Multiple species of morels occur in landscape settings

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Abstract: Morels (*Morchella* spp.) are popular ascomycete fungi due to their culinary value. Recent advancements in molecular identification have spurred interest in the ecology and genetics of these edible fungi. Most morels are known to inhabit forest settings and may have mycorrhizal associations with trees. Two species of morels, *M. importuna* and *M. rufobrunnea*, are associated with urban landscape settings and *M. rufobrunnea* has been cultivated, suggesting that it has a saprotrophic nutritional mode. As part of a graduate class project at Washington State University, morels collected from landscape settings in the Pacific Northwest of the United States and British Columbia, Canada were identified using sequence data. Five phylogenetic species were identified and two species have not been previously reported from urban settings. Of the three species previously reported from landscape settings, one represents the first report of this species from a North American landscape setting, and another is only the third specimen collected of an as of yet unnamed species. Our findings suggest that a diverse range of morels occur in landscape and urban settings.

Key words: Canada, distribution, ecology, *Morchellaceae*, mulch morel, United States.

Introduction

Morels (*Morchella* spp.) are some of the most highly prized edible macrofungi. Morels are thought to have originated in the Holarctic over 120 million years ago and are now distributed worldwide (O'Donnell et al., 2011). Nineteen phylogenetic lineages of North American morels identified by O'Donnell et al. (2011) were formally described as species by Kuo et al. (2012). More recently, Richard et al. (2015) reconciled the North American species described by Kuo et al. (2012) with those described by Clowez (2010). The majority of North American species has been reported from forested and other "non-urban" environments and may have mycorrhizal associations with trees (Dahlstrom et al., 2000).

So-called "landscape" or "mulch morels" are of particular interest and have been identified and characterized based on their ecological niche (Kuo et al., 2012; Mann and Mann, 2014; Ondřej et al., 2011). Two species of morels, *M. rufobrunnea* and *M. importuna,* are the most common species inhabiting landscape settings (Guzmán and Tapia, 1998; Kuo, 2008; Kuo et al., 2012; Mann and Mann, 2014). Such landscapes include bark or mulched beds, gardens, planters, lawns, and roadsides (Guzmán and Tapia, 1998; Kuo, 2008; O'Donnell et al., 2011). Many morels are found in association with trees (Kuo et al., 2012) and are suspected to form mycorrhizal relationships (Dahlstrom et al., 2000), however, both *M. rufobrunnea* and *M. importuna* are suspected saprotrophs due to their profundity in landscapes and apparent lack of association with trees (Mann and Mann, 2014). Despite the reported prevalence of these two species from urban environments (Guzmán and Tapia, 1998; Kuo, 2008; Kuo et al., 2012; Mann and Mann, 2014), a few morel species have been found to inhabit both landscape and forest settings (Kuo et al., 2012; Ondřej et al., 2011). As part of a spring 2014 class project in "Advanced Fungal Biology," a graduate-level plant pathology course at Washington State University (WSU), we characterized specimens of morels collected from landscape and urban settings using DNA-sequence data. Our aim was to determine the diversity and ecology of landscape morels present at several sites in Washington State, Idaho, and British Columbia.

Materials and Methods

Morel fruiting bodies were collected from April to May 2011-2013 from landscape settings including lawns, landscaping bark and rocks, and mulched plant beds in Washington, Idaho, and British Columbia. Specimens were placed on paper overnight at room temperature (20-25˚C) to generate spore prints, and then allowed to dry at room temperature prior to storage. Voucher specimens were deposited in WSU's Charles Gardner Shaw Mycological Herbarium (accessions in Fig. 1). Single-ascospore cultures were grown on potato dextrose agar and (PDA) (BD, Franklin Lakes, New Jersey) amended with penicillin (500 units/L) and streptomycin (500 µg/L) to inhibit bacterial contamination. If the specimen lacked mature ascospores, a culture was initiated from surface sterilized stipe tissue. Subcultures for DNA extraction were grown on sterile dialysis membrane (SpectraPor, VWR Scientific, Radnor, Pennsylvania) overlaying PDA amended with antibiotics (BD, Franklin Lakes, New Jersey). Mycelia were gently scraped from the membranes after 5-7 days, frozen, and lyophilized. DNA extraction, PCR amplification, sequencing, and phylogenetic analysis followed the methods described in

