Revision of pyrophilous taxa of *Pholiota* described from North America reveals four species—*P. brunnescens, P. castanea, P. highlandensis*, and *P. molesta*

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Revision of pyrophilous taxa of Pholiota described from North America reveals four species—P. brunnescens, P. castanea, P. highlandensis, and P. molesta

P. Brandon Matheny [a], Rachel A. Swenie[a], Andrew N. Miller [b], Ronald H. Petersen [a], and Karen W. Hughes[a]

aDepartment of Ecology and Evolutionary Biology, University of Tennessee, Dabney 569, Knoxville, Tennessee 37996-1610; bIllinois Natural History Survey, University of Illinois Urbana Champaign, 1816 South Oak Street, Champaign, Illinois 61820

ABSTRACT

A systematic reevaluation of North American pyrophilous or "burn-loving" species of Pholiota is presented based on molecular and morphological examination of type and historical collections. Confusion surrounds application of the names P. brunnescens, P. carbonaria, P. castanea, P. fulvozonata, P. highlandensis, P. molesta, and P. subsaponacea, with multiple names applied to a single species and multiple species described more than once. Molecular annotations using nuc rDNA ITS1-5.8S-ITS2 (internal transcribed spacer [ITS] barcode) and RPB2 (RNA polymerase II second largest subunit) are used to aid in application of these names in a phylogenetic context. Based on ITS molecular annotations of 13 types, the following heterotypic synonymies are proposed: P. highlandensis (syn. P. carbonaria and P. fulvozonata); P. molesta (syn. P. subsaponacea); and P. brunnescens (syn. P. luteobadia). In addition, we observed that the species P. castanea, known previously only from the type collection in Tennessee, is found commonly on burned sites near the Gulf Coast and other southeast regions of the United States. Overall, the pyrophilous trait is evolutionarily derived in Pholiota. Endophytic and endolichenic stages were deduced for P. highlandensis, the most widely distributed of the pyrophilous Pholiota. As a result, we introduce the "body snatchers" hypothesis that explains the maintenance of some pyrophilous fungi in ecosystems as endophytes and/or endolichenic fungi. Photographs, taxonomic descriptions, and a dichotomous key to pyrophilous species of Pholiota that occur in North America are presented.

INTRODUCTION

In their monographic work on the genus Pholiota (Fr.) P. Kumm. from North America, Smith and Hesler (1968) documented seven pyrophilous species—that is, fungi that produce reproductive structures exclusively on burned or heated areas in post-fire habitats and that otherwise are rare, undetected, or absent from pre-fire fungal communities (Seaver 1909; El-Abyad and Webster 1968; Gochenauer 1981; Carpenter and Trappe 1985). The authors treated two previously described species, P. carbonaria A.H. Sm. and P. highlandensis (Peck) Quadr. & Lunghini, and described five new species—P. brunnescens A.H. Sm. & Hesler, P. fulvozonata A.H. Sm. & Hesler, P. luteobadia A.H. Sm. & Hesler, P. molesta A.H. Sm. & Hesler, and P. subsaponacea A.H. Sm. & Helser. All seven species were ascribed to section Carbonicola A.H. Sm. & Hesler, also published as new in the same work, configured around species that produce basidiomes on burned soil, with a viscid pileus and conspicuous pleurocystidia, and typified by P. fulvozonata. Smith and Hesler (1968) emphasized differences in veil color and development, taste, basidiome size, and pileus color to distinguish the

seven species. However, four of the new taxa—P. fulvozonata, P. luteobadia, P. molesta, P. subsaponacea—were described from single collections and thus documented with little data on their morphology, geographic distribution, and overall ecology.

Our investigation of pyrophilous Pholiota was spurred by collections identified as P. highlandensis on burned soil after the Chimney Tops 2 fire that occurred in the Great Smoky Mountains National Park (GSMNP) of eastern Tennessee during Nov and Dec 2016. Pholiota highlandensis was originally described by Peck (1872) from New York (as Agaricus (Naucoria) highlandensis) on burned ground. We observed basidiomes of P. highlandensis at multiple burned sites in the GSMNP as soon as the first spring (Apr 2017) after the Chimney Tops 2 fire, continuing into winter and spring of 2018. It is arguably the most common fungus in the recently burned areas of the GSMNP. Blast sequences of the nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region produced from samples of our collections revealed that sequences labeled with different names—P. brunnescens, P. carbonaria, P. flavida, in addition to P. highlandensis, as well as several insufficiently named endophytes and endolichenic

fungi—were ascribed to our application of the name P. highlandensis with high (99%) ITS sequence similarity. To resolve the taxonomy of P. highlandensis and related pyrophilous species, we obtained the type collections and supporting materials ascribed to those species in section Carbonicola and other relevant groups in Smith and Hesler (1968).

Our objectives were thus as follows: (i) to annotate type and other historical collections with the ITS region; (ii) examine the distribution of "burn-loving" Pholiota in a molecular phylogenetic context; (iii) to resolve species-level boundaries among pyrophilous species of Pholiota using a combination of molecular phylogenetic analyses and morphological and ecological data; and (iv) arrive at a better understanding of the ecology and evolution of burn species of Pholiota in North America.

From an ecological perspective, it is not entirely clear how pyrophilous fungi enter or are maintained in burned or excessively heated ecosystems, whether by dispersal, reduced competition, evolution of spore dormancy, heat tolerance, fire-associated mutualisms, or evading detection in pre-fire systems (Seaver 1909; Petersen 1970; Bruns et al. 2005; Robinson et al. 2008; McMullan-Fisher et al. 2011; Baynes et al. 2012). As Petersen (1970) points out, it is necessary to distinguish maintenance of mycelial or vegetative states apart from conditions that stimulate sexual reproductive stages. Here, we summarize four hypotheses and the predictions each makes regarding the appearance of pyrophilous fungi in burned or heated systems and suggest some tests for these predictions.

MATERIALS AND METHODS

Field sampling.—Recently collected fresh specimens were made at low elevation (590–850 m) in the GSMNP near Gatlinburg at various burned localities and burn intensities at Baskins Creek Trail (35.67912, −83.477662), Cove Hardwood Nature Trail (35.695481, −83.575472), Twin Creeks Trail (35.687855, −83.501176), Two Mile Lead Trail (35.673746, −83.497506; also referred to as "Two Mile Trace"), and Old Sugarlands Trail (35.671862, −83.510796) between Apr 2017 and May 2018. These sites are characterized by mixed hardwood forests, some dominated by Tsuga canadensis with a Rhododendron understory, and ridges dominated by stands of Pinus pungens (Welch and Waldrop 2001) mixed with P. rigida and Kalmia latifolia, or to a lesser extent Rhododendron, understory (Whittaker 1956).

Samples of basidiomes were documented using Kornerup and Wanscher (1967), Munsell Soil Color

Charts (1954), or Ridgway (1912). Gross morphological details in the taxonomic descriptions were made from notes on fresh materials, from notes present in historical collections, or supplemented with descriptions from Smith and Hesler (1968). "L" refers to the number of lamellae that reach the stipe. Specimens were dehydrated and preserved at TENN (herbarium abbreviations follow Thiers [continuously updated]) and are available on the Mycology Collections Portal (MyCoPortal 2018). MO-numbered vouchers refer to observations on Mushroom Observer (http://mushroomobserver.org/).

Type collections were obtained by loan from MICH and NYS and supplemented by examination of types and other historical collections held at TENN and WTU.

Microscopy.—Anatomical observations were made from fresh material, where possible, or from sections of dried basidiomes rehydrated in 5% KOH, at times mixed with Congo Red (Clémençon 2009). Mean values of basidiospore length, width, and Q-value (spore length divided by width in profile view) are italicized. Measurements of basidiospores in parentheses fall outside two standard deviations from the mean. Otherwise, measurements of cellular features and line drawings follow Braaten et al. (2014). Basidiospores and other microscopic features were examined from at least three specimen vouchers per species. Basidiospore measurements were made from n total spores sampled from y collections.

Single-basidiospore isolation.—Deposits of basidiospores were collected from fresh samples of P. highlandensis and diluted in sterile water. Aliquots of basidiospore suspensions were plated on malt extract agar (MEA) and examined daily for germination. Early germinating spores were isolated on a small block of agar and subcultured on MEA for microscopic examination and in baby food jars containing 30 mL potato dextrose (PD) broth (Difco, Detroit, Michigan). Resulting mycelia on MEA were examined for presence/absence of clamp connections and conidial production.

DNA extractions, PCR, and sequencing.—Small pieces of lamella fragments or mycelial mats from cultures were placed in 40-µL solutions of Extract-N-Amp (Sigma-Aldrich, St. Louis, Missouri; Truong et al. 2017), incubated at 95 C for 10 min, and then mixed with equal volumes of 3% bovine serum albumin (BSA) dilution solution to extract DNA. Polymerase chain

reaction (PCR) and sequencing were performed following Sánchez-García et al. (2014) using primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). DNA was extracted from 10–20 mg of dried basidiome tissue from types and historical collections using a "High Performance" HP Fungal DNA Kit (Omega Bio-Tek, Norcross, Georgia). For these collections, ITS1 and ITS2 were amplified and sequenced separately as in Ammirati et al. (2007). DNA extractions of 19th century material were performed in a laminar-flow hood apart from standard DNA extractions. Dried tissues were incubated at 65 C for 24–72 h before proceeding with the protocol outlined in the HP Fungal DNA Kit and using filtered pipette tips.

Primers b6F and b7.1R were used to amplify the most variable region of the gene RPB2, which encodes the second largest subunit of RNA polymerase II (Matheny 2005). As these are degenerate primers, we designed nondegenerate primers near the same original primer sites to amplify Pholiota-only RPB2 sequences based on an alignment of Strophariaceae Singer & A.H. Sm.: PhRPB2-6F: 5′-GCC GAA ACC CCA GAA GGT CAA GC-3′; PhRPB2-7R: 5′-CTG GTT ATG ATC TGG GAA GGG-3′. ITS and RPB2 sequence chromatograms were assembled in Sequencher 5.0.1 (Gene Codes Corporation, Ann Arbor, Michigan). DNA sequences produced during this work were deposited in GenBank.

