Mycologia, 104(5), 2012, pp. 1200–1205. DOI: 10.3852/11-409

Armillaria altimontana, a new species from the western interior of North America

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Abstract: *Armillaria altimontana*, previously considered North American biological species (NABS) X, is described as new. To date, it appears that *A. altimontana* prefers higher-elevation, mesic sites within the dry, conifer forest zone of western interior North America. This species has been found on hardwoods and conifers and is associated most commonly with *Abies*-dominated forest types in southern British Columbia, Washington, Oregon, Idaho and northern California. Partial elongation factor 1-alpha (*tef1*) sequences were generated from six isolates of *A. altimontana* originating from three locations in northern Idaho. Phylogenetic analyses of all 10 North American *Armillaria* species were carried out with maximum parsimony and maximum likelihood. Results indicate that isolates of *A. altimontana* formed a monophyletic group and clustered with *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. nabsnona*, which is in agreement with recent phylogenetic studies of *Armillaria*.

Key words: *Abies*, armillaria, elongation factor 1-alpha, root disease

Introduction

Ten species of *Armillaria* are known to occur in North America and their identities have been confirmed from multiple studies utilizing a combination of morphological, biological and phylogenetic species concepts (Anderson and Ullrich 1979, Anderson and Stasovski 1992, Burdsall and Volk 1993, Kim et al. 2006, Ross-Davis et al. 2012). One species, North American biological species (NABS) X, has remained undescribed due to the scarcity of basidiocarps. Since its designation as a unique species, NABS X has undergone rigorous study to confirm its species status using cultural and molecular techniques (Banik and Burdsall 1998; Kim et al. 2000, 2001; Ross-Davis et al. 2012).

Submitted 3 Dec 2011; accepted for publication 23 Mar 2012.
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NABS X is known from the western interior of North America, where it has been reported infrequently. Overall, this species is known to inhabit forests in southeastern British Columbia, eastern Washington, northern Idaho, eastern Oregon and northern California (Anderson and Ullrich 1979; Morrison et al. 1985; Baumgartner and Rizzo 2001; Ferguson et al. 2003; Kim et al. 2006, 2010). While it has been suggested that NABS X likely occurs as far south as Colorado (Hunt et al. 2011), *A. ostoyae* was the only species identified from a collection of 379 isolates in southwestern Colorado campgrounds (Worrall et al. 2004) and from 103 isolates collected from Colorado, Wyoming and South Dakota (Wu et al. 1996). Further investigation of Wyoming forests reported no collections of NABS X (Blodgett and Lundquist 2011). Collectively these surveys suggest that the southern limit of the geographic range of NABS X includes the Cascade Range in California and the northern Rocky Mountains in Idaho and Montana.

NABS X has been collected on both conifers and hardwoods (Morrison et al. 1985, McDonald et al. 1987), although it is not believed to be an aggressive pathogen. However, like other infrequently encountered *Armillaria* species (e.g. *A. gemina*), further study is required to properly ascertain virulence in natural settings. Across its range NABS X has been found most often inhabiting higher elevation sites dominated by *Abies* species (Morrison et al. 1985, McDonald et al. 1987, Baumgartner and Rizzo 2001, Ferguson et al. 2003, McDonald et al. 2011). In northern California, NABS X was collected in the southern Cascade and Great Basin ranges at 1600–2000 m but could not be located in *Abies*-dominated forests in the Sierra Nevada and Coast Range (Baumgartner and Rizzo 2001). Kim et al. (2010) located NABS X in cedar/hemlock (*Thuja plicata/Tsuga heterophylla*) and grand fir (*A. grandis*) forest types in the eastern Cascade Range in Washington, which taken together with the studies mentioned above suggests NABS X prefers mesic sites within the dry, western interior region. Although little is known about the specific ecological preferences of NABS X, *A. ostoyae* usually is found co-occurring with this species (Ferguson et al. 2003, Kim et al. 2010).