Those pursuing collection of morels for the table from landscape and urban settings should proceed with caution. Morels and other mushrooms are known bioaccumulators of heavy metals (Cocci et al., 2006). A study published in FUNGI in 2010 showed arsenic and lead accumulation in morels collected in abandoned apple orchards previously treated with commercial pesticides (Shavit and Shavit, 2010). Furthermore, there is at least one report of a morel fruiting following an application of the herbicide atrazine (Goldway et al., 2000). Thus it is advisable to consume landscape morels sparingly or not at all unless one has intimate knowledge of the history of the fruiting site including any past chemical applications or toxic contamination due to the potential for contamination of the fruiting body.

Carris et al. (2015). Four loci were used: translation elongation factor 1-α (EF1-α), the 28s ribosomal subunit (28s), RNA polymerase II largest subunit (RPB1), and RNA polymerase II second largest subunit (RPB2). Representative sequences were deposited in GenBank (accessions in caption of Fig. 1 – page 30).

Results and Conclusions

Our analysis reveals that the diversity of morel species occurring in landscape settings is likely much greater than is currently recognized. Eleven specimens of morels collected from landscape settings belonged to five different phylogenetic species of morels (Fig. 1) based on the results of our phylogenetic analysis. Seven of the specimens, collected from Salt Spring Island, British Columbia, Moscow, ID, and Pullman, WA, were identified as *M. importuna*. *Morchella importuna* was collected from different types of landscapes including landscape gravel, mulch, and a grassy lawn. Other specimens were identified as *M. brunnea*, *M. populiphila*, *M. snyderi*, and an unnamed species corresponding to phylogenetic lineage Mel-8 (Kuo et al., 2012; O'Donnell et al., 2011). Specimens of *M. brunnea* and *M. populiphila* were characterized as part of another study focusing on the occurrence of the asexual stage of morels and false morels (Carris et al., 2015), and represented the first report of *M. brunnea* and *M. populiphila* from landscape settings. *Morchella brunnea* was described by Kuo et al. (2012) as occurring under hardwoods (*Acer* and *Quercus*) and likely also occurs in non-burned conifer forests in the Pacific Northwest. The *M. brunnea* collections in this study were fruiting in landscape bark and an adjacent lawn in a residential yard in Pullman, WA (Carris et al., 2015). Nearby woody plants included species of