DNA alignment and phylogenetic analyses.—ITS sequences from GenBank with >93% similarity to Tennessee sequences of P. highlandensis produced primarily in studies by Holec and Kolařík (2014), Holec et al. (2014, 2016), and Siegel et al. (2015) were downloaded and aligned in Clustal X 2.0.9 (Larkin et al. 2007). The aligned sequences were manually adjusted in MacClade 4.08 (Maddison and Maddison 2005). All nucleotide sites were included in phylogenetic analyses. Sequences of RPB2 from Pholiota and an outgroup Hypholoma (Fr.) P. Kumm. based on Matheny et al. (2006, 2015) were manually aligned with newly produced RPB2 sequences. Two RPB2 sequences were retrieved from the genomes of P. conissans (Fr.) M.M. Moser and Hypholoma sublateritium (Fr.) Quél. using the BLASTn function of the Pholiota_conissans 20151025 and Hypholoma_sublateritium 20110822 assemblies (Grigoriev et al. 2012; Nordberg et al. 2014). After inspection for strongly supported intergene conflict (maximum likelihood [ML] bootstrap values >70% for conflicting internodes), we combined 47 ITS sequences with 29 RPB2 sequences to form a two-gene supermatrix. ITS sequences were

added to the supermatrix if from a type collection (e.g., P. chocenensis) or from taxa represented by one sequence (e.g., P. marangania).

jModelTest 2 (jmodeltest.org; Guindon and Gascuel 2003; Darriba et al. 2012) was used to select the best-fit model of DNA substitution for the ITS data set according to the Akaike information criterion for maximum likelihood (ML) and Bayesian inference (BI) analyses. These were conducted in RAxML 8.0.0 (Stamatakis 2014) and MrBayes 3.2.6 (Ronquist et al. 2012). For the RPB2 analyses, the alignment was partitioned by codon position and modeled separately as in Matheny (2005); only coding regions were used. One thousand bootstrap replicates were performed in ML analyses. Five million generations were run in the BI analyses, sampling trees from the posterior distribution every 5000 generations. Two independent runs were performed using default chain and heating parameters. To insure convergence between the two runs, the standard deviation of split frequencies and potential scale reduction factors (PSRFs) for all model parameters were observed following recommendations in the user manual. Posterior probabilities (PPs) were calculated after discarding the first 25% of sampled trees. Tree files were viewed in FigTree 1.4.0. ML bootstrap values >70% and BI PP >0.95 were considered as strong support. DNA alignments and tree files are available at http://mathenylab.utk.edu/Site/Alignments_%26_Data_Sets.html and TreeBASE (submission no. 22384). For the ITS data set, Pholiota squarrosa was used for rooting purposes following Holec et al. (2014). Hypholoma was used for rooting purposes in the combined ITS and RPB2 supermatrix as explained above.

RESULTS

In this study, 133 ITS and 29 RPB2 sequences were analyzed. Of these, 99 are new (78 ITS, 21 RPB2) (TABLE 1), including 13 ITS sequences from holotype and isotype collections. A GTR+I+G model was selected as best fit for the ITS alignment, which included 108 taxa and 711 sites. This model permits six unique substitution types and provides a parameter for a proportion of invariable sites and gamma-distributed rate heterogeneity across four rate categories. A GTR+I+G model was applied to each codon in the RPB2 exon alignment. The supermatrix contained a total of 1435 sites. Two independent Bayesian runs converged easily on the ITS and supermatrix data sets based on the standard deviation of split frequencies reaching <0.01. PSRFs were 1.0 or very close to 1.0 for all model parameters; 15002 trees were sampled from the posterior distribution to calculate PPs.

Table 1. DNA sequences analyzed in this study.

Species	Specimen voucher or strain (herbarium)	Geographic origin	ITS	RPB2
Hypholoma australe	PBM3481 (PERTH 08241856)	Western Australia	HQ832446	HQ832434
Hypholoma sublateritium	JS031107 (CUW)/v1.0		AY818349	20110822 assembly
Hypholoma subviride	TJB10226 (CORT)	Belize	HQ222023	HQ832435
Pholiota baeosperma	TFB7383 (TENN 054431)	Chile	MG735312	MF978357
	TENN 054993	Argentina	KY559332	MF978336
Pholiota brunnescens	AHS3525 (holotype MICH 11657)	Oregon	MG735292	—
	AHS3462a (paratype MICH 30237)	Oregon	MG735285	—
	AHS55353 (paratype MICH 30236)	Oregon	MG740766	—
	AHS74092 (paratype MICH 30234)	Idaho	MG735291	—
	AHS9350 ("molesta" TENN 028871)	California	MG740767MG735268	—
	AHS19891 ("highlandensis" MICH 30482)	Oregon	MG735286	—
	AHS43222 (holotype of Pholiota luteobadia MICH 11688)	Michigan	MG735289	
	GAL137545 ("highlandensis" WTU 21130)	Alaska	MG735298	MG923687
	JFA10092 (WTU 010349)	Washington	MG740768MG735269	MH360724
	MTS4953b (WTU 010343)	Washington	MG735305	MG923688
	PBM527 (WTU 001032)	Washington	MG735299	MG923689
	PBM1408 (WTU 010337)	Washington	MG735270	MG923690
	PBM3057 (TENN 063855)	California	MG735314	MG923699
	Stz9021 ("Flammula carbonaria" WTU 10354)	Washington	MG735303	MG923691
	TFB6242 ("highlandensis" TENN 052897)	Mexico	KF871789	MG923692
	2013071701 Pinus densiflora dead wood	Japan	LC100010	—
	Uncultured fungus Baikal Lake	Russia	KY991007	—
Pholiota castanea	DPL7769 (TENN 071878)	Texas	MH016952	MG923702
	TENN 020269 (holotype Pholiota castanea)	Tennessee	HQ222025	—
	TENN 003958 ("carbonaria Fr.")	Louisiana	MG735301	—
	TENN 010128 ("carbonaria Fr.")	Tennessee	MG740769MG735271	—
	TENN 016152 ("carbonaria Fr.")	Florida	MG740770MG735272	—
	TENN 018845 ("carbonaria Fr.")	Tennessee	MG740771MG735273	—
	TENN 021329 ("carbonaria Fr.")	Tennessee	MG735302	—
	TENN 021600 ("carbonaria Fr.")	Florida	MG735274	—
	TENN 021647 ("carbonaria Fr.")	Tennessee	MG740772MG735275	—
	Stz21915 ("highlandensis WTU 53296)	Mississippi	MG735308	—
	X-35 washed organic particles from mineral soil (A horizon) conifer forest	Michigan	KC176331	—
Pholiota chocenensis	GAL2659 ("cf. highlandensis" WTU 43920)	Norway	MG735304	—
	PRM 895066	Czech Republic	HG007985	—
	11237 ("decussata" holotype Pholiota chocenensis)	Italy	JF908583	—
Pholiota conissans	395/CIRM-BRFM 674		JF908575	20151025 assembly
Pholiota ferrugineolutescens	AHS9393 (isotype TENN 028897)	California	HQ222026	—
Pholiota gallica	Herb.Chevassut 3478 (holotype MPU)	France	HG007988	—
	PRM 933232 (isotype Pholiota highlandensis var. citronosqumulosa)	France	LN889967	—
	PRM 933233	France	LN889968	—
	PRM 933234	France	LN889969	—
Pholiota highlandensis	AHS9342 (paratype Pholiota carbonaria MICH 30243)	California	MG735293	—
	AHS9500 (holotype Pholiota carbonaria MICH 11663)	California	MG735288	—
	AHS48449 ("carbonaria" MICH 30263)	Washington	MG735294	—
	AHS73887 (holotype of Pholiota fulvozonata MICH 5316)	Idaho	MG735290	—
	ASI 24032 ("carbonaria")	Korea	AY251301	—
	Fire184 Pinus pungens root—environmental sample	Tennessee	MH348870	MH360725
	Fire285 (TENN 072178)	Tennessee	MH348871	MH360726
	Fire387 (TENN 072212)	Tennessee	MG735295	MH360727
	Fire409 (TENN 072234)	Tennessee	MH348872	MH360728
	I12F-00262 ("Fungal sp." from Peltigera neopolydactyla)	China	KC131245	—
	NYSf1468.1 (isotype of Agaricus highlandensis)	New York	MH016956	—
	Orton_3049 ("brunnescens" E 55717)	United Kingdom (see Orton 1977)	HG007977	—
	PBM4071 (TENN 071535)	Tennessee	MG735311	—
	PBM4085 (TENN 071544)	Tennessee	MG735310	MH360729
	PRM 887239	Czech Republic	HG007974	—
	PRM 888152	Czech Republic	HG007976	—
	PRM 895180	Czech Republic	HG007975	—
	RAS133 (TENN 071504)	Tennessee	MG663261	MG923693
	SGLMf39 (as "mixta" Taxus globosa bark)	Mexico	EU715686	—
	Stz5629 ("Flammula carbonaria" WTU 48127)	Michigan	MG735307	—
	TENN 022978	Alabama	MG740773MG735276	—
	TFB6477a (TENN 053396)	Virginia	MG735321	—
	TFB14663 (TENN 069394)	Tennessee	MG735280	—
	TFB14667 (TENN 069398)	Tennessee	MG735283	—

(Continued)

Table 1. (Continued).

Species	Specimen voucher or strain (herbarium)	Geographic origin	ITS	RPB2
	TFB14668 (TENN 069399)	Tennessee	MG735282	—
	TFB14710 (TENN 069436)	Tennessee	MG735281	—
	UC 1999437 ("carbonaria")	California	KF878376	—
	Unknown liverwort (Cheiloejeunea) endophyte	Peru	EU685975	—
	VL292 (from xylem of burned Pinus mugo)	Lithuania	JF440578	—
	861 ("carbonaria" from root systems in mixed Fraxinus excelsior stands with rich soils and badly damaged by fungal attacks)	Lithuania	GU934596	—
Pholiota lenta	R. Tuomikoski s.n. (H, IBUG)	Finland	AY281022	—
	7100—Venice Museum of Natural History	Italy	JF908582	—
Pholiota lubrica	PRM 857179	Slovakia	HG007984	—
	PRM 899117	Czech Republic	HG007986	—
	PRM 915546	Czech Republic	HG007987	—
Pholiota marangania	HLepp856	A.C.T., Australia	MG735320	—
Pholiota mixta	PBM2499 (TENN 062357)	Massachusetts	MH016953	MG923703
	PRM 897292	Czech Republic	HG007978	—
	PRM 909924	Czech Republic	HG007979	—
	TENN 020591 (holotype Pholiota virescentifolia)	Tennessee	HQ222029	—
	UC 1999438 ("virescentifolia")	North Carolina	KF878379	—
Pholiota molesta	AHS65008 (holotype Pholiota molesta MICH 5323)	Idaho	MG740774MG735277	—
	AHS65008 (isotype TENN 028830)	Idaho	MG735296	MG923694
	AHS74095 (holotype Pholiota subsaponacea MICH 5332)	Idaho	MG735287	—
	JFA9246 ("highlandensis" WTU 10719)	Washington	MG735297	MG923695
	MTS4953a ("highlandensis" WTU 10954)	Washington	MG735309	MG923696
	PBM480 ("carbonaria" WTU 10328)	Washington	MG735306	MG923697
	SAR85/286 ("carbonaria" WTU 53356)	California	MG740775MG735278	—
	SAT0712409 ("highlandensis" WTU 1767)	Washington	MG735279	—
	UC 1998624 ("highlandensis")	California	KC122891	—
	UC 1999269 ("highlandensis")	California	KC122892	—
	IHBF2282 ("flavida")	India	MF326631	—
Pholiota multicingulata	PBM3124 (PDD 97861)	New Zealand	HQ832449	HQ832440
Pholiota nubigena	PBM504 ("Nivatogastrium nubigenum" WTU)	California	DQ494679	—
Pholiota olivaceophylla	MICH 290502 (holotype)	California	KF878381	—
Pholiota polychroa	PBM2866 (TENN 062649)	Louisiana	MG735317	—
Pholiota sp.	SCK05-5-7-zp19 from Ophiocordyceps sinensis	China	HM439572	—
Pholiota sp.	TFB8644 (TENN 055366)	Argentina	KY559343	MF978347
Pholiota sp.	TFB12842 (TENN 060957)		MG735318 MG735319	
Pholiota spumosa	PBM2849 (TENN 062637)	Massachusetts	MG735316	MG923698
	PRM 901623	Czech Republic	HG007980	—
	PRM 885615	Czech Republic	HG007982	—
	None cited ("alabamensis")	China	JF961356	—
	TFB6574 (TENN 054555)	Russia	MG735322	—
	UC 1998527	California	KC122879	—
	UC 1999270	California	KC122884	—
	TFB6868 (TENN 054603)	New York	MG735323	—
	TFB6889 (TENN 054616)	South Carolina	MG735324	—
	TENN 004259	Tennessee	MG735300	—
	TFB6500 (TENN 054542)	New York	MG735325	—
	TFB6901 (TENN 054622)	North Carolina	MG735326	—
	TFB6902 (TENN 054623)	North Carolina	MG735327	—
	618 ("flavida")	Italy	JF908576	—
	3533	Italy	JF908577	—
	TFB6033 (TENN 054523)	Washington	MG735328	—
	TFB6899 (TENN 054620)	Georgia	MG735329	—
Pholiota "spumosa"	K41	Latvia	KR072505	—
Pholiota squarrosa	PBM2735 (TENN 062547)	Colorado	DQ494683	—
Pholiota velaglutinosa	AHS9285 (isotype TENN 028851)	Oregon	MH016954	—
	UC 1859567	California	KC122877	—
	AHS56192 (isotype of Pholiota rubronigra TENN 028840)	California	MH016955	—