Outside North America, NABS X has not been found in Japan or China (Ota et al. 1998, Qin et al. 2007) or Europe (Guillaumin et al. 1993). Therefore, it appears that NABS X is unique to western North America and inhabits a narrow set of forest types. The

TABLE I. Characteristics of *Armillaria altimontana* isolates used to generate partial *tef1* sequences

Isolate code[a]	Year collected	Host/substrate	Origin	GenBank accession no.
D82[b]	1988	Unknown stump	Deception Creek Experimental Forest, Kootenai County, ID	JN944611
D83	1988	Unknown log	Deception Creek Experimental Forest, Kootenai County, ID	JN944610
D84	1988	Unknown	Deception Creek Experimental Forest, Kootenai County, ID	JN944609
POR100[b,c]	1983	Unknown	Poirier Creek, Bonner County, ID	JN944606
Mac4	1992	*Abies grandis*	Clearwater National Forest, Clearwater County, ID	JN944608
Mac6	1992	*Alnus* sp.	Clearwater National Forest, Clearwater County, ID	JN944607

[a] All isolates collected by Geral I. McDonald and previously identified as described in Banik and Burdsall (1998).
[b] Isolates used in Kim et al. (2000, 2006) and Ross-Davis et al. (2011).
[c] Designated as holotype and housed at CFMR.

goal of this study was to characterize the morphological features of *Armillaria* NABS X basidiocarps and to formally describe this new species.

MATERIALS AND METHODS

Isolates used.—Basidiocarps of NABS X were collected by G.I. McDonald 1981–1993 and were archived in the USDA Forest Service, Center for Forest Mycology Research (CFMR) herbarium in Madison, Wisconsin. Both haploid and diploid cultures were generated from these fruiting bodies and have been maintained in the CFMR culture collection (TABLE I).

Six isolates that originated from three locations in northern Idaho were chosen for the species description. All six were identified as NABS X, using mating analyses with haploid testers (Banik and Burdsall 1998). Two of the six were characterized further based on molecular markers (IGS1, *tef1* and AFLP genotyping) and nuclear weights (Kim et al. 2000, 2006). Because there are few distinctive morphological characters among *Armillaria* species (e.g. Guillaumin et al. 1993) and because NABS X is known from few collections from throughout a limited geographic range, NABS X is the last North American biological species to be described. Analyses of multicopy genes (e.g. nuclear rDNA loci) have not provided strong support for the species description of NABS X (e.g. Anderson and Stasovski 1992, Kim et al. 2006). As such, the nuclear locus elongation factor subunit 1-alpha (*tef1*) was used, which is a confirmed single-copy gene in *Armillaria* (Baumgartner et al. 2010) and has been proven useful for delineation of North American *Armillaria* species (see Maphosa et al. 2006, Brazee et al. 2011, Ross-Davis et al. 2012). Isolate characteristics are described (TABLE I).

DNA extraction, PCR and sequencing.—Genomic DNA was obtained from individual isolates as described by Brazee et al. (2011). PCR was performed with 5 × Green GoTaq reaction buffer and GoTaq DNA polymerase (Promega, Madison, Wisconsin). GoTaq reaction buffer was diluted to a 1 × working concentration and 0.25 units of GoTaq DNA polymerase were used per reaction. Primers EF595F and EF1160R (Kauserud and Schumacher 2001) were used at a final concentration of 0.4 μM, and each dNTP (Promega, Madison, Wisconsin) had a final concentration of 200 μM. Thermo-cycler conditions were: initial denaturing at 94 C for 2 min, 40 cycles of denaturing at 94 C for 30 s, annealing at 55 C for 45 s and extension at 72 C for 80 s and a final extension step of 72 C for 10 min.

Before sequencing, PCR products were viewed on 1% agarose gels stained with ethidium bromide to confirm the presence of a single amplicon and then diluted 1 : 10 with DNA-grade water. Isolates were sequenced with the Big Dye Sequencing Kit 3.1 on an ABI 3130xl capillary sequencer (Applied Biosystems, Foster City, California) at the University of Wisconsin Biotechnology Center. GenBank accession numbers (JN944606–JN944611) are listed (TABLE I).