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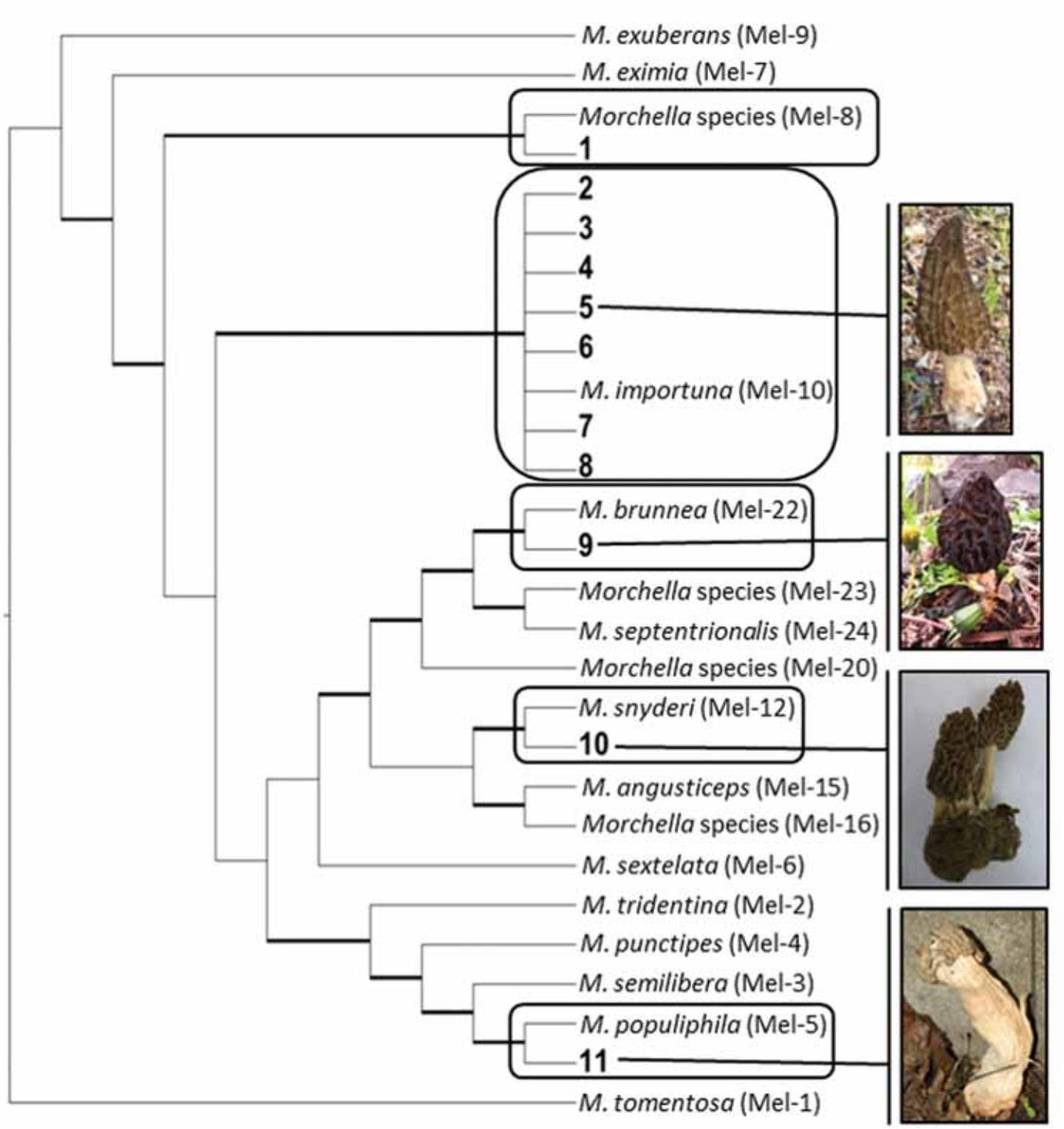

Figure 1. Cladogram describing phylogenetic relationship among various morel species from the *M. elata* clade (Kuo et al., 2012; O'Donnell et al., 2011) and landscape morels reported in this study with *M. tomentosa* as the outgroup. Five clades (circled) are represented from specimens genotyped in this study. Specimens, designated by numbers from 1 through 11, were collected from landscape settings in: 1. WSP 72734, Pullman, WA, USA (landscape bark mulch); 2. WSP 72735, Salt Spring Island, BC, Canada (landscape gravel); 3. WSP 72733, Moscow, ID, USA (landscape bark mulch); 4-5. WSP 72736 and 72737, Salt Spring Island, BC, Canada (landscape gravel); 6. WSP 72356, Pullman, WA, USA (WSU Food Science and Human Nutrition Building, landscape bark mulch). 7. WSP 72732, Pullman, WA, USA (WSU Campus Police Station, landscape bark mulch). 8. Pullman, WA, USA (WSU Compton Union Building, landscape bark mulch; specimen not available). 9. WSP 72719, Pullman, WA, USA (residential yard, landscape bark mulch). 10. WSP 72738, Pullman, WA, USA (WSU Ensminger Pavilion, landscape bark mulch). 11. WSP 72720, Pullman, WA, USA (landscape bark mulch and lawn). Photos of selected specimens are included. Bolded lines represent bootstrap values and posterior probabilities greater than 70 and 95, respectively. Unique sequences from this study were deposited in GenBank under accession numbers KP842238-KP842261. Sequences for specimen numbers 6, 9 and 11 were deposited in GenBank separately by Carris et al. (2015). Supplementary sequences were obtained from Kuo et al. (2012), O'Donnell et al. (2011) and, Taşkin et al. (2012).

Mel-8 is considered a "black morel," a group distinguished by the presence of a vallécula, where the bottom of the cap hangs slightly over the stipe (Bunyard, 2013). Ridges on this species are vertically-aligned on a conical-shaped, slightly rounded cap. It is difficult to distinguish this species from *M. brunnea* based on morphology alone.

M. importuna is considered the "typical" landscape morel as it seems to be the most common in landscape settings. Also a "black morel," this morel can be recognized by its vertically-arranged, deep pits with ladder-like, horizontally-aligned ridges.

M. brunnea is also a "black morel" and closely resembles Mel-8 and its eastern U.S. look-alike, *M. angusticeps*. The cap is nearly conical with vertically-aligned pits, however, without the ladder-like ridges of *M. importuna*

M. snyderi can be easily confused with the "yellow morel" due to its pale color when immature, however, yellow morels do not have a vallécula which is present in *M. snyderi*. This morel turns dark brown as it matures and has a characteristic stipe which becomes increasingly ridged and pocketed with age.

M. populiphila has only been recently distinguished from its look-alike, *M. semilibera* or the "half-free morel," due to its cap that overhangs significantly over the stipe without attachment. When young, the cap is pale colored, but matures dark brown to black.

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Betula, *Euonymus,* and *Thuja*. *Morchella populiphila* has been described as associated with *Populus trichocarpa* in riverbeds in western North America (Kuo et al., 2012) and has also been reported from Spain under introduced *Populus* (Richard et al., 2015). *Morchella populiphila* in the present study was collected from a residential yard and a city park in Pullman, WA (Carris et al., 2015). *Populus* is not present at either of the Pullman, WA collection sites for *M. populiphila*, indicating that this species has a broader ecological niche than is recognized in recent treatments (Kuo et al. 2012; Richard et al., 2015). *Morchella snyderi* was described as occurring in non-burned conifer forests in western North America (Kuo et al., 2012), and is one of the most common species of morels in northern Idaho, where it can be found fruiting in large numbers in logged sites (Carris et al., 2015; Pagliacci et al., 2011). The specimen of *M. snyderi* included in this study was growing in bark mulch in the Horticulture and Landscape Architecture Garden next to the Ensminger Pavilion on the WSU campus. Over 20 different fruiting bodies, all immature, were collected from this site in late April to early May, 2013. Based on morphological similarity to the specimen that was sequenced, most of these fruiting bodies were also *M. snyderi*. In addition to the WSU landscape specimens collected for this study, the only other report of *M. snyderi* from a landscape setting was from a mulched bed in the Czech Republic (Ondřej et

al., 2011). Interestingly, the unnamed species (Mel-8) was also collected from the WSU Ensminger Pavilion site later in the season, indicating that at least two different species were fruiting in the same landscape setting. The WSU Ensminger Pavilion specimen is the third time this unnamed species has been collected. The first collection was made in 1990 by Nancy Smith Weber at a post-fire forest site in Oregon (specimen NSW 6190; O'Donnell et al., 2011; Kuo et al., 2012). The second collection of Mel-8 was under an incense cedar in an old apple orchard in a northern California backyard (see "*Morchella* species Mel-8" MushroomExpert.com). Little can be concluded about the ecology of this species with so few reported findings, but the diversity of habitats from which the three known specimens were collected is intriguing. As more genetic data become available for landscape morel specimens, it will be interesting to see if the distinction between urban and forest morels continues to break down, as suggested by the diversity of species observed in this study.

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