Note. New sequences and newly molecularly annotated holotypes and isotypes are in bold. Herbarium abbreviations follow Thiers [continuously updated].

Our results indicated a poor understanding of the taxonomy and ecology of pyrophilous Pholiota, confused morphological species concepts encompassing multiple morphological species, and overly inflated taxonomic estimates of burn-associated Pholiota in North America (FIG. 1; see also Siegel et al. (2015) for Pholiota in general). Of the seven species placed in section Carbonicola by Smith and Hesler, we now recognize three as separate and autonomous—P. highlandensis, P. molesta, and P. brunnescens—but discovered a novel pyrophilous association for the species P. castanea, described originally from a single collection in Tennessee on soil and rotten wood. We found that section Carbonicola was paraphyletic.
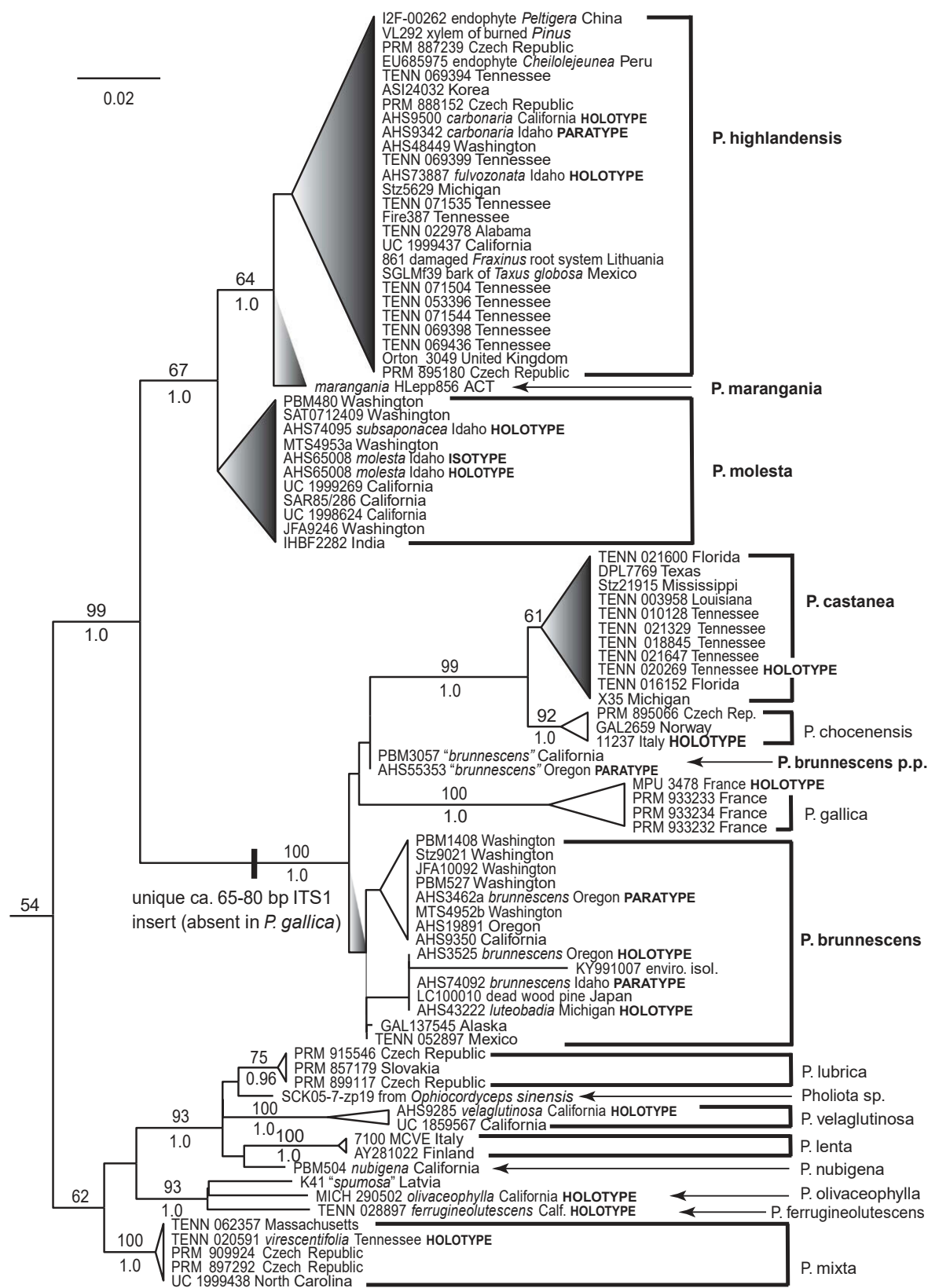
Figure 1. ML phylogeny of ITS data from pyrophilous species of Pholiota in section Carbonicola and their closest relatives in section Spumosae. Values above branches indicate ML bootstrap support, whereas those below are Bayesian posterior probabilities. Pyrophilous species-level clades are indicated by gray gradient fills. Taxon labels of pyrophilous species are in bold. The scale bar reflects the number of expected nucleotide substitutions per site. Pholiota squarrosa, used to root the tree, and samples of P. spumosa, the sister lineage to the clade shown, have been pruned.

In phylogenetic analyses of the ITS data (FIG. 1), sequences from the type collections of P. carbonaria and P. fulvozonata clustered with numerous sequences of P. highlandensis. This clade was weakly supported, however, and included sequences mislabeled as P. mixta (Fr.) Kuyper & Tjall.-Beuk. and P. brunnescens. Several environmental sequences from endophytes and endolichenic fungi also clustered with P. highlandensis isolated from the bark of a yew in Mexico (EU715686), from the xylem of a burned pine in Lithuania (JF440578), from the thallus of the lichen Peltigera Willd. in China (KC131245), and from an unknown endophyte of the liverwort Cheilolejeunea (Spruce) Schiffn. in Peru (EU685975). In addition, one sequence of P. highlandensis was recovered from a putative ecto-mycorrhizal root tip on a Pinus pungens seedling (Fire184) in Jun 2017. The sister lineage to P. high-landensis was the Australian pyrophilous species P. marangania (Grgur.) Matheny & Bougher, represented here by a single ITS sequence from material collected in the Australian Capital Territory (A.C.T.).

A third species-level group was recovered from an assemblage of sequences variously labeled as P. carbo-naria, P. highlandensis, P. flavida (Schaeff.) Singer, and the type sequences of P. molesta and P. subsaponacea. This grouping is labeled P. molesta. The inclusive clade of P. molesta, P. marangania, and P. highlandensis clustered with measures of moderate support (67% bootstrap, 1.0 PP; FIG. 1).

The sister group to the clade containing P. molesta, P. marangania, and P. highlandensis included four spe-cies, i.e., the pyrophilous P. brunnescens and P. castanea and two nonpyrophilous associates, P. chocenensis Holec & M. Kolařík and P. gallica Holec & M. Kolařík. Pholiota brunnescens, however, was recovered as a nonmonophyletic group because two ITS sequences marked by polymorphisms (from PBM3057 and AHS55353) did not cluster with the clade that includes the holotype. This result was contradicted by the RPB2 gene tree (not shown) where the RPB2 sequence of PBM3057 clustered with all other P. brun-nescens sequences. Nonetheless, the remaining ITS sequences of P. brunnescens did form a monophyletic group and included the holotype of P. luteobadia from Michigan and two environmental sequences from Japan and Russia. Pholiota castanea was recovered as mono-phyletic with moderate support (61% ML bootstrap only; FIG. 1) but included several sequences from col-lections cited as P. highlandensis in Smith and Hesler (1968). Pholiota castanea was most closely related to the nonpyrophilous species P. chocenensis with strong measures of support. Within this mixed ecological grouping, three species are characterized by a unique

ca. 65−80-bp insert in the ITS1 region, first reported by Holec et al. (2014). This insert was lacking in P. gallica, and its presence was polymorphic within P. brunnes-cens. The origin of the insert appears to be a duplica-tion of an upstream fragment.

Phylogenetic analyses of combined ITS and RPB2 (FIG. 2) revealed strong to moderate support for the monophyly of P. highlandensis, P. molesta, P. brunnes-cens (without PBM3057), and P. castanea. Pholiota marangania was strongly supported as the sister group to P. highlandensis. The analyses also strongly supported two subgroups, one including P. highlanden-sis, P. marangania, and P. molesta, and a second strongly supported subgroup of P. brunnescens, P. cas-tanea, P. chocenensis, and P. gallica. Overall, the five pyrophilous Pholiota species formed a strongly sup-ported monophyletic group together with the nonburn species P. gallica and P. chocenensis. Phylogenetic trees from both ITS and the ITS+RPB2 data sets supported the derived status of the pyrophilous trait in Pholiota, as nonpyrophilous Pholiota formed paraphyletic enti-ties in which pyrophilous lineages evolved.

Basidiospore germination of P. highlandensis was very low. Only five single-basidiospore isolates from P. highlandensis were recovered. However, cultures derived from these isolates lacked clamp connections, typical of homokaryons, and produced chains of con-idia. All five cultures were confirmed as P. highlanden-sis by ITS sequencing.

TAXONOMY

Pholiota brunnescens A.H. Sm. & Hesler, North Am Species Pholiota:286. 1968. FIGS. 3A, 4A
= Pholiota luteobadia A.H. Sm. & Hesler, North Am Species Pholiota:284. 1968.