Sequence alignment and phylogenetic analysis.—Partial *tef1* sequences were aligned in MAFFT 6 (Katoh et al. 2005) using the L-INS-i method. Heterozygous positions were coded as ambiguous characters. Phylogenetic reconstructions were carried out in MEGA 5 (Tamura et al. 2011) with maximum parsimony and maximum likelihood, as described by Brazee et al. (2011). Additional partial *tef1* sequences of *Armillaria* isolates originating from North America, generated previously, were included to compare NABS X to the remaining nine North American *Armillaria* species (Maphosa et al. 2006, Brazee et al. 2011, Ross-Davis et al. 2012). Four additional isolates, originating from South Dakota and Wyoming (Blodgett and Lundquist 2011), also were included (with GenBank accession numbers in parenthesis): (i) *A. gallica*, isolate PB-33-1 (JQ677994); (ii) *A. sinapina*, isolate BLH-14-1 (JQ677998); and (iii–iv) *A. ostoyae*, isolates AF-2-1 (JQ678003) and WS-4-1-3 (JQ678013). All trees were rooted by the outgroup method with *Schizophyllum commune* (GenBank accession number X94913).

Microscopic analyses.—Macroscopic descriptions are based on dried specimens of three collections. Because of its

limited distribution and rare occurrence, as detailed above, there are few collections of basidiomata available for examination. Color terms are approximations, while numerical color designations are from Kornerup and Wanscher (1978). Microscopic structures were observed with an Olympus BH-2 compound microscope; freehand sections of dried fungal material were rehydrated in 70% EtOH and mounted in 3% KOH and Melzer's reagent. Basidiospore length and width were measured (n = 20) and used to calculate the proportion of basidiospore length to width (*Q*). The species description includes the range, mean and standard deviation of the basidiospore dimensions and the mean *Q* values (Q_m).

RESULTS

Phylogenetic analysis.—Amplification of the *tef1* gene region resulted in a single amplicon of approximately 550 bp. The phylogenetic tree of partial *tef1* sequences shows that ML/MP grouped eight *A. altimontana* isolates into a monophyletic clade with strong BS (100/100%; FIG. 1). Overall, this species grouped with *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. nabsnona* with low BS separating the groups (FIG. 1). Meanwhile, isolates of *A. sinapina*, *A. gemina* and *A. ostoyae* formed a distinct cluster, with the latter two species grouping together with high BS (97/96%; FIG. 1). Finally, *A. mellea* and *A. tabescens* grouped distal to all other species.

TAXONOMY

Armillaria altimontana Brazee, B. Ortiz, Banik, and D.L. Lindner, sp. nov. FIG. 2
MycoBank MB 563757

Basidiomata tricholomatoid, pileus plane in mature specimens, up to 3 cm broad in dried samples, rust brown (5E) with cream-colored scales and short fibrils present. Context 0.5–1 mm thick. Dried stipe 4–7 cm long, 8–10 mm broad at base and narrowing to 3–5 mm at the apex; annulus present, cottony, white to cream, robust and thick on two specimens examined, while difficult to observe on the remaining specimen. Rhizomorphs monopodial, and thin, cylindrical on attached substrates.

Basidiospores 7.2–11.7 × 5.4–6.3 µm (n = 20, 9.1 ± 1.5 × 5.8 ± 0.4; Q_m = 1.6 ± 0.2), broadly elliptical, smooth, guttulate, hyaline in KOH; nonamyloid in Melzer's reagent (FIG. 2). Basidia 34.2–55.8 × 6.3–10 µm, cylindro-clavate, four-sterigmate, hyaline in KOH; sterigmata 2.7–4.5 µm long (FIG. 2). Marginal cells among the basidia 24.3–50.4 × 5.4–8.1 µm, clavate to cylindro-clavate, hyaline in KOH (FIG. 2). Pleurocystidia absent. Pileipellis a tangled layer of repent to erect hyphae 4–18 µm broad, subparallel and hyaline in KOH. Hyphae of the pileal scales 6.3–20 µm broad, multiseptate, thin to moderately thick-walled, hyaline or yellowish in KOH. Clamp connections absent.

