1 **Contributions of North American endophytes to the phylogeny,**
2 **ecology, and taxonomy of Xylariaceae (Sordariomycetes,**
3 **Ascomycota)**

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Abstract

The Xylariaceae (Sordariomycetes) comprise one of the largest and most diverse families of Ascomycota, with at least 85 accepted genera and ca. 1,343 accepted species. In addition to their frequent occurrence as saprotrophs, members of the family often are found as endophytes in living tissues of phylogenetically diverse plants and lichens. Many of these endophytes remain sterile in culture, precluding identification based on morphological characters. Previous studies indicate that endophytes are highly diverse and represent many xylariaceous genera; however, phylogenetic analyses at the family level generally have not included endophytes, such that their contributions to understanding phylogenetic relationships of Xylariaceae are not well known. Here we use a multi-locus, cumulative supermatrix approach to integrate 92 putative species of fungi isolated from plants and lichens into a phylogenetic framework for Xylariaceae. Our collection spans 1,933 isolates from living and senescent tissues in five biomes across the continental United States, and here is analyzed in the context of previously published sequence data from described species and additional taxon sampling of type specimens from culture collections. We found that the majority of strains obtained in our surveys can be classified in the hypoxylid and xylaroid subfamilies, although many also were found outside of these lineages (as currently circumscribed). Many endophytes were placed in lineages previously not known for endophytism. Most endophytes appear to represent novel species, but inferences are limited by potential gaps in public databases. By linking our data, publicly available sequence data, and records of ascomata, we identify many geographically widespread, host-generalist clades capable of symbiotic associations with diverse photosynthetic partners. Concomitant with such cosmopolitan host use and distributions, many xylariaceous endophytes appear to inhabit both living and non-living plant tissues, with potentially important roles as saprotrophs. Overall, our study reveals major gaps in the availability of multi-locus datasets and metadata for this iconic family, and provides new hypotheses regarding the ecology and evolution of endophytism and other trophic modes across the family Xylariaceae.

1. Introduction

Fungi are one of the most diverse and ecologically important clades of life (Hammond, 1995; Agrios, 2005), yet only a tiny fraction of their estimated diversity has been discovered and described (i.e., < 5%; Hawksworth, 1991; Hawksworth, 2001; Mueller and Schmit, 2007). Many of the ‘missing fungi’ – species filling the gap between the number thought to exist (1.5 to 9 million species, Cannon, 1997; Hawksworth, 1991; Hawksworth, 2001; O'Brien et al., 2005) and those described to date (ca. 99,000 species; Blackwell, 2011) – are microfungi living in cryptic symbioses (Agrios, 2005; Blackwell, 2011; Hawksworth, 1991; Hyde, 2001). In particular, an enormous amount of yet-unknown diversity is thought to occur as endophytes, which inhabit apparently healthy, above-ground tissue of all major lineages of land plants (i.e., Class 3 endophytes, sensu Rodriguez et al., 2009), and as endolichenic fungi, which occur in symptomless lichen thalli in close association with the photobiont (i.e., the algal or cyanobacterial partner in lichen thalli; Arnold et al., 2009; Li et al., 2007; Suryanarayanan et al., 2005; U'Ren et al., 2012; U'Ren et al., 2014).

The majority of foliar endophytes and endolichenic fungi (hereafter, globally referred to as endophytes) are members of the Pezizomycotina (see Rodriguez et al., 2009), with particular diversity in the Dothideomycetes, Eurotiomycetes, Leotiomycetes, Pezizomycetes, and Sordariomycetes (e.g., Arnold et al., 2009; Bálint et al., 2015; Chen et al., 2015; Davey et al., 2013; Tedersoo et al., 2013; U'Ren et al., 2012; U'Ren et al., 2014; Wang et al., 2006; Zimmerman and Vitousek, 2012). These endophytes occur in ecosystems ranging from hot deserts to wet forests, to arctic tundra (e.g., Arnold et al., 2009; Davis et al., 2003; Del Olmo-Ruiz and Arnold, 2014; Gazis and Chaverri, 2010; Higgins et al., 2007; Massimo et al., 2015; Suryanarayanan et al., 2011; U'Ren et al., 2012; Zimmerman and Vitousek, 2012). Individual plants and lichen thalli can harbor phylogenetically diverse endophytes, with significant turnover across the geographic ranges of their hosts (e.g., Fisher et al., 1994; Fisher et al., 1995; Higgins et al., 2014; U'Ren et al., 2012; Vaz et al., 2014; Zimmerman and Vitousek, 2012). Endophytes in plants can play important ecological roles, mediating defense against pathogens and herbivores