Pileus 15−70 mm wide, broadly convex to plano-convex with an incurved to decurved margin, becoming plane with age or retaining a low umbo; surface viscid to slightly so or glutinous, margin even, at first sparsely decorated with small, whitish veil remnants forming evanescent fibrillose squamules, with age smooth or finely innately fibrillose in appearance, occasionally tearing at the margin; center orange brown (5YR 5/8−4/8) or apricot brown, margin brown to reddish brown (5YR 4/6; Prout's Brown, Tawny-Olive, Snuff Brown, Chestnut Brown), hygrophanous with the margin fad-ing to light reddish brown (2.5YR 5/8; Apricot Orange), when dry surface dark red to dark vinaceous red with 5% KOH; context pallid, pale yellow, or dingy watery brown, rather thick and up to 5 mm thick under the disc; odor and taste mild, not remarkable, or slightly disagreeable. Lamellae adnate but seceding or adnexed,
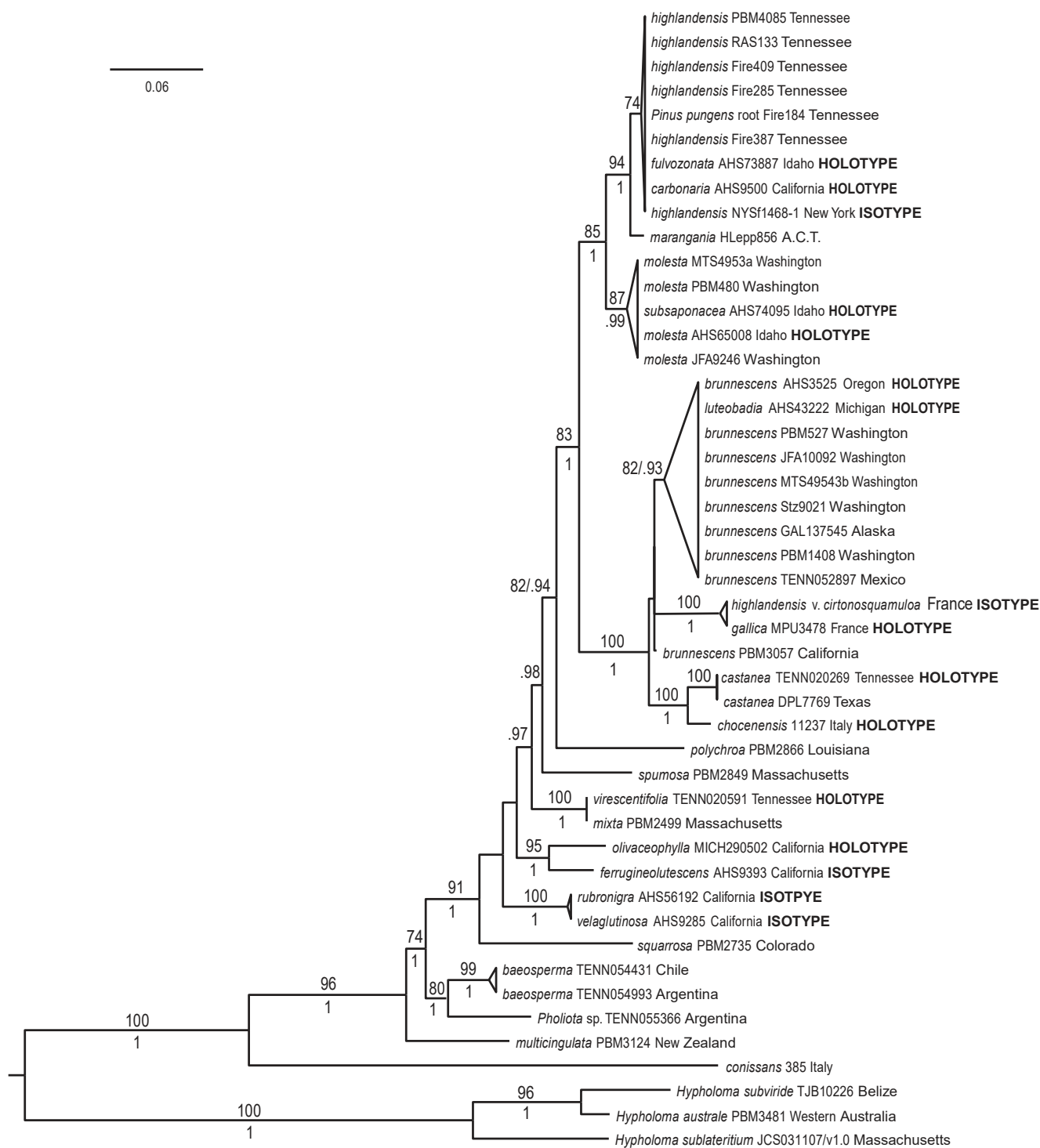
0.06

highlandensis PBM4085 Tennessee
highlandensis RAS133 Tennessee
highlandensis Fire409 Tennessee
highlandensis Fire285 Tennessee
Pinus pungens root Fire184 Tennessee
highlandensis Fire387 Tennessee
fulvozonata AHS73887 Idaho **HOLOTYPE**
carbonaria AHS9500 California **HOLOTYPE**
highlandensis NYSf1468-1 New York **ISOTYPE**
marangania HLepp856 A.C.T.
molesta MTS4953a Washington
molesta PBM480 Washington
subsaponacea AHS74095 Idaho **HOLOTYPE**
molesta AHS65008 Idaho **HOLOTYPE**
molesta JFA9246 Washington
brunnescens AHS3525 Oregon **HOLOTYPE**
luteobadia AHS43222 Michigan **HOLOTYPE**
brunnescens PBM527 Washington
brunnescens JFA10092 Washington
brunnescens MTS49543b Washington
brunnescens Stz9021 Washington
brunnescens GAL137545 Alaska
brunnescens PBM1408 Washington
brunnescens TENN052897 Mexico
highlandensis v. cirtonosquamuloa France **ISOTYPE**
gallica MPU3478 France **HOLOTYPE**
brunnescens PBM3057 California
castanea TENN020269 Tennessee **HOLOTYPE**
castanea DPL7769 Texas
chocenensis 11237 Italy **HOLOTYPE**
polychroa PBM2866 Louisiana
spumosa PBM2849 Massachusetts
virescentifolia TENN020591 Tennessee **HOLOTYPE**
mixta PBM2499 Massachusetts
olivaceophylla MICH290502 California **HOLOTYPE**
ferrugineolutescens AHS9393 California **ISOTYPE**
rubronigra AHS56192 California **ISOTPYE**
velaglutinosa AHS9285 California **ISOTYPE**
squarrosa PBM2735 Colorado
baeosperma TENN054431 Chile
baeosperma TENN054993 Argentina
Pholiota sp. TENN055366 Argentina
multicingulata PBM3124 New Zealand
conissans 385 Italy
Hypholoma subviride TJB10226 Belize
Hypholoma australe PBM3481 Western Australia
Hypholoma sublateritium JCS031107/v1.0 Massachusetts

Figure 2. ML phylogeny of a supermatrix of ITS and RPB2 sequences of Pholiota and the outgroup Hypholoma. ML bootstrap values >70% and Bayesian PPs >0.90 are shown. Types are indicated in bold font. The scale bar reflects the number of expected nucleotide substitutions per site.

close to crowded with several tiers of lamellulae, up to 8 mm deep; at first whitish, becoming pale grayish brown (10YR 6/3) or dull cinnamon to dull gray brown (10YR 5/4) or yellowish brown; edges pallid when young but concolorous with the faces in age, even in appearance. Stipe 15–60(–90) × (4–)5–10 mm,

equal, (extreme) apex pruinose and pallid, below pale yellow or citrine-yellow due to fibrillose-scaly veil remnants, veil sometimes observed as a fibrillose-submembranous zone near the apex, surface staining tawny in age or where handled; context solid, becoming hollow, pallid to pale dingy yellow when water soaked.

Figure 3. Basidiomes of four pyrophilous species of Pholiota from North America. A. Pholiota brunnescens (SAT-Nov-1992; photo by Steve A. Trudell). B. Pholiota castanea (DPL7760; photo by David P. Lewis). C. Pholiota highlandensis (PBM4085; photo by P. Brandon Matheny). D. Pholiota molesta (MGW s.n.; photo by Michael G. Wood). Bars = 1 cm.

Basidiospores 6.0–6.8–7.5 × 4.0–4.3–5.0 µm, Q 1.33–1.58–1.80(–1.88) (n = 60/3), smooth, ellipsoidal to ovate, apices subconical or obtuse (not truncate) and with a very small indistinct germ pore, apiculus small and indistinct; thick-walled to slightly thick-walled, walls ca. 0.50–0.75 µm thick; yellowish brown to brown in KOH, umbrinous (10YR 4/4) in deposit. Basidia 18–21 × 6–7 µm, 4-sterigmate, urniform, hyaline. Pleurocystidia 60–80 × 11–15 µm, frequent, projecting well beyond the hymenium, fusiform-ventricose with long cylindrical necks or tapered upward, thin-walled, apices obtuse to swollen, hyaline to pale yellowish. Cheilocystidia similar to but shorter than pleurocystidia, many fusiform. Caulocystidia 33–112 × 10–40 µm, often clustered or in tufts, restricted to the extreme apex of the stipe, shape variable, clavate, fusiform, saccate, clavate-mucronate, or cylindrical, thin-walled, hyaline, the apices occasionally with short rostrate extensions. Pileipellis composed of two distinct layers: (i) suprapellis an ixocutis of narrow, smooth, interwoven, cylindrical, hyaline hyphae, these mostly 2–6 µm wide; and (ii)

subpellis tawny to dark rusty brown in mass, composed of parallel cylindrical hyphae, these mostly 5–17 µm wide, thin-walled but with coarse or distinctive bands of incrustations. Lamella trama parallel, hyphae cylindrical or narrowly so, pale yellow to hyaline in mass, subhymenium filamentous and gelatinous. All hyphae inamyloid. Clamp connections present.

Ecology and distribution: Caespitose or gregarious on burned soil, occasionally on burned wood, in the Pacific Northwest ranging from Alaska to northern California (type from Oregon), south to Mexico, eastward to Michigan, also east Asia (Japan, Russia). May to Dec.