Holotype.—United States, Idaho: Bonner County, Poirier Creek. Herbarium specimen POR100 designated as the holotype, was recovered on an unknown host in autumn 1983 (collector G.I. McDonald). Dried herbarium sample and live culture have been maintained at the CFMR herbarium (POR100), and a partial *tef1* sequence has been deposited in GenBank (accession number JN944606). Isotype deposited at BPI (BPI number 883541).

Additional specimens.—United States, Idaho: Kootenai County, Deception Creek Experimental Forest. Isolate D82 was recovered on an unknown tree stump in autumn 1988 (collector G.I. McDonald). United States, Idaho. Kootenai County, Deception Creek Experimental Forest. Isolate D83 recovered on an unknown log in autumn 1988 (collector G.I. McDonald). Dried herbarium samples and live cultures of both specimens are maintained at the CFMR herbarium (D82; D83), and partial *tef1* sequences have been deposited in GenBank (accession numbers in TABLE I).

Habitat.—Found on hardwoods and conifers in higher elevation forests, especially in forest types dominated by *Abies* species.

Known distribution.—Known from the Cascade, Great Basin and Rocky Mountain ranges in western North America (California, British Columbia, Idaho, Oregon and Washington).

Etymology.—Referring to its occurrence in high elevation mountains (alti = high; montana = mountain).

DISCUSSION

This study documents the characters of *Armillaria altimontana*, previously known as NABS X, the only known unnamed *Armillaria* species in North America to currently lack a formal description. *Armillaria altimontana* has been recognized as a distinct biological species for more than 30 y (see Anderson and Ulrich 1979). Given the ecological and economic importance of many species in genus *Armillaria*, the formal naming of this species should aid future research and identification. The geographical range and site preferences of *A. altimontana* are among the more distinctive characteristics of this species. In particular, this species appears to prefer mesic, higher elevation forests in the western interior that support *Abies* species. *Armillaria* species distribution studies have documented *A. altimontana* in forests typically dominated by *A. ostoyae* (Baumgartner and Rizzo 2001, Ferguson et al. 2003, Kim et al. 2010). Important macroscopic characters that could be used to distinguish *A. altimontana* from *A. ostoyae* include

