by collembola reduced nitrogen translocation within the mycelium but did not increase nitrogen released into the soil (Tordoff et al. 2011). Bebber et al. (2011) studied the impact of experimental nitrogen addition on decomposition of beech wood blocks in forest soil by *H. fasciculare* and *Phanerochaete velutina*. Both species caused greater mass loss and completely decayed the baits more frequently with nitrogen added at a rate equivalent to 2.8 $kg \cdot ha^{-1} \cdot y^{-1}$. Wood decay fungi are vital and interconnected with the environment during decomposition of wood and play a major role in the health of an ecosystem.

Other researchers have had an interest in determining what species of fungi are found in the soil habitat at different depths, and in the general diversity of soil Basidiomycota. Jumpponen et al. (2010) used a 454 pyrosequencing approach to study the fungal communities in tallgrass prairie soils from 0–60 cm in depth. Among over 14 500 fungal sequences recovered, of which Basidiomycota represented 54%, were an estimated 1500 operational taxonomic units (OTUs); both diversity and the abundance of most individual OTUs decreased with depth beyond 10 cm. Lim et al. (2010) also used pyrosequencing to study fungal communities in soils 5–10 cm deep from three islands in the Yellow Sea. Approximately 70% of the nearly 10 000 sequences obtained were of Basidiomycota, the majority of which were ectomycorrhizal

and wood rotting fungi. O'Brien et al. (2005) used a strategy of PCR followed by cloning and sequencing of ribosomal DNA to study the fungal communities of the litter and organic layers and A and B soil horizons in mixed hardwood and pine forests in North Carolina. There were no clear trends in richness or diversity with increasing depth, the Basidiomycota representing approximately 10%–65% of the sequences obtained from each horizon. Lynch and Thorn (2006) also used a PCR, cloning and sequencing approach to study the diversity of basidiomycetes in Michigan agricultural soils from soil cores (2.5 cm diameter × 5–15-cm depth) and found 231 species-level OTUs among 409 unique basidiomycete sequences recovered. Porter et al. (2008) assessed the diversity of soil-dwelling Agaricomycotina in a temperate hemlock (*Tsuga*) forest by both periodic sampling of fruiting bodies and PCR, cloning and sequencing of ribosomal DNA from soil samples. The 132 species found as fruiting bodies and the 66 OTUs found as DNA sequences represented predominantly complementary (nonoverlapping) taxa. All of these floristic studies detected considerable diversity of basidiomycetes from soils yet, remarkably, neither *H. lateritium* nor the genus *Hypholoma* were reported in any of them.

This study is the first to demonstrate the same species of basidiomycete, the common and widespread *H. lateritium*, in bole wood and into the organic and mineral soils. Such wood decay basidiomycete fungi could translocate nutrients, providing a means to recycle and retain nutrients in terrestrial ecosystems.

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Robert O. Blanchard passed away on 15 September 2010 at his home in Durham, N.H. Robert earned his PhD in Mycology from the University of Georgia for his research with Ascomycete fungi. Robert taught several courses, served as advisor to numerous graduate students, and served in various leadership roles at the University of New Hampshire for over 33 years until his retirement in 2005. This paper is a tribute to his dedication to education and to this research as the first author's MS and PhD advisor.

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