Specimens examined: MEXICO. TLAXCALA: Montaña la Malinche, Volcan National Park, on charcoal, 12 Jul 1993, K.W. Hughes TFB6242 (TENN 052897). USA. ALASKA: Fairbanks, North Star Borough, near (on?) burned stump, 31 Jul 1992, R. Treu RT1610 (GAL137545; WTU 021130). CALIFORNIA: Del Norte County, Crescent City, 3 Dec 1937, A.H. Smith (TENN 028871 as Pholiota

molesta); Mendocino County, Mendocino Woodlands Camp, 16 Nov 2008, P.B. Matheny PBM3057 (TENN 063855). IDAHO: Bonner County, Upper Priest River, on burned area, 11 Oct 1966, A.H. Smith AHS74092 (paratype MICH 30234). MICHIGAN: Emmet County, Wilderness Park, gregarious in a burned area, 24 Sep 1953, A.H. Smith AHS43222 (holotype of Pholiota luteobadia MICH 11688). OREGON: Lake Tahkenitch, on burned area, 18 Nov 1935, A.H. Smith AHS3525 (holotype MICH 11657); Jackson County, on burned area, 6 Nov 1956, A.H Smith AHS55353 (paratype MICH 30236); Lane County, Siltcoos Lake, on burned area, 13 Nov 1935, A.H. Smith AHS3462a (paratype MICH 30237). WASHINGTON: Chelan County, Leavenworth area, Eight Mile Road, Tumwater Campground, on burned ground in conifer forest, 17 May 1997, P.B. Matheny PBM527 (WTU 010342); Kittitas County, Gulf Course Road, exit 78 Interstate 90, densely caespitose on charred remains in logged conifer forest, 1 May 1999, P.B. Matheny PBM1408 (WTU 010337); Pend Oreille County, above Tiger Meadow, ca. 1000 m, 18 May 2002, M.T. Seidl MTS4953b (WTU 010343); San Juan County, Friday Harbor Biological Station, 13 Nov 1954, D.E. Stuntz Stz9021 (WTU 010354 as Flammula carbonaria); Gifford Pinchot National Forest, Iron Creek Campground, on burned wood, 3 Nov 1989, J.F. Ammirati JFA10092 (WTU 010349).

Commentary: The gross morphological description is a composite from Smith and Hesler (1968) and data from recent collections. Pholiota brunnescens is best characterized by the relatively large basidiomes compared with other pyrophilous Pholiota, the often caespitose to gregarious habit (FIG. 3A), the presence of large caulocystidia at the extreme apex of the stipe (FIG. 4A), and the tawny staining stipe surface in age
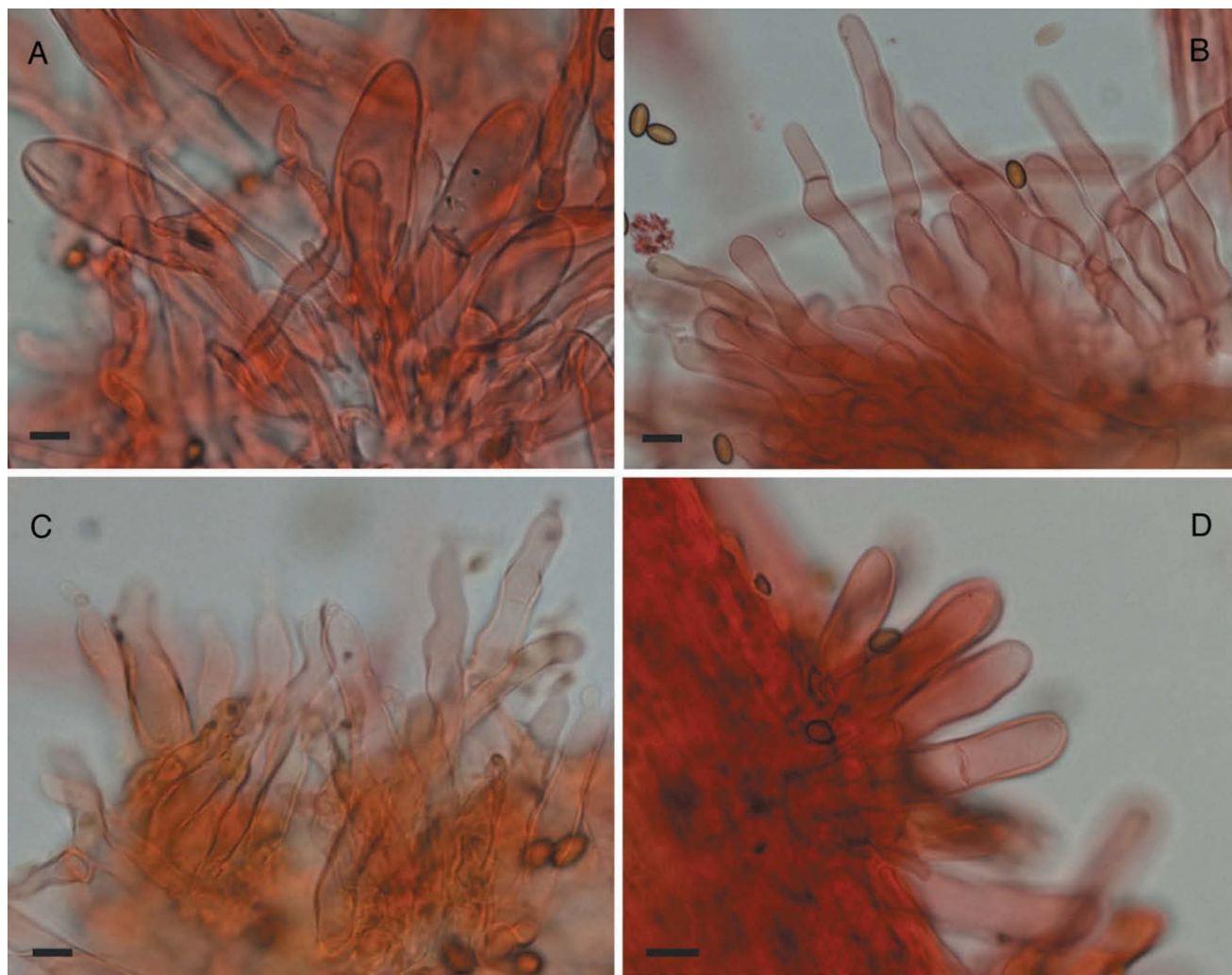

Figure 4. Caulocystidium variation among pyrophilous Pholiota from North America. Cells stained in mixture of 5% KOH and Congo Red. A. Pholiota brunnescens (PBM527). B. Pholiota castanea (Stz21915). C. Pholiota highlandensis (RAS133). D. Pholiota molesta (JFA9246). Bars = 10 μm.

or where handled, hence the epithet. The species can co-occur on burned sites together with basidiomes of P. molesta in western North America. Pholiota molesta, however, differs from P. brunnescens by its often shorter and squat basidiomes, nonstaining stipe surface with scattered pale brown bands of veil material, and smaller caulocystidia. Pholiota brunnescens appears most common in western North America, but is confirmed here from Japan and eastern Russia based on phylogenetic analysis of environmental sequences, and extends southward into volcanic regions of Mexico. One collection, the holotype of P. luteobadia, the ITS sequence of which is nearly identical (see below) to that of the holotype of P. brunnescens, was recorded from Michigan, the only confirmed record to date of this species from the upper Midwest. Because the names P. brunnescens and P. luteobadia have equal priority (both have the same effective publication date), we favor application of the name P. brunnescens because it is much more widely used.

European material identified as P. brunnescens (Orton 3049, Orton 1977; E55717, Holec 2001) is P. highlandensis (FIG. 1). Misidentification of this material led Holec et al. (2014) to suggest a possible synonymy of P. brunnescens with P. highlandensis. However, P. brunnescens differs from P. highlandensis generally by the larger basidiomes and often caespitose habit, among other characters. Indeed, P. brunnescens is more closely related to a suite of species most of which have a unique ca. 65–80-bp insert in the ITS1 region. These closely related species vary in their ecology, but all appear to produce basidiomes on soil (or on charred remains), and include two nonpyrophilous associates, P. gallica and P. chocenensis, and the pyrophilous P. castanea. The clade containing these four species is sister to the clade containing P. highlandensis, P. marangania, and P. molesta (FIGS. 1, 2). To date, P. brunnescens has yet to be confirmed from Europe despite earlier reports (Orton 1977; Holec 2001).

Murrill (1917) attributed several other heterotypic synonyms to P. brunnescens excluded by Smith and Hesler (1968): Agaricus ascophorus Peck, Naucoria subvelosa Murrill, and Hebeloma peckii House. We are unsure of the status of these synonyms, not having examined their types. Singer (1969) transferred A. ascophorus to Pholiota and reports this species from South America.

ITS sequences from the holotypes of P. brunnescens and P. luteobadia are the same, aside from three sites that are polymorphic. We suspect that P. luteobadia, based on a single collection, was described from immature material.

Pholiota castanea A.H. Sm. & L.R. Hesler, North Am Species Pholiota:235. 1968.   FIGS. 3B, 4B
= Pholiota highlandensis sensu Smith & Hesler (1968) pro parte.

Pileus 10–50 mm wide, convex to expanded-convex, with age at times depressed-concave; surface smooth, viscid, pellicle separable nearly to the disc, margin even; chestnut brown to reddish brown or yellowish red (5YR 4/6–4/8–5/8; Chestnut, Pecan Brown, to Mikado Brown) or dark brown (10YR 4/3; Bister), not hygrophanous, when dry surface dark red to dark vinaceous red with 5% KOH; context thin, tough or firm, whitish or watery white; odor and taste mild or none. Lamellae sinuate, adnate, to subdecurrent, close to crowded, medium to rather broad; pallid to yellowish when young, becoming pale tawny with brown spots, to Pinkish Buff, and finally umbrinous (Snuff Brown to Tawny-Olive); edges serrulate, slightly eroded, or even in appearance. Stipe 20–60 mm × 2–6 mm, equal or tapered downward, flexuous, pallid or dingy; surface dry, scattered fibrillose, fibrillose-reticulated, becoming somewhat fibrillose-scaly; veil webby, yellowish, evanescent, at times not observed; context solid or stuffed, becoming hollow, tough.

Basidiospores 6.0–6.9–7.5(–8.0) × 3.5–4.0–4.5(–5.0) μm, Q = (1.33–)1.51–1.75–1.89(–2.00) (n = 60/3), smooth, ellipsoidal to subovate, with subconical to obtuse apices, germ pore present and indistinct; yellowish brown to dull cinnamon in KOH, paler cinnamon in Melzer's, umbrinous or Snuff Brown in mass. Basidia 17–21 × 5–6 μm, 4-sterigmate, urniform, hyaline. Pleurocystidia 47–70 × 8–17 μm, fusiform-ventricose or bottle-shaped (sublageniform) to skittle-shaped, with long cylindrical necks, thin-walled, apices obtuse to swollen, hyaline. Cheilocystidia similar to pleurocystidia but shorter. Caulocystidia 28–65 × 7–10 μm, cylindrical to subcylindrical, occasionally utriform or slenderly clavate, in tufts or clusters at the extreme stipe apex, thin-walled, hyaline. Pileipellis an ixocutius resting on a bright golden brown hypodermium; the suprapellis composed of gelatinous, narrowly cylindrical hyphae, these mostly 2–4 μm wide, thin-walled, hyaline or ochraceous, smooth or incrusted; hypodermium composed of cylindrical and many inflated hyphae, these up to 30 μm wide, incrusted, with thickened walls; mounts of pileipellis sections noticeably turn reddish in 5% KOH. Lamella trama parallel, pale yellowish in mass, hyphae cylindrical and mostly 5–12 μm wide, smooth, thin-walled, flanked by a gelatinous subhymenium. All hyphae inamyloid. Clamp connections present.

Ecology and distribution: Gregarious to caespitose on burned debris, burned soil, and charcoal, more

common near the Gulf of Mexico and in southeastern states (type from Tennessee), northward to Michigan, Nov to Apr.