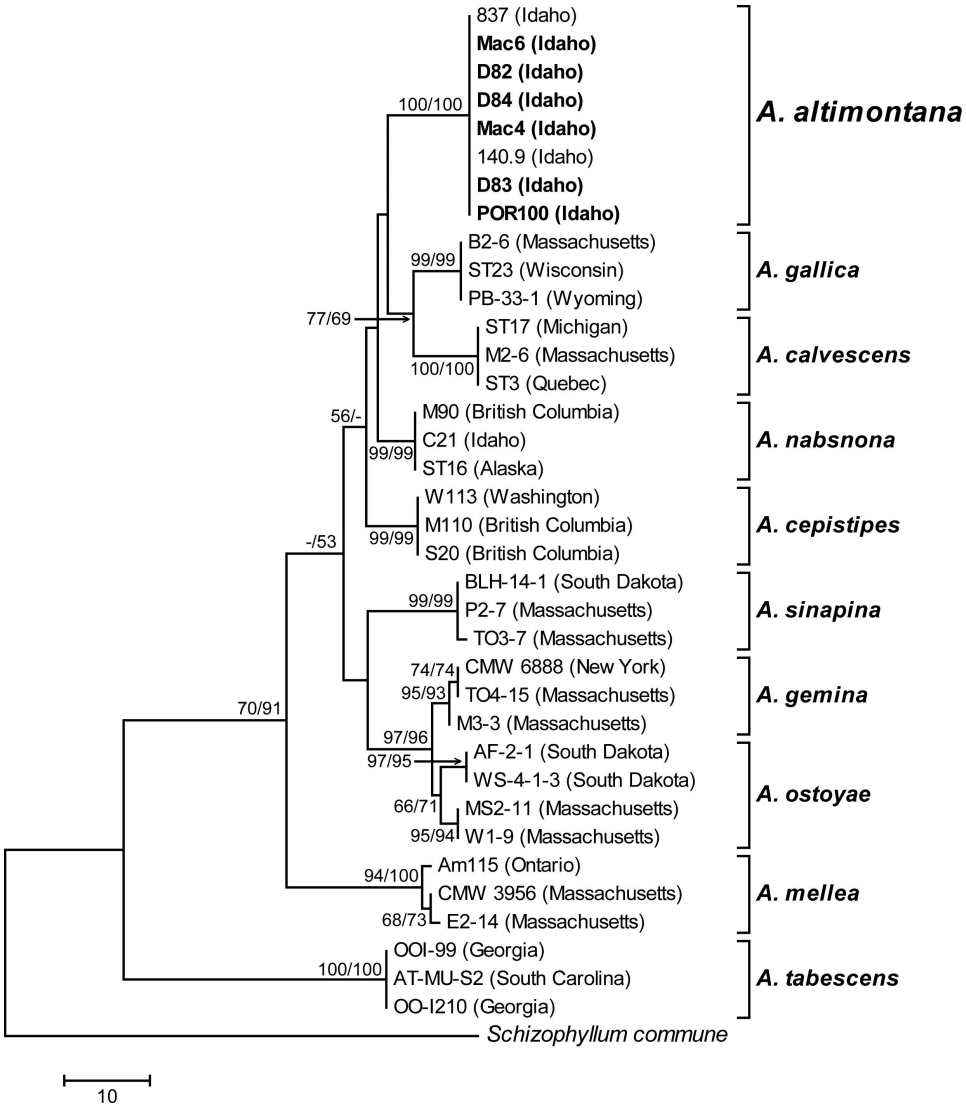

FIG. 1. Consensus tree of North American *Armillaria* species based on partial *tef1* sequences using MP with gaps and missing data excluded from the analysis. Consensus BS values (1000 replicates) for ML and MP with values greater than 50% are next to the nodes. Isolate codes are followed by the state/province of origin. Codes for isolates of *A. altimontana* generated in this study appear in boldface, with accession numbers listed in TABLE I.

the swollen base of the stipe and monopodially branched rhizomorphs of the former. Additional macroscopic characters of the studied specimens, such as the presence and the size of scales and fibrils, along with the brown to reddish brown pileus, and the consistency of the annulus are not distinctive enough to distinguish *A. altimontana* from *A. ostoyae*.

However, the predominance of *A. altimontana* in higher elevation, conifer-dominated forests, along with the presence of distinct scales on the pileus can aid in distinguishing this species from four of the five remaining species of *Armillaria* in western forests. Specifically, *A. cepistipes*, *A. gallica*, *A. mellea* and *A. nabsnona* typically have pilei that lack scales and when present are often small (Burdsall and Volk 1993, Bérubé and Dessureault 1989). Also, some specimens of *A. cepistipes* can have a double annulus, which is a distinctive character for this species (Antonin et al. 2009). *Armillaria nabsnona* is found only in lower elevation coastal sites of the Pacific Northwest, especially in *Alnus*-dominated forests (Volk and Burdsall 1996), while *A. mellea* also is most abundant in hardwood-dominated forest types and has a pileus that is consistently smooth and honey-colored (Bérubé and Dessureault 1989). The last remaining species, *A. sinapina*, can have dark scales on the pileus and produces both bulbous stipes and monopodially branched rhizomorphs, but this species also usually has distinctive yellow flecks on the pileus,