and influencing host responses to abiotic stressors such as drought (Arnold et al., 2003; Arnold and Engelbrecht, 2007; Costa Pinto et al., 2000; Estrada et al., 2013; Mejía et al., 2008; Schardl et al., 2004). They are increasingly recognized as a major source of novel metabolic products for use in medicine, agriculture, and industry (Ding et al., 2009; Deyrup et al., 2007; Fan et al., 2014; Jiménez-Romero et al., 2008; Paranagama et al., 2007; Staniek et al., 2008; Strobel et al., 1997; Strobel and Long, 1998; Wu et al., 2011) and as an important but under-studied aspect of plant and lichen biology.

Studies over the last four decades have revealed an extremely high richness of xylariaceous endophytes (Xylariales, Sordariomycetes, Pezizomycotina) (e.g., Petrini and Petrini, 1985; Rogers, 2000). They inhabit the living tissues of phylogenetically diverse plants, including conifers, angiosperms, ferns, lycophytes, and bryophytes in a diverse range of biogeographic provinces (e.g., Arnold et al., 2009; Brunner and Petrini, 1992; Carroll and Carroll, 1978; Davey et al., 2014; Davis et al., 2003; Del Olmo-Ruiz and Arnold, 2014; Higgins et al., 2007; Okane et al., 2012; Petrini and Petrini, 1985; Petrini et al., 1995). They also occur frequently in taxonomically diverse lichens encompassing diverse growth forms (e.g., foliose, fruticose, crustose) and substrates (e.g., terricolous, saxicolous, epiphytic) (Arnold et al., 2009; Petrini and Petrini, 1985; Suryanarayanan et al., 2005; Wu et al., 2011). Some xylariaceous endophytes persist in leaf litter, reflecting abilities to decompose lignocellulose and the capacity of some species to directly infect decaying leaves, potentially circumventing the need for an endophytic life stage (Osono, 2002; 2005; 2006).

Many endophytic Xylariaceae remain sterile in culture or reproduce only asexually on standard media (Stadler et al., 2013), precluding identification based on teleomorphic characters such as stromata features and ascospore number (see Petrini and Petrini, 1985; Rogers, 1979a; 2000; Rogers et al., 2002). Anamorphic cultures can be classified based on conidiophore branching and the nature of conidiogenous cell proliferation (Ju and Rogers, 1996), as well as cultural characteristics such as growth rates and color (Petrini and Petrini, 1985). However, anamorphic characters alone often lack sufficient information for species-level identification (Petrini and Petrini, 1985; Stadler et al., 2013). As a result, estimates of species boundaries and

taxonomic placement of endophytes frequently are assigned on the basis of BLAST comparisons of barcode sequences (i.e., nuclear ribosomal internal transcribed spacers and 5.8S; ITS rDNA) in GenBank. This approach is often problematic due to inconsistencies in levels of interspecific ITS rDNA variation among taxonomic groups (Nilsson et al., 2008), misidentified sequences in public databases (Bridge et al., 2003; Harris, 2003; Peršoh et al., 2009; Vilgalys, 2003), and problems posed by under-representation of fungal biodiversity in public databases, such that the closest named BLASTn hit is not always the closest relative (see Gazis et al., 2012; U'Ren et al., 2009). Misassignment of unknown sequences can erroneously expand or contract the family concept over time, alter taxonomic concepts for genera and species (e.g., *Xylaria hypoxylon*; see Peršoh et al., 2009), and confound ecological inferences for newly discovered strains for which only ITS rDNA or other single-locus data are available.