Specimens examined: USA. FLORIDA: Alachua County, Gainesville, on charcoal in thin woods, 23 Jan 1944, W.A. Murrill (TENN 016152); Wakulla County, Apalachicola State Park, Smith Creek, on burned soil and charcoal, 24 Dec 1954, L.R. Hesler (TENN 021600). LOUISIANA: Baton Rouge, on ground (burned debris not evident), 7 Dec 1957, B. Lowy (TENN 003958). MISSISSIPPI: Hancock County, Waveland, Buccaneer State Park, 8 Jan 1982, D.E. Stuntz Stz21915 (WTU 053296). TENNESSEE: Johnson County, Shady valley, "on decaying log" but specimens have soil attached to the base (burned debris not evident), 15 Apr 1934, A.J. Sharp & Underwood (TENN 004260) (identification putative); Anderson County, Claxton School, on charred wood and burned soil, 10 Jan 1937 (TENN 010128); Morgan County, near Wartburg, on soil among charcoal, chips, and sawdust, 14 Nov 1948, L. R. Hesler (TENN 018845); Knox County, near Knoxville, on soil and rotten wood, 11 Dec 1951, A.J. Sharp (holotype TENN 020269); Great Smoky Mountains National Park, Cades Cove, on burned over soil, 18 Apr 1954, L.R. Hesler (TENN 021329); similar locality as previous, on burned wood and burned soil, 5 Mar 1955, T.H. Campbell (TENN 021647). TEXAS: Newton County, Bleakwood, Lewis residence, gregarious to caespitose on burned ground, 27 Nov 2006, D.P. Lewis DPL7769 (TAES, TENN 071878).

Notes: The original description of P. castanea is based on a single collection made "on soil and rotten wood" in Knoxville, Tennessee. To this we have incorporated notes made by Hesler from TENN 018845 and TENN 021329, two of several collections attributed to P. highlandensis in Smith and Hesler (1968) but conspecific with P. castanea based on ITS phylogenetic analyses (FIG. 1). According to our evaluation, it is evident that P. castanea represents an additional pyrophilous species of Pholiota, one not previously recognized as such by the original authors. As the species was not observed during the first year following the Nov 2016 Chimney Tops fire in the Great Smoky Mountains National Park, we suspect that P. castanea is a later successional pyrophilous species. Pholiota castanea differs from P. highlandensis most readily by the darker reddish–colored pileus and the presence of a large insert (ca. 65–80 bp) in the ITS1 region. These features, in combination with the presence of noticeably large or inflated subpellis hyphae, distinguish P. castanea from other pyrophilous Pholiota.

Pholiota castanea is most closely related to the recently described P. chocenensis (Holec et al. 2014) from central and southern Europe. Both species form separate monophyletic groups, but bootstrap support for the P. castanea lineage is not strong based on ITS sequences only (FIG. 1). Sequences from the holotype in Tennessee and a sample from Texas do cluster with strong support in the supermatrix data set (FIG. 2). Pholiota chocenensis is a nonpyrophilous associate that produces basidiomes on soil. Thus, it appears that ecological differences would serve to separate the two species. However, the presence of burned debris was not noted (perhaps missed) or could not be confirmed for all collections with ITS sequences that cluster as P. castanea (viz., TENN 003958, TENN 004260, TENN 020269). The Michigan sample of P. castanea (FIG. 1) is based on an environmental sequence (KC176331) obtained from mineral soil in a conifer forest (Thorn et al. 1996). No reference to burned substrates was mentioned in this study.

Pholiota highlandensis (Peck) Quadr. & Lunghini, Quad Accad Naz Lincei 264:111. 1990. FIGS. 3C, 4C

≡ Agaricus (Naucoria) highlandensis Peck, Ann Rep N Y St Mus Nat Hist 24:67. 1872.

≡ Flammula highlandensis (Peck) Peck, Ann Rep N Y St Mus Nat Hist 50:138. 1897.

≡ Gymnopilus highlandensis (Peck) Murrill, North Am Fl 10(3):197. 1917.

≡ Pholiota highlandensis (Peck) Singer, Lilloa 25:343. 1952 (inval. Art. 41.1).

≡ Pholiota highlandensis (Peck) A.H. Smith & Hesler, North Am Species Pholiota:287. 1968 (inval. Art. 41.1).

= Agaricus carbonaria Fr., Obs Myc 2:33. 1818.

≡ Flammula carbonaria (Fr.: Fr.) P. Kumm., Führ Pilzk:82. 1871.

≡ Flammula carbonaria (Fr.: Fr.) Quél., Mém Soc d'Émul Montbéliard 5:232. 1872 (nom. illegit. Art. 53.1).

≡ Inocybe carbonaria (Fr.: Fr.) Roze, Bull Soc Bot Fr 23:113. 1876.

≡ Dryophila carbonaria (Fr.: Fr.) Quél., Enchir Fung:70. 1886.

≡ Gymnopilus carbonarius (Fr.: Fr.) Murrill, Mycologia 4:256. 1912.

≡ Flammula spumosa var. carbonaria (Fr.: Fr.) Konr. & Maubl., Icon Select Fung 6(4):107.

≡ Pholiota carbonaria (Fr.: Fr.) Singer, Lilloa 22:517. 1951 (nom. inval. Art. 33.2).

= Pholiota carbonaria A.H. Sm., Mycologia 36:253. 1944.

≡ *Pholiota carbonicola* A.H. Sm., Mycologia 36:254. 1944 (nom. illegit. Art. 61.3).

= *Pholiota fulvozonata* A.H. Sm., North Am Species Pholiota:280. 1968.

Pileus 3–30 mm wide, obtusely conical to convex, becoming plano-convex and eventually plane with age, umbo absent, margin incurved in youth becoming decurved; surface viscid, smooth in appearance and lacking any veil material; light brown, brown, to strong brown (7.5YR 4/4–5/6–5/8; Sayal Brown, Buckthorn Brown, Ochraceous-Tawny, to Cinnamon-Brown), margin entire, edge with a fibrillose partial veil; brownish at first but then becoming dark vinaceous red with 5% KOH, when dry surface red to dark vinaceous red with 5% KOH; context pallid; odor not remarkable or somewhat musty, taste same. *Lamellae* adnexed to adnate, moderately close (25–30 L) with several tiers of lamellulae, pale brown (10YR 6/3) in youth, becoming light yellowish brown to yellowish brown (10YR 5/3–5/4) or brown (10YR 4/3) at maturity; edges pallid but not appearing fimbriate, medium to broad, easily splitting in two where carefully torn. *Stipe* 10–40(–65) × 1–4(–5) mm, even, cylindrical, occasionally flexuous but often straight, occasionally tapered downward; partial veil present only when young, evanescent, lacking any pigmentation; pale yellowish overall or pallid to creamy above and pale brown (10YR 7/3) below, with age brownish yellow (10YR 6/6) nearly throughout; surface dry, pruinose only at the extreme apex, with scattered fibrils below or with an overall silky-fibrillose appearance, typically not scaly or with light brown (5E8) small fibrillose scales; context solid or stuffed and becoming hollow with a white interior, cortex dull yellowish or colored like the surface.

Basidiospores 6.0–7.2–8.0(–8.5) × 4.0–4.3–4.5(–5.0) μm, Q = 1.44–1.66–1.92(–2.00) (n = 42/3), ellipsoidal to ovate in profile, apices obtuse to subconical, germ pore and apiculus small and indistinct, thick-walled (walls ca. 0.5–0.75 μm thick), yellowish brown. *Basidia* 18–22 × 6–7 μm, 4-sterigmate, cylindrical, clavate, or urniform, hyaline. *Pleurocystidia* 49–66 × 10–14 μm, easily projecting from hymenium; fusiform, fusiform-ventricose, sublageniform, to skittle-shaped, with elongated cylindrical necks, apices obtuse or swollen, thin-walled, hyaline. *Cheilocystidia* similar to pleurocystidia but shorter, fusiform, ventricose, or utriform. *Caulocystidia* 30–61 × 5–11 μm, in tufts or clusters at the extreme apex, cylindrical, subcylindrical, fusiform, occasionally flexuous or sublageniform, apices obtuse but occasionally with short rostrate extensions. *Pileipellis* an ixocutis above a bright rusty brown–pigmented subpellis; ixocutis hyphae narrowly cylindrical, these mostly 2–4 μm wide, many with fine incrustations, hyaline or ochraceous; subpellis hyphae cylindrical, mostly 5–15 μm wide, thin-walled, incrusted.

Lamella trama hyphae parallel, cylindrical, many 5–7 μm wide, hyaline; subhymenium filamentous and gelatinous. All hyphae inamyloid. Clamp connections present.

Ecology and distribution: Scattered singly to gregarious, less often in clusters of few to several basidiomes, generally not caespitose, on burned ground, in charcoal pits, or on tephra deposits, occasionally in troops on extensive burns, eastern North America (type from New York), California, Mexico, South America (Peru), Europe, and Asia (China), also reported from Argentina, Brazil, and Chile (Coimbra 2015), occurring Apr to Dec or year-round, 1–2 y post-fire.

Specimens examined: USA. ALABAMA: DeKalb County, near DeSoto Park, on burned-over soil, 4 Apr 1959, A.J. Sharp (TENN 022978). CALIFORNIA: Del Norte County, Crescent City, in burn area, 7 Dec 1935, A.H. Smith (holotype of *Pholiota carbonaria* MICH 11663); Del Norte County, Crescent City, on burned area, 3 Dec 1937, AHS9342 (paratype of *Pholiota carbonaria* MICH 30243). MICHIGAN: Cheboygan County, Colonial Point, on charred ground, 13 Sep 1949, D.E. Stuntz (WTU 048127). IDAHO: Boundary County, upper Priest Lake region, gregarious on burned (partially) chip dirt, 3 Oct 1966, A.H. Smith AHS73887 (holotype of *Pholiota fulvozonata* MICH 5316). NEW YORK: Top of one of the "Highlands," near Highland Falls, 26 Jun 1872, C.H. Peck (holotype of *Agaricus highlandensis* NYSf 1467, isotype NYSf 1468). TENNESSEE: Great Smoky Mountains National Park (GSMNP), Cherokee Orchard area, adjacent to Science Center Road, Twin Creeks, in burn area inside edge of a hole, 4 Apr 2017, K.W. Hughes TFB14663 (TENN 069394); GSMNP, Cherokee Orchard area, adjacent to NEON site, on burned soil, 12 Apr 2017, K.W. Hughes TFB14667 (TENN 069398); same locality and date as previous, K.W. Hughes TFB14668 (TENN 069399); GSMNP, Cherokee Orchard area, Old Sugarlands Trail, on burned ground under dead Rhododendron, 29 Apr 2017, P.B. Matheny PBM4085 (TENN 071544); same locality and date as previous, on burned ground under dead Rhododendron, B.P. Looney BPL916 (TENN 071508); same locality and date as previous, on burned ground, R.A. Swenie RAS133 (TENN 071504); GSMNP, Baskins Creek Trail, on burned ground, 30 May 2017, K. Johnson TFB14710 (TENN 069436); GSMNP, Baskins Creek watershed overlook, Roaring Fork Nature Trail, burned Pinus pungens stand, P. pungens root sample (seedling no. 7), 20 Jun 2017, K.W. Hughes Fire184 (environmental sample); GSMNP, Twin Creeks Trail, in high burn area on burned soil, 16 Aug 2017, A. Case & W. Thayer Fire285 (TENN 072178); GSMMP, Cove Hardwood Nature Trail, in low burn area, 17 Oct

2017, A.N. Miller Fire409 (TENN 072234); GSMNP, Baskins Creek Trail, on burned ground, 16 Dec 2017, K. Hughes & A. Case Fire448 (TENN 072249); same locality as previous, 16 Dec 2017, K. Hughes & A. Case Fire449 (TENN 072250); GSMNP, Cherokee Orchard, Two Mile Lead Trail, on burned ground (16 mo old) in heavily burned Pinus-dominated forest, 14 Mar 2018, J. Kalichman MO311845 (TENN 073642); GSMNP, Baskins Creek Trail, in Pinus pungens–dominated burn, 7 May 2018, S.R. Warwick PBM4148 (TENN 074419). VIRGINIA: Grayson County, Whitetop Mountatin, 1676 m, in charcoal pit in Picea-Fagus forest, 23 Sep 1993, S.C. McCleneghan TFB6477a (TENN 053396).

Notes: The gross morphological description presented here is based on observations of fresh basidiomes collected in Tennessee and Virginia. Considerable morphological variation is expressed by P. highlandensis with respect to basidiome size and presence/absence of fibrillose scales and their pigmentation on the pileus and stipe. Collections of P. highlandensis made at burned sites in the GSMNP throughout 2017 and 2018 consistently lacked scales on the pileus and stipe, and the basidiomes for most collections were small (pileus <20 mm wide, stipe <3 mm thick). However, small, light brown fibrillose scales were observed on the stipe in TENN 053396 collected in Virginia from a charcoal pit. The pigmentation of the scales, when present, varies from light brown to hazel or ferruginous (as in the holotype of P. carbonaria; see illustration in Siegel and Schwarz 2016) or dark russet brown (as in the holotype of P. fulvozonata). In terms of variation in basidiome size, P. carbonaria produces a pileus 20–40 mm wide and a stipe 4–6 mm wide. Despite these morphological differences, collections of P. carbonaria are nearly identical at the ITS locus and cluster into a species-level lineage with P. highlandensis (FIG. 1). In Europe, Holec (2001) described the pileus of P. highlandensis with fugacious whitish to yellowish floccose veil remnants at the margin, and the stipe covered more or less with distinct whitish, cream, pale yellow, lemon yellow to bright yellow floccose to fibrillose-scaly veil remnants. In age the scales may be dusted with rusty brown–colored basidiospores, thus imparting a ferruginous appearance. In a pairwise comparison, ITS sequences of the types of P. carbonaria and P. highlandensis are the same except for two polymorphic positions.

Kauffman (1918) included two different taxa under the name Flammula carbonaria (Fr.) P. Kumm. (Singer 1969). His description is probably a composite or based on the cited larger taxon that was not always found on charred remains. Nonetheless, Kauffman did emphasize small basidiomes (pileus 20–30 mm diam) of one of the two forms occurring on burned ground. We interpret this to be P. highlandensis. Singer (1969) interpreted Kauffman's Flammula carbonaria pro parte as Pholiota ascophora (Peck) Singer, considered by Murrill (1912) as synonymous with P. highlandensis (as Gymnopilus highlandensis (Peck) Murril). Singer's report of P. ascophora includes the presence of chrysocystidia in specimens collected on burned debris from northern regions of Chile and Argentina. It seems likely Singer's taxon is not P. highlandensis owing primarily to the presence of chrysocystidia; however, we have not examined the type of Pholiota ascophora (Agaricus ascophorus Peck).

The following Smith collections cited in Smith and Hesler (1968) from New York could not be located at MICH or TENN: AHS3617, AHS7929, and AHS19894. In addition, this work erroneously listed two collections (AHS19891, AHS55353) from New York. Both are from Oregon, and AHS55353 is cited as a paratype of P. brunnescens (Smith and Hesler 1968). Fortunately, the type collection of Pholiota highlandensis (Agaricus highlandensis) is in good condition, and the ITS sequence produced from one of two portions of the isotype (NYSf1468.1) makes it easily interpretable, thus precluding the designation of an epitype.

Pholiota highlandensis exhibits endophytic and endolichenic stages in various plants and lichens. Such a broad association with diverse land plants and lichens could explain the broad geographic distribution of P. highlandensis and its primary successional status in patchily burned areas.

Holec (2001) considered P. luteobadia a heterotypic synonym of P. highlandensis; however, P. luteobadia, as demonstrated here based on the ITS sequence of the holotype, is a heterotypic synonym of P. brunnescens. Holec (2001) also suggested a possible synonymy between P. highlandensis and Flammula carbonaria var. gigantea J.E. Lange.

The name Pholiota carbonaria A.H. Sm. has a confusing nomenclatural history and should not be confused with Agaricus carbonarius Fr.: Fr. Smith (1944) first published the name Pholiota carbonaria A.H. Sm. but mistakenly referred to his new species in the observations section of that work under two names as P. carbonaria and "P. carbonicola." The binomial P. carbonaria A.H. Sm. is clearly intended by Smith as the name of the new species, and "P. carbonicola" should be viewed as an orthographic error. Singer (1951), however, referred to the combination of "Pholiota carbonaria (Fr.) Singer (non A. H. Smith)," but this is invalid (International Code of Nomenclature for algae, fungi, and plants [ICN], Art. 33.1-2) as a "correction of the original spelling of a name … does not affect its

date" per the ICN. Singer (1952, 1962) tried to attribute "Pholiota carbonicola" to A. H. Smith as a correction for what he interpreted as an erroneously referred P. carbonaria A.H. Sm. However, Singer's "correction" is superfluous. Smith and Hesler (1968) list the name "Pholiota carbonicola" Singer (Singer 1962:"543") as a synonym of P. carbonaria A.H. Sm. However, this reference is erroneous in two ways: (i) Singer (1962) referenced the name P. carbonicola on page 554, not 543; and (ii) Singer attributed P. carbonicola to A.H. Sm., not himself, as a putative orthographic correction.

TENN 004259 from Shady Valley, Tennessee, was cited under P. highlandensis; however, ITS data from this collection support the identity as P. spumosa (Fr.) Singer (FIG. 1). ITS sequences from the holotypes of P. carbonaria and P. fulvozonata are identical except for one polymorphic site.

Pholiota marangania is very similar morphologically to P. highlandensis but occurs in Australia (Grgurinovic 1997, as Inocybe marangania Grgur.; Matheny and Bougher 2010). ITS data support its placement as the sister group to P. highlandensis (FIGS. 1, 2). We are not aware of how to readily distinguish the two species morphologically. Pholiota marangania is hardly differentiated microscopically from P. highlandensis (Matheny and Bougher 2010) and is known by us only from two collections, one the type and the other sequenced here. Grgurinovic (1997) illustrated pleurocystidia from the type with crystalliferous apices; however, this feature was not observed by Matheny and Bougher (2010). On occasion, the necks of pleurocystidia did appear granular incrusted.

Two infraspecific taxa have been described in P. highlandensis, P. highlandensis f. persicina (P.D. Orton) Holec (Holec 2001) and P. highlandensi var. citronosquamulosa Maire ex Bidaud & Borgarino. The latter is synonymous with P. gallica (Holec et al. 2016).

Pholiota molesta A.H. Sm. & Hesler, North Am Species Pholiota:284. 1968. FIGS. 3D, 4D

= Pholiota subsaponacea A.H. Sm. & Hesler, North Am Species Pholiota:282. 1968.

Pileus 10–40 mm wide, broadly convex to convex with an inrolled margin, becoming plane or slightly depressed; surface smooth to radially appressed-fibrillose, or margin bare, when young with scant fibrillose veil material, viscid to glutinous; pale pinkish buff or orange brown (6D8) when young, becoming dull brown (ca. 4A4) or dark brown, when dry surface dark vinaceous red with 5% KOH; context pliant, white to watery brownish, olivaceous or no reaction with $FeSO_4$; odor and taste mild or disagreeable (somewhat soapy farinaceous). Lamellae sinuate to adnate becoming adnexed; close to crowded, narrow, whitish to gray-white (5B2), dull cinnamon to wood brown; edges white or concolorous, even to uneven. Stipe 15–70 × 3–10 mm, equal; veil evanescent, brown; ground color white with pale yellowish brown– or cinnamon-colored bands of fibrils or fibrillose-scales, soon rusty over the base (but not within), coated up to the apical veil zone with a dull cinnamon sheath that breaks up into patches, apex whitish and pruinose or silky; context hollow.

Basidiospores 6.0–7.2–8.0 × 4.0–4.2–4.5(–5.0) μm, Q = (1.33–)1.46–1.72–2.00 (n = 41/2), ellipsoidal to ovate in profile, apices obtuse to subconical, germ pore and apiculus small and indistinct, thick-walled (walls ca. 0.5–0.75 μm thick), yellowish brown. Basidia 22–30 × 5–6 μm, 4-sterigmate, urniform, hyaline. Pleurocystidia 45–66 × 10–12 μm, frequent, projecting easily from the hymenium, fusiform-ventricose to skittle-shaped, with elongated necks, apices obtuse or swollen, thin-walled, hyaline. Cheilocystidia similar to pleurocystidia but shorter, often fusiform. Caulocystidia 38–70 × 10–13 μm, cylindrical, subcylindrical, slenderly fusiform, or clavate, in tufts or clusters at extreme stipe apex, thin-walled, hyaline. Pileipellis an ixocutis resting on a bright rusty brown–pigmented subpellis; the ixocutis composed of interwoven, narrowly cylindrical, smooth, thin-walled, hyaline hyphae, these mostly 2–3 μm wide; subpellis (originally reported as a hypoderm) composed of coarsely incrusted cylindrical hyphae, these mostly 6–15 μm wide with slightly thickened walls. Lamella trama of parallel hyphae, pale yellowish in mass, with a gelatinous subhymenium. All hyphae inamyloid. Clamp connections present.

Ecology and distribution: Singly or in pairs on charred remains in conifer forests, in US western states (Washington, Idaho (type), California), also northern high-elevation Himalayas, India (FIG. 1), May–Jun and Oct.

Specimens examined: USA. CALIFORNIA: Sierra County, Yuba Pass, gregarious on soil and burned woody debris in selectively logged and burned over Abies magnifica forest, 10 Jun 1985, S.A. Redhead SAR85/286 (WTU 53356). IDAHO: Granite Twin Lakes, near McCall, on burned area, 22 Jun 1962, A. H. Smith AHS65008 (holotype MICH 05323, isotype TENN 028830); boundary County, upper Priest Lake region, gregarious on burned area, 11 Oct 1966, A.H. Smith AHS74095 (holotype of Pholiota subsaponacea MICH 5332). WASHINGTON: Kittitas County, Table Mountain, Reecer Canyon Road (FS 35), on soil among charred remains under high-elevation conifers (Abies, Pinus, Larix), 25 May 1997, P.B. Matheny PBM480 (WTU 10328).

Table 2. Four hypotheses that explain the colonization of burned systems by pyrophilous fungi and predictions each hypothesis makes.

Description	Dispersal hypothesis	Dormancy hypothesis	Heat tolerance hypothesis	Body snatchers hypothesis
Selected references	Gochenaur (1981) Wicklow (1975, 1988) Allen (1987) Dahlberg (2002) Morris et al. (2016)	Warcup and Baker (1963) El-Abyad and Webster (1968) Wicklow (1988) Taylor and Bruns (1999) Claridge et al. (2009)	Gochenaur (1981) Peay et al. (2009) Carlsson et al. (2012)	Baynes et al. (2012) This study
Hypothesis	Absent from pre-fire systems but colonize burned systems after aerial dispersal of spores from other burned systems	Present in pre-fire system as dormant below-ground storage organs or spore banks, which germinate and become reproductive in response to fire or heat	Present in pre-fire systems but possess adaptations to mycelia or spores that enable survival and post-fire colonization	Present in pre-fire system as endophytes or endolichenic fungi but become reproductive in burned systems
Predictions	DNA and RNA transcripts not evident in pre-fire systems	DNA evident in pre-fire soil samples; RNA transcripts should not be evident	DNA and RNA transcripts evident in pre-fire systems in soils or wood	DNA and RNA transcripts evident from plants or lichens in pre-fire systems, not soils or wood
	Spores have higher germination rates in heated treatments; spores should be present in air samples	Spores and sclerotia germinate under heated or burned conditions	Rates of spore germination or mycelial growth higher in heat, alkaline, or other carbonicolous treatments compared with controls	Mycelia grow in response to death or stress of plant host; spores germinate without heat or burned treatment
	Low genetic diversity due to high gene flow	High genetic diversity due to structured populations or low genetic diversity due to slow reproductive cycles	High genetic diversity as fire-adapted traits are subject to selection	Low genetic diversity due to high gene flow (dispersal of plant or lichen propagules) or slow reproductive cycles

Notes: Collections of P. molesta can vary in basidiome size and habit. Several, including the type, generally feature a squat appearance with the pileus as wide or wider than the stipe is long (FIG. 3D). However, other collections (e.g., JFA9246) are difficult to distinguish from typical basidiomes of P. highlandensis because of a small pileus (<20 mm wide), slender stipe (2–3 mm wide), and nonsquat appearance. In such cases, ITS or RPB2 sequences are necessary to distinguish the two taxa. The name P. molesta has not been widely applied in North America because collections of it have often been referred to as P. carbonaria or P. highlandensis.

ITS sequences from the holotypes of P. molesta and P. subsaponacea are identical except for one site difference and nine polymorphic positions in a pairwise comparison. Because the names P. molesta and P. subsaponacea have equal priority (both have the same effective publication date), we favor application of the name P. molesta because it is easier to recall and the etymology (Latin, "troublesome" or "irksome") more relevant than P. subsaponacea (Latin, "almost soapy," in reference to taste).

KEY TO PYROPHILOUS SPECIES OF PHOLIOTA IN NORTH AMERICA

Note that in some cases, because of morphological variation, it may not be possible to always delineate P. molesta apart from P. highlandensis. DNA verification may be necessary.

1. Pileus reddish brown to yellowish red, common near the Gulf Coast and in the southeast.............. .... P. castanea
1′. Pileus light brown, brown, or strong brown, more widely distributed or in western regions 2
2. Stipe brunnescent; caulocystidia large, 40–120 × 10–40 µm, basidiomes often caespitose.................. .... P. brunnescens
2′. Stipe not brunnescent; caulocystidia smaller than above; basidiomes not caespitose......................... 3
3. Habit thin, stipe often <4 mm thick, throughout North America but more common in the east..... .... P. highlandensis
3′. Habit often squat, stipe often >4 mm thick, western North America... P. molesta

DISCUSSION

We found that molecular annotations, using the ITS region as a barcode, of some 70 historical collections including 13 types (8 holotypes and 5 isotypes) distinguish at least four pyrophilous species of Pholiota in North America—P. brunnescens, P. castanea, P. highlandensis, and P. molesta. A fifth pyrophilous species, P. marangania from Australia, is autonomous as well based on phylogenetic analysis and geographic distribution. The combination of ITS with RPB2 resulted in strong to moderate support for all pyrophilous species with more than a single sample. Moreover, several inclusive groupings were strongly supported, and the pyrophilous trait is derived within Pholiota. Apart from

methods of phylogenetic recognition, several gross morphological features also help to distinguish the four North American pyrophilous species as emphasized in the taxonomic key.

Most microscopic features were not useful for distinguishing the subjects of this study. None of the pyrophilous species could be consistently distinguished based on characters such as basidiospore size and shape, hymenial cystidial features, and hymenophoral trama traits. We did observe differences in the size of caulocystidia, which is noticeably larger in P. brunnescens compared with P. castanaea, P. highlandensis, and P. molesta (FIG. 4) as originally observed by Smith and Hesler (1968). In addition, the subpellis of P. castanea is composed of a hypodermium of many inflated hyphae up to 30 μm wide. The subpellis structure of the other three North American burn species examined reveals a mixture of cylindrical to somewhat inflated hyphae, these generally no more than 15 μm wide. Dried pilei and sections of the pileipellis of P. castanea noticeably changed to a dark vinaceous red color in 5% KOH compared with other taxa. Dried pilei of P. highlandensis changed to brown then dark vinaceous red with KOH (see also Kuo 2006).

Of the four pyrophilous species, P. highlandensis, based on numerous observations from the GSMNP, tends to produce small basidiomes, with the pileus often <20 mm in diameter and the stipe <3 mm wide. This observation is consistent with the holotype (see also Holec 2001). However, other taxa demonstrated here to be conspecific with P. highlandensis, viz., P. carbonaria (type from California), can produce larger basidiomes, with the pileus reaching up to 30 mm wide or larger (see also Orton [1977] as 'P. brunnescens'). Pholiota molesta tends to produce basidiomes with a squat stipe; however, some collections deviate from this and resemble small forms of P. highlandensis. Pholiota brunnescens does not produce small basidiomes. The latter is also distinguished by the often caespitose habit.

To date, we are aware of little previously published successional data regarding pyrophilous Pholiota in North America (Carpenter et al. 1987). Some works have presented patterns of fungal succession on burns outside North America (Petersen 1970; Robinson et al. 2008; Gassibe et al. 2011), and successional data have been published for mycorrhizal fungi in western North American burn systems (Stendell et al. 1999; Treseder et al. 2004; Bruns et al. 2005; Glassman et al. 2016) and in Europe (Dahlberg 2002). It is clear that P. highlandensis is an early successional burn or "phoenicoid" species consistent with general observations of tephra deposits in the western United States (Carpenter et al. 1987), Denmark (Petersen 1970), and more specifically in

burned pine stands and Cistus landscapes in Spain (Gassibe et al. 2011; Hernández-Rodríguez et al. 2013, as Pholiota carbonaria). We observed basidiomes as soon as approximately 4 mo (early Apr) after the Chimney Tops 2 fire was contained in the GSMNP, continuing throughout 2017 and the first half of 2018. Motiejūnaitė et al. (2014) also recorded basidiomes of P. highlandensis during the first 2 y following a fire in pine plantations in Lithuania.

Historical records indicate the presence of P. castanea on burned and nonburned sites in southeastern states of the United States, including in the GSMNP, but no collections of it were observed 1.5 y after the Chimney Tops 2 fire. We thus conclude that P. castanea is probably a later successional pyrophilous species. We have no successional data for P. brunnescens and P. molesta. The authors have collected the latter in a charcoal pit or small patchy burn in what was otherwise an intact conifer forest in the Pacific Northwest. Basidiomes of P. brunnescens can be found on burns during morel season in western states in our experience, but clear successional data are lacking. Basidiomes of both P. brunnescens and P. molesta were collected simultaneously in western burned areas as well.

Regarding its early successional appearance in burned systems, Petersen (1970) hypothesized that P. highlandensis (as P. carbonaria), among other species, is involved in decomposition of dead or dying roots, utilizing the presence or release of various organic compounds from the roots. This hypothesis is strengthened somewhat by the detection of this species (as P. carbonaria) from nonburned root systems in mixed Fraxinus excelsior stands with rich soils badly damaged by fungal attacks (Bakys et al. 2011). In this latter study, P. highlandensis was implicated in cambium necrosis.

The austral "burn-loving" P. marangania is poorly known in Australia (Matheny and Bougher 2010) but could be reported as P. highlandensis (Claridge et al. 2009). Robinson et al. (2008) reported an unidentified Pholiota rather frequently on first-year burned sites in southwest Australia and in a few successive years after that. Specimen vouchers produced from these reports should be examined to determine if these represent P. marangania. At least two Australian field guides depict P. highlandensis (Fuhrer 2009; Gates and Ratkowsky 2016), both characterized by pinkish brown to brown squamules on the stipe surface. We suspect that both could represent P. marangania because P. highlandensis has yet to be confirmed from Australia with genetic data.

Of the five pyrophilous species documented here, one—P. highlandensis—most likely completes part of its life cycle as an endophytic and/or endolichenic fungus (U'Ren et al. 2012; Suryanarayanan and Thirunavukkarasu 2017) in association with lichens, bryophytes, and possibly pine and yew trees. At this time, based on best available data,

P. highlandensis also has the widest geographic distribution occurring in the Americas, east Asia, and Europe and was the first and only Pholiota to occur on burns in the GSMNP in 2017–2018.

Whereas some pyrophilous fungi colonize burn systems by aerial dispersal of spores (Wicklow 1975; Allen 1987), or persist in ecosystems in dormant states (Warcup and Baker 1963; El-Abyad and Webster 1968; Wicklow 1988) such as spore banks (Taylor and Bruns 1999) or as subterranean sclerotia or pseudosclerotia (McMullan-Fisher et al. 2011), or are capable of persistence despite fire disturbance due to adaptations of heat tolerance (Peay et al. 2009; Carlsson et al. 2012), we suggest that P. highlandensis is maintained in pre-fire systems as an endophyte and/or endolichenic fungus in a fire-associated symbiosis (Baynes et al. 2012; TABLE 2). Response to fire probably includes hyphal proliferation from lichen thalli or plants on burned soils and subsequent basidiome production in absence of competition. We describe this type of symbiosis as the "body snatchers" hypothesis, in reference to a science fiction novel of the same name by Jack Finney (Finney 1955) where aliens invade human bodies. If accurate, this predicts that other pyrophilous fungi could also exhibit endophytic and/or endolichenic life stages, as described in Baynes et al. (2012), perhaps persisting somatically for long periods of time between burn events.

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ORCID

P. Brandon Matheny ⓘ http://orcid.org/0000-0003-3857-2189
Andrew N. Miller ⓘ http://orcid.org/0000-0001-7300-0069
Ronald H. Petersen ⓘ http://orcid.org/0000-0002-3170-5770

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