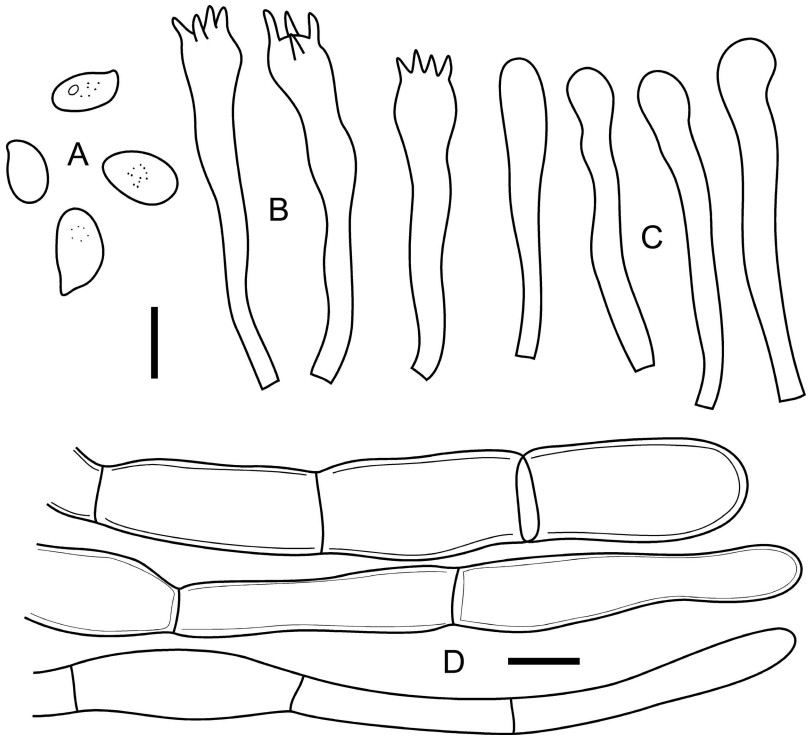

FIG. 2. Microscopic features of *Armillaria altimontana* from the holotype (POR100). A. Basidiospores. B. Basidia. C. Cells among basidia. D. Hyphae of the pileal scales. Bar = 10 μm.

annulus and stipe, which are remnants of the universal veil (Burdsall and Volk 1993).

A potentially important microscopic character that could distinguish *A. altimontana* from other *Armillaria* species is larger basidiospores, a trait observed in roughly 10% of all basidiospores examined from two of the three specimens used in this study. However, basidiospores up to 12 μm long have been recorded for *A. gallica* in Europe (Antonin et al. 2009), although descriptions of *A. gallica* from North America do not mention such large spore sizes (Burdsall and Volk 1993). In any case, this suggests that additional North American species also could produce large basidiospores. Ultimately macro- and microscopic characters are unreliable for ecological studies where delimitation of individual species is necessary. In those instances, the use of partial *tef1* sequences is the only way to confidently identify species of *Armillaria* at present. Analysis of partial *tef1* sequences for eight isolates of *A. altimontana* produced a well supported, monophyletic group and supported findings that this species groups within the same cluster as *A. calvescens*, *A. cepistipes*, *A. gallica* and *A. nabsnona* (Kim et al. 2006, Ross-Davis et al. 2012).

Currently *A. altimontana* has been collected infrequently across its range and the full geographic extent and ecology of this species in the western interior of North America remains in doubt. Future studies of *Armillaria* species distribution in the southern Rocky Mountains (Utah, Colorado, Arizona and New Mexico) should help determine whether *A. altimontana* occupies these regions.

ACKNOWLEDGMENTS

We thank Dr Geral McDonald and Dr Ned Klopfenstein for providing information about the isolates used in this study and Dr. Shaun Pennycook for assistance with choosing the species epithet. Two anonymous reviewers provided valuable comments on a previous version of this manuscript.

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