As currently circumscribed, Xylariaceae comprise one of the largest and most diverse families of filamentous Ascomycota, with at least 85 accepted genera and an estimated 1,343 accepted species (Eriksson, 2006; Kirk et al., 2008; Stadler et al., 2013). Traditionally two subfamilies are recognized: Hypoxyloideae and Xylarioideae (Dennis, 1961). Currently recognized species include saprotrophs occurring in wood, litter, soil, and dung, and a few plant pathogens that cause canker diseases (e.g., *Entoleuca mammata* on *Populus*), root rots (e.g., *Xylaria mali* on *Malus*), and needle blight (e.g., *Hypoxylon herpotrichoides* on *Pseudotsuga* and *Picea*) in agricultural and natural systems (Edwards, 2003; Martin, 1967; Rogers, 1979a; 2000; Rogers and Ju, 1996; Whalley, 1985). Although several described species are close matches with endophytes in BLAST comparisons of ITS rDNA sequences, the taxonomic placement of those endophytic isolates rarely is investigated based on multi-locus phylogenetic analyses (but see Bills et al., 2012; Pažoutová et al., 2010b; Visser et al., 2009). To our knowledge, few novel species of Xylariaceae have been described solely as endophytes from anamorphic cultures (but see Worapong et al. [2001] and González et al. [2009] for description of *Muscodor* spp.).

Given the captivating ascomata morphologies (i.e., spore-bearing structures resulting from sexual reproduction) and ecological importance of *Xylaria* and related taxa, the Xylariaceae have long received attention from mycologists. A particularly rich tradition of morphological

systematics (e.g., Dennis, 1957; Hawksworth and Whalley, 1985; Ju et al., 1993; Ju and Rogers, 1996; Ju et al., 1998; Læssøe et al., 1989; Miller, 1961; Möller, 1901; Pouzar, 1985a; 1985b; Rogers, 1981; Rogers and Ju, 1996; Rogers et al., 1997a; 1997b) is increasingly complemented by chemotaxonomic (e.g., Fournier et al., 2010; Læssøe et al., 2010; 2013; Stadler and Fournier, 2006; Stadler et al., 2008; 2010) and molecular approaches (e.g., Bills et al., 2012; Hsieh et al., 2005; 2010; Jaklitsch et al., 2014; Peršoh et al., 2009; Whalley, 1996). Several genera have been updated or monographed recently (e.g., Daranagama et al., 2015; Rogers et al., 2002; Peršoh et al., 2009; Stadler et al., 2014) and NCBI contains nucleotide data for >3,000 isolates representing ca. 442 recognized species (as of August 2015). Phylogenetic analyses support monophyly of the family as presently circumscribed (Tang et al., 2009), but numerous studies suggest that many recognized genera and species may not be monophyletic (see Daranagama et al., 2015; Hsieh et al., 2005; 2010; Pažoutová et al., 2010b), and many genera require taxonomic revisions (Stadler et al., 2013). Integrating previously unknown strains into a robust phylogenetic framework for the Xylariaceae can provide insight into the taxonomic circumscription at the family and infrafamilial levels, illustrate previously unknown connections between anamorphic and teleomorphic species, and inform evolutionary relationships, host ranges, distributions, major ecological modes, and diversity of major clades, previously known taxa, newly found strains, and the family as a whole.

The goal of this study was to address the impact of a large collection of endophytic and saprotrophic fungi on the circumscription of the Xylariaceae and infrafamilial taxa. Our work takes advantage of 1,933 newly cultured isolates representing 92 putative species, which were collected from living photosynthetic tissues of angiosperms, conifers, ferns, lycophytes, bryophytes, and lichens, as well as senescent and decomposing leaves of selected woody plants in five biomes across the continental United States (U'Ren et al., 2010; 2012). Here, we place these strains in a multi-locus phylogenetic framework in conjunction with additional sequencing of type specimens from culture collections and previously published sequence data from described species. We then address the following questions: (1) Does the inclusion of newly cultured isolates alter current phylogenetic hypotheses regarding the delimitation of Xylariaceae and the relationships and circumscription of xylariaceous taxa? (2) What is the classification of these

isolates? (3) Do they represent novel species or anamorphs of previously described teleomorph species? (4) How can these cultures expand our knowledge of the host affiliations, substrate use, geographic distribution, and phylogenetic diversity of the Xylariaceae? In addressing these questions we provide an overview of currently available metadata for members of the Xylariaceae, and highlight emergent patterns regarding ecological modes across this diverse, important, and morphologically compelling family.

2. Materials and Methods

2.1. Field surveys

As part of a larger study investigating the diversity and distributions of endophytic fungi, living leaves and healthy lichen thalli were collected systematically in five sites representing distinct environmental, biological, and biogeographic regions across the continental United States (U'Ren et al., 2012). Sites were located in the Madrean Sky Island Archipelago of southeastern Arizona (AZC); the Appalachian Mountains of western North Carolina (NCH); sub-tropical scrub forest in Florida (FLA); Beringian tundra and boreal forest in the Seward Peninsula ecoregion of western Alaska (AKN); and inland, subalpine tundra in the Interior Highlands of east-central Alaska (AKE). Endophytes were cultured from surface-sterilized tissues of living, apparently healthy plants (angiosperms, conifers, lycophytes, ferns, and bryophytes) and lichens (with diverse mycobionts, substrates, and growth forms) as described in U'Ren et al. (2012). In each site fungi also were cultured concurrently from surface-sterilized tissues of senescent leaves in the canopy of selected woody plants (i.e., dead plant leaves, DP) and leaf litter (i.e., fallen leaves of the same species, FP) (U'Ren et al., 2010; U'Ren, 2011). Classifying fungi broadly as “endophytes” or “saprotrophs” based on the condition of the tissue from which they are isolated is insufficient to adequately define their ecological roles (U'Ren, 2011). However, for the purposes of this study fungal OTU isolated from living host tissues (either plant or lichen) are referred to as endophytes (even if isolates were found in non-living tissues as well), whereas fungal OTU isolated only from

non-living plant tissues (i.e., DP and/or FP) are referred to as saprotrophs. Each isolate is maintained as an axenic voucher in sterile water at the Robert L. Gilbertson Mycological Herbarium at the University of Arizona (ARIZ) (Supplemental Table 1).

2.2. Sequencing the ITS-partial LSU rDNA of field-collected strains

Overall, the field surveys described above generated 6,784 cultures, which were screened by DNA sequencing for preliminary taxonomic placement. Methods for DNA extraction, PCR amplification, DNA sequencing and sequence editing followed U'Ren et al. (2010). Briefly, DNA was extracted from each isolate using phenol:chloroform:IAA (Arnold and Lutzoni, 2007). The nuclear ribosomal internal transcribed spacers and 5.8S (i.e., ITS rDNA) were amplified by PCR with ca. 500 bp of the adjacent nuclear ribosomal large subunit (LSU rDNA) as a single fragment using primers ITS1F/LR3 or ITS5/LR3 (Gardes and Bruns, 1993; Vilgalys and Hester, 1990; White et al., 1990). Amplicons were sequenced bidirectionally with the above primers using Applied Biosystems BigDye® Terminator v3.1 cycle sequencing kits (Applied Biosystems 3730xl DNA Analyzer; Foster City, CA, USA) at the University of Arizona Genetics Core. The software applications *phred* and *phrap* (Ewing and Green, 1998; Ewing et al., 1998) were used to call bases and assemble contigs with automation provided by the ChromaSeq package in Mesquite (Maddison and Maddison, 2011; <http://mesquiteproject.org>). Base calls were verified by visual inspection of chromatograms in Sequencher v. 4.5 (Gene Codes, Ann Arbor, MI).

Sequences were assembled into groups by first generating a distance matrix in ESPRIT (Sun et al., 2009) based on pairwise Needleman-Wunsch alignments for all sequence pairs with *k*-mer distances less than 0.5, followed by clustering using the furthest neighbor algorithm in *mothur* (Schloss et al., 2009). Groups were defined by 100%, 99%, and 95% sequence similarity as a proxy for delimiting genotypes (100%, 99%) and putative species (95%) following U'Ren et al. (2009) and Liggenstoffer et al. (2010). A single representative sequence for each 95% similarity group (hereafter, operational taxonomic unit, OTU) was queried against the curated ITS rDNA sequence database at the Alaska Fungal Metagenomics Project

(<http://www.borealfungi.uaf.edu/>) using BLASTn (Altschul et al., 1990) to estimate taxonomic affiliation. Overall, 92 OTU and 245 unique genotypes (based on 95% and 100% sequence similarity, respectively) had top BLASTn hits to taxa identified as Xylariaceae. These OTU comprise a total of 1,933 isolates (Supplemental Table 1).

2.3. Multi-locus sequencing of field-collected strains

A single isolate from each 95% OTU (with the exception of one OTU with two isolates in different 99% OTU) was selected for morphological examination and multi-locus sequencing (Table 1). The resulting set of 92 OTU included 39 OTU (131 isolates) found only in living plant tissues or lichen thalli (i.e., endophytes), 44 OTU (1,780 isolates) found in both living tissues and dead or fallen leaves (here, treated as endophytes), and 9 OTU (22 isolates) found only in dead or fallen leaves (here treated as saprotrophs).

Based on previously published multi-locus studies of Hypoxyloideae (Hsieh et al., 2005) and Xylarioideae (Hsieh et al., 2010), we focused on three protein-coding genes (β -tubulin, α -actin and *RPB2*). β -tubulin and α -actin were amplified by PCR using primer pairs T1/T22 or T11/T22 (O'Donnell and Cigelnik, 1997) and ACT-512F/ ACT-783R (Carbone and Kohn, 1999), respectively. Approximately 1 kb of the gene encoding the RNA polymerase II second-largest subunit (*RPB2*) was amplified with the primer pair fRPB2-5F/ fRPB2-7cR (Liu et al., 1999). Each 25 μ l reaction contained a final concentration of 5 ng of genomic DNA, 0.6 μ M forward and reverse primers, 0.08 mg/ml Bovine serum albumin (BSA), and 1X REDTaq® ReadyMix (Sigma-Aldrich, St. Louis, MO, USA). Because the majority of samples (i.e., 63 out of 92) failed to amplify using previously published PCR protocols for *RPB2* (Liu et al., 1999) we performed a two-step touchdown PCR following U'Ren et al. (2007) for the remaining isolates. After initial denaturation at 94° C for 5 min, 30 cycles of touchdown PCR were performed (denaturation at 94° C for 1 min, annealing for 1 min with a 0.5° C/cycle decrement starting at 60° C, and an extension at 72° C for 1 min), followed by 20 cycles of regular PCR (95° C for 1 min, 45° C for 1 min, 72° C for 1 min, and a final extension step for 5 min at 72° C). After an initial β -tubulin PCR with eight isolates

revealed no amplification with previously published protocols (O'Donnell and Cigelnik, 1997), the two-step touchdown protocol was used to amplify all isolates. Negative controls, which contained all components except DNA templates, were included in parallel.

PCR products were evaluated by staining with SYBR Green I (Molecular Probes, Invitrogen, Carlsbad, CA, USA) after electrophoresis on a 1% agarose gel. When positive amplicons yielded single bands, PCR products were sequenced directly as described below. When isolates displayed multiple bands or weak amplification, PCR products were cloned using the Strataclone PCR Cloning Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions, except that one-half the recommended reagent volumes were used for each reaction. After blue/white screening, successfully transformed colonies were transferred to new plates and incubated an additional 24 h to increase colony size. Five positive clones per isolate were amplified in secondary PCR with primers M13F and M13R. Up to five amplicons per isolate were selected for sequencing. PCR products were cleaned by adding 1 µl of ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) to 20 µl of PCR product and incubating for 60 min at 65°C followed by 15 min at 85°C. Following a 1:2 dilution, PCR products (either directly from initial PCR or from secondary PCR from cloning) were sequenced and edited as described above.

Bidirectional sequences were assembled and edited as described above. Edited consensus sequences were queried against NCBI using BLASTn to estimate taxonomic placement. Protein-coding sequences were subject to a BLASTx search to determine the reading frame and the start/stop positions of each exon when applicable (i.e., β -tubulin and α -actin).

2.4. Sampling of previously described taxa

Available sequence data (as of January 2015) for ITS rDNA, LSU rDNA, β -tubulin, α -actin, and *RPB2* were downloaded from NCBI or the AFTOL database (www.aftol.org) for 293 species (representing 429 accessions) of Xylariomycetidae. These species represent families of Xylariales proposed by Smith et al. (2003) and revised by Senanayake et al. (2015), providing the basis for establishing the family boundaries of Xylariaceae: Apiosporaceae (2 species),

Cainiaceae (1 species; see Jeewon, 2002), Diatrypaceae (32 species), Graphostromataceae (1 species), Hyponectriaceae (4 species), Lopadostomaceae (1 species), Pseudomassariaceae (1 species), Xylariaceae (208 species), as well as Xylariales *incertae sedis* isolates (6 species) (Supplemental Table 2). Several putative members of Xylariales were not included due to a lack of sequence data for protein-coding genes: Coniocessiaceae (García et al., 2006), Vialaeaceae (Shoemaker et al., 2013), Melogrammataceae (see Senanayake et al., 2015), and Iodosphaeriaceae (see Senanayake et al., 2015). Families previously classified within Xylariales, but recently proposed for placement in the Amphisphaeriales, also were included (Amphisphaeriaceae [33 species] and Clypeosphaeriaceae [4 species]; Supplemental Table 2; see Senanayake et al., 2015). *Ophiostoma ulmi*, *O. piliferum*, and *O. stenoceras* were used to root the tree following Huhndorf et al. (2004) and Tang et al. (2007).

In addition, 26 putative species of Xylariaceae were obtained from the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre (Utrecht, Netherlands; Supplemental Table 2). Cultures were chosen to represent genera and species that were lacking molecular data in public databases at the time (June 2011), focusing on major clades of Xylariaceae. On receipt, cultures were immediately plated on 2% malt extract agar and grown at room temperature for 1-2 weeks. Once sufficient mycelium was present, DNA was extracted and ITS rDNA-partial LSU rDNA, β -tubulin, α -actin, and *RPB2* were PCR-amplified and sequenced as described above. Sequence data generated for this study have been deposited in GenBank under accession numbers KU683751-KU684310.

2.5. Sequence alignment and topological incongruence tests

ITS rDNA and ITS-partial LSU rDNA sequences were analyzed using Fungal ITS extractor (Nilsson et al., 2010) to create separate fasta files for ITS1, 5.8S, ITS2, and LSU rDNA. LSU rDNA sequences were aligned according to the secondary structure of *Saccharomyces cerevisiae* as described in Miadlikowska et al. (2006). 5.8S sequences were aligned with MUSCLE 3.8.31 (Edgar, 2004) using default parameters and then edited manually. Alignments

for protein-coding genes were first done at the amino acid level using MUSCLE as implemented in Mesquite (Maddison and Maddison, 2011). Aligned amino acids for β -tubulin, α -actin and *RPB2* were then used as a guide for nucleotide alignments using the “Align DNA to Protein” option (see <http://mesquitealign.wikispaces.com/Automated+Alignment>) in Mesquite. If necessary, nucleotide alignments were manually adjusted using the “Nucleotide with AA color” option in Mesquite. For LSU and *RPB2*, ambiguously aligned nucleotides (sensu Lutzoni et al., 2000) were delimited manually and excluded from subsequent analyses (Supplemental Table 3). ITS1 rDNA, ITS2 rDNA, and introns from β -tubulin were too divergent to be reliably aligned at such a broad taxonomic level; therefore, we tested the impact of excluding these regions vs. including them in analyses after recoding as non-DNA characters using Principal Coordinates Analysis (PCoA) as implemented in PICS-Ord (Lücking et al., 2011).

Preliminary maximum likelihood (ML) analyses were performed on a total of six single-locus datasets (recoded ITS1, 5.8S, and recoded ITS2 rDNA; 5.8S rDNA; LSU rDNA; *RPB2*; β -tubulin exons; and β -tubulin exons plus recoded introns) at the nucleotide level using RAxMLHPC-MPI-SSE3 version 7.7.6 (Stamatakis, 2006) on the Mobyle SNAP Workbench version 1.0.5 (Monacell and Carbone, 2014). Optimal tree and bootstrap searches were conducted with the default rapid hill-climbing algorithm for 1000 replicates with GTR substitution model (Rodríguez et al., 1990) and gamma distribution approximated with four categories in all analyses. Recoded ITS1 rDNA, ITS2 rDNA, and introns from PICS-Ord were analyzed as unordered characters with the GTR substitution model following recommendations in Lücking et al. (2011). For each protein-coding gene, subsets of partitions were defined with the program PartitionFinder v.1.1.0 (Lanfear et al., 2012), the greedy search option, and the Bayesian information criterion (BIC) for model selection.

To identify topological incongruence among the resulting single-locus trees, a reciprocal 70% ML bootstrap support criterion was applied (Mason-Gamer and Kellogg, 1996). Briefly, a conflict was considered significant if taxa in a strongly-supported monophyletic clade (i.e., with $\geq 70\%$ bootstrap value) based on one locus were strongly supported as non-monophyletic (i.e., $\geq 70\%$ bootstrap value) in another single-locus tree. No significant conflict was detected between

single-locus tree topologies with and without recoded data (i.e., β -tubulin exons vs. β -tubulin exons plus recoded introns; ITS1 rDNA, ITS2 rDNA recoded plus 5.8S rDNA vs. 5.8S rDNA only), thus the single-locus alignments including the recoded data were concatenated into a single supermatrix for phylogenetic analyses.

2.6. Phylogenetic analyses to delimit Xylariaceae and place newly cultured strains within Xylariomycetidae

The following seven subsets (obtained with PartitionFinder) were analysed with RAxML as described above to infer relationships within Xylariomycetidae: (1) 5.8S, β -tubulin first codon position, and LSU rDNA; (2) *RPB2* first codon position; (3) *RPB2* second codon position; (4) *RPB2* third codon position; (5) β -tubulin second codon position; (6) β -tubulin third codon position; and (7) recoded ITS1 rDNA, ITS2 rDNA, and β -tubulin introns. The final concatenated dataset for Xylariomycetidae containing 520 terminal taxa (including three *Ophiostoma* spp. as the outgroup) has been deposited in TreeBASE (XXXXX). Sequences for α -actin were not included in these analyses because they were not available in GenBank for Xylariomycetidae representatives other than Xylariaceae.

Once the taxonomic boundaries for Xylariaceae were estimated using this concatenated supermatrix for Xylariomycetidae (Supplemental Fig. 1), non-Xylariaceae taxa were removed from each alignment (except *Diatrype disciformis* and *Eutypa lata*, which were chosen to root subsequent trees based on the topology of the Xylariomycetidae phylogeny and the availability of multi-locus data; Supplemental Fig. 1; Supplemental Table 2). Although preliminary analyses placed *Graphostroma platystoma* (the sole species in Graphostromataceae; Barr et al., 1993) in a well-supported clade with *Biscogniauxia arima*, *B. marginata*, *B. granmo*, and *B. simplicior* (a placement that agrees with morphological similarity between anamorphs of *G. platystoma* and hypoxylid Xylariaceae), it was removed due to uncertainty regarding its affinities to Xylariaceae (see Senanayake et al., 2015) and low quality sequence data for non-ribosomal loci (see Supplemental Table 2). Additionally, isolates considered previously to be within the Xylariaceae,

but which appeared outside of the monophyletic Xylariaceae in these analyses, also were removed (i.e., *Anthostomella torosa*, *Dicyma funiculosa*, *D. pulvinata*, and 13 newly isolated OTU tentatively identified as Xylariaceae based on BLASTn; Supplemental Tables 1-2; Table 1). Because analyses were performed prior to the reclassification of *Seynesia* in the Cainaceae (see Senanayake et al., 2015), these analyses included *Seynesia erupens* as well as two endophyte OTU (clades E1-E2) that are potentially outside Xylariaceae. As described below, their resulting placement is not in conflict with recent studies (see Senanayake et al., 2015).

2.7. Phylogenetic analyses of Xylariaceae using a cumulative supermatrix approach

When taxon sampling was narrowed to focus on putative Xylariaceae, a total of 79 putative species defined at 95% ITS-partial LSU rDNA sequence similarity from our collections (representing 1,815 isolates total) remained in the analysis. The family-level focus decreased the prevalence of ambiguous regions in the *RPB2* and LSU rDNA alignments, and alignments were adjusted to gain additional phylogenetically informative characters (Supplemental Table 3). For each individual locus, ML analyses and assessment of topological incongruence were performed as described above. No significant conflict was detected between single locus tree topologies with and without recoded data, such that all recoded data were kept in the concatenated dataset. However, conflict among different loci resulted in the removal of seven taxa (FL0975, NC1612, *Nemania diffusa* AT-113, *N. aenea* JF02118, *N. serpens* AT-114, *N. chestersii* JF04024, and *Xylaria* sp. XT09003). For four additional taxa, single sequences that were in conflict with other loci were removed (*RPB2* FL0933; β -tubulin FL0016, FL0804, and *Xylaria escharoidea* 658; Supplemental Table 4). Conflicting sequences potentially represent alternative copies of β -tubulin (see Keeling et al., 2000; Landvik et al., 2001) or contaminants. After removing conflicting sequences single-locus analyses were repeated to assess congruence.

Following the assessment of congruence, the single-locus alignments were concatenated into a single supermatrix for subsequent ML analysis. The supermatrix contained 77 putative species (representing 1,778 isolates total and 78 terminal taxa) from our collections as well as

209 previously described taxa. DNA partitions for the five-locus supermatrix were analyzed in PartitionFinder using the parameters described above. The following seven subsets were specified for the RAxML analysis: (1) α -actin first codon position, α -actin second codon position, and β -tubulin second codon position; (2) third codon position for both α -actin and β -tubulin; (3) 5.8S rDNA, α -actin introns (for which 15 bp could be reliably aligned), β -tubulin first codon position, and LSU rDNA; (4) *RPB2* first codon position; (5) *RPB2* second codon position; (6) *RPB2* third codon position; and (7) recoded β -tubulin introns, α -actin introns, ITS1 rDNA and ITS2 rDNA. The final concatenated alignment containing 367 terminal taxa has been deposited in TreeBASE (XXXXXX).

Because only a subset of taxa within Xylariaceae were represented by all five loci (Table 1; Supplemental Table 2), we examined the effect of adding taxa with an increasing amount of missing data using a cumulative supermatrix approach (following Miadlikowska et al. 2006; 2014; and Gaya et al., 2012). Four individual datasets were analyzed: (1) taxa containing a minimum of four loci (i.e., taxa with 5 loci sequenced + taxa with 4 loci sequenced, i.e., hereafter refer to as 5 + 4); (2) taxa containing a minimum of three loci (i.e., 5 + 4 + 3); (3) taxa containing a minimum of two loci (i.e., 5 + 4 + 3 + 2); and (4) all taxa (5 + 4 + 3 + 2 + 1). For each dataset, the appropriate partition subsets were defined with PartitionFinder using the parameters described previously. The subsets for the first and third datasets were the same seven as used for the supermatrix containing all taxa (i.e., 5 + 4 + 3 + 2 + 1 dataset; see above). The 5 + 4 + 3 data set had an additional subset for the third codon position of β -tubulin (Supplemental Table 5).

ML analyses for all four datasets were conducted with RAxML as described above. Majority-rule consensus trees (70%) were built in Mesquite based on sets of 1,000 bootstrap trees generated with RAxML for the four concatenated datasets. The Mesquite module Hypha (Oliver et al., 2013; see also Miadlikowska et al., 2014) was used to integrate support values derived from all applicable consensus trees onto each internode of the best tree derived from the complete concatenated dataset (i.e., 5 + 4 + 3 + 2 + 1) (Fig. 1). No significant conflict was detected among these trees except at the very tip of the H1 clade (Fig. 1).

Phylogenetic diversity (i.e., the sum of all the edge lengths in the subtree given by the tip subset; PD) of Xylariaceae taxa was calculated in R (R Core Team) with the package caper (Orme et al., 2013) and the topology generated from ML analysis of the 5 + 4 + 3 + 2 + 1 supermatrix. To assess whether newly collected isolates significantly increased the phylogenetic diversