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First report of the post-fire morel *Morchella exuberans* in eastern North America

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ABSTRACT

Reports of true morels (*Morchella*) fruiting on conifer burn sites are common in western North America where five different fire-adapted species of black morels (Elatia Clade) have been documented based on multilocus phylogenetic analyses. Fruiting of post-fire morels in eastern North America, by comparison, are rare and limited to a report from Minnesota in 1977 and eastern Ontario in 1991. Here, nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) sequences were used to identify the post-fire morel that fruited in great abundance the year following the 2012 Duck Lake Fire in the Upper Peninsula of Michigan and after the 2016 large-scale fire in the Great Smoky Mountains National Park in Tennessee as *M. exuberans*. A preliminary phylogenetic analysis suggests that the collections from eastern North America may be more closely related to those from Europe than from western North America, Europe, and China.

INTRODUCTION

Five fire-adapted species of true morels (*Morchella*) fruit abundantly in western North America, especially the first season after a forest fire. These post-fire morels are all early-diverging lineages of the Elata Clade (i.e., black morels), and their fruiting appears to be restricted to conifer burn sites (Du et al. 2012a; Richard et al. 2015). In late May of 2012, a lightning fire (the Duck Lake Fire) burned over 21,000 acres in Luce County of the Upper Peninsula of Michigan at the mouth of the Two Hearted River. A large portion of the area comprised plantations of *Pinus banksiana*, which were severely burned, and subsequently salvage cutting removed many trees. Visits to the site in early Jun 2013 revealed a massive fruiting of a black morel in logged and uncut areas that had experienced intense fire (FIG. 1). Three years later, in a fire attributed to aggravated arson, the Great Smoky Mountains National Park (GSMPNP) suffered its first large-scale fire in Nov 2016, in which ~11,000 acres in the northern part of the Park between Gatlinburg and Chimney Tops in Tennessee were scorched. During a survey of post-fire fungi in slightly to severely burned areas, a black morel in the Elata Clade was discovered on 13 Apr 2017 throughout a severely burned area near Baskins Creek Trail dominated by table mountain pine (*Pinus pungens*) and Rhododendron (FIG. 2). In this paper, internal transcribed spacer (ITS) nuc rDNA (ITS1-5.8S-ITS2 = ITS) and multilocus DNA sequence data were used to identify the post-fire morel on the conifer burn sites in Michigan and Tennessee as *M. exuberans*.

MATERIALS AND METHODS

Six fruiting bodies from the Duck Lake Fire site were collected and deposited into MICH as a dried specimen (MICH-F-139093). A culture designated UM884 was obtained from tissue removed from the inside of the cap of one of these specimens by plating onto potato dextrose agar. Culture UM884 was stored for 5 y at 4 °C on a slant of malt extract agar before the DNA analysis described below and is now deposited in the US Department of Agriculture Agricultural Research Service (USDA-ARS) Culture Collection as NRRL 66726. Fruiting bodies from the
GSMNP fire site were collected under charred P. pungens, photographed, and dried (FIG. 2). A voucher from this collection was deposited in the fungarium of the Illinois Natural History Survey as ILLS 81091. In addition, several freshly collected fruiting bodies were used to obtain spore deposits by incubating them overnight at room temperature wrapped in wax paper. Pure cultures of collection ILLS 81091 were obtained by germinating ascospores overnight on 3% water agar supplemented with antibiotics following a published protocol (O’Donnell et al. 2011). Approximately 30 colonies 1–3 mm in diameter from ILLS 81091 were stored in the O’Donnell laboratory as KOD1548 and KOD1549 at –80°C in a cryogen consisting of 10% skim milk and 1% dimethyl sulfoxide (DMSO). Colonies were obtained by culturing the strains in 50 mL of yeast-malt broth (3 g yeast extract, 3 g malt extract, 5 g peptone, 20 g dextrose per liter; Difco, Detroit, Michigan) in 300-mL Erlenmeyer flasks on a rotary shaker set at 200 rpm/min and 25°C for 4–5 d. Strain KOD1548 was also deposited as NRRL 66725 where it and NRRL 66726 are available for distribution upon request (https://nrrl.ncaur.usda.gov/). DNA was extracted from both a dried fruiting body (PBM4081) in the Hughes laboratory at University of Tennessee (UT) and from NRRL 66725 in the Miller laboratory at University of Illinois Urbana-Champaign (UIUC) and sequenced for the entire ITS region. In
addition, partial DNA sequence data were obtained from RNA polymerase largest and second largest subunits (RPB1 and RPB2), translation elongation factor 1-α (TEF1), and domains D1 and D2 of the nuc rDNA 28S subunit from cultures of the post-fire morels from the GSMNP (NRRL 66725) and Michigan (NRRL 66726). Maximum parsimony (MP) phylogenetic analyses were conducted with PAUP* 4.0b10 (Swofford 2003) as previously described (O’Donnell et al. 2011). DNA sequences were deposited in GenBank under accession numbers MF981025–MF981033, and the NEXUS file and most-parsimonious tree were deposited in TreeBASE (accession number S21567 and tree number Tr106895).

RESULTS AND DISCUSSION

ITS sequence data generated from a dried fruiting body (PBM4081) and a culture (NRRL 66725) of the post-fire morel collected in the GSMNP were identical (763 bp) and had 100% identity (760/760 bp) to M. exuberans from Sweden (GenBank accession KM587918; Richard et al. 2015). This identification is definitive based on previous work that demonstrated that ITS data distinguish M. exuberans from all other morels (Du et al. 2012b). We also compared ITS + 28S rDNA sequence data on NRRL 66725 from the GSMNP and NRRL 66726 from the Upper Peninsula of Michigan and found that they were 99.8% identical (1310/1312 bp). These two strains differed at only two nucleotide positions within the ITS1, which confirms their identity as M. exuberans (Du et al. 2012b). In addition, partial DNA sequence data were obtained from RPB1, RPB2, and TEF1 from M. exuberans NRRL 66725 and NRRL 66726 so that these eastern North American collections could be compared with M. exuberans from western North America, Europe, and China. Maximum parsimony analysis of multilocus DNA data from 13 transcontinental collections of M. exuberans, and two of M. eximia from western North America selected for rooting the phylogeny, suggests that the eastern North American collections are more closely related to specimens from Europe than from the other regions sampled (FIG. 3).

Morchella exuberans was first formally described based on a collection from a burned site in Emigrant Gap, California (Clowez 2012). This name has priority over the heterotypic synonym M. capitata proposed later the same year for a post-fire collection in Oregon (Kuo et al. 2012). Prior to receiving a formal Latin binomial, this species was reported using an informal ad hoc nomenclature as Morchella sp. Mel-9 from Turkey (Taşkin et al. 2010), Oregon, USA (O’Donnell et al. 2011), Yunnan, China (Du et al. 2012a), and Sweden (Taşkin et al. 2012). Loizides et al. (2016) recently reported M. exuberans from a burned Turkish pine (P. brutia) forest in Cyprus. The current study represents the first published report of this species

**Figure 3.** One of 416 most-parsimonious trees (MPTs) inferred from maximum parsimony (MP) analysis of a four-gene data set comprising 13 sequences of Morchella exuberans rooted on two sequences of M. eximia. Gray highlight is used to identify the collections of M. exuberans from eastern United States, which grouped with two collections from Sweden with 92% bootstrap support (BS). Numbers above nodes represent MP-BS based on 1000 pseudoreplicates of the data. ANK = herbarium Turcicum Ankara; CI = consistency index; HKAS = cryptogamic herbarium Kunming Institute of Botany; M = morel genomic DNA accessioned in O’Donnell laboratory; NRRL = ARS Culture Collection; PIC = parsimony-informative character; RI = retention index; S = Swedish Museum of Natural History.
from eastern North America. *Morchella exuberans* is one of five fire-adapted morels from conifer burn sites documented from western North America, all nested in the Elata Clade. These include *M. tomentosa* (*Mel*-1), *M. sextelata* (*Mel*-6), *M. eximia* (*Mel*-7 and later synonym *M. septimelata*), and an undescribed species reported as *Mel*-8 (O’Donnell et al. 2011).

Reports of post-fire morels in eastern North America are rare, with published reports only from Minnesota and Ontario. *Morchella angusticeps* was reported in a Minnesota mixed jack pine (*P. banksiana*) and black spruce (*Picea mariana*) forest in 1977, 1 year after a wildfire (Apfelbaum et al. 1984). In the second report, a black morel identified as *M. conica* was collected on a conifer burn site in May 1991 following a prescribed burn the year before at the Petawawa National Forestry Institute in eastern Ontario, Canada (Duchesne and Weber 1993). The true identity of the morels reported in these two studies cannot be verified using molecular systematic data because vouchers were not deposited in a fungarium. However, the identifications are viewed as unconfirmed because *M. angusticeps* is not known to fruit on burn sites and the name *M. conica* is illegitimate at the rank of species because it is a superfluous name for *M. continua* Tratt., in violation of Article 52 of the International Code of Nomenclature for Algae, Fungi, and Plants (McNeill et al. 2012; Richard et al. 2015).

Given the large number of *Morchella* species and their morphological plasticity, we recommend that ITS sequence data be used where possible to obtain a definitive identification, as done here for *M. exuberans* (Du et al. 2012b). However, multilocus DNA sequence data are required to differentiate some closely related species, especially those in the species-rich *M. elata* species complex (Du et al. 2012a; Taškın et al. 2012). Lastly, results of the present study highlight the importance of vouchered fungarium specimens, which are essential for developing a comprehensive North America MycoFlora (http://www.northamericanmycoflora.org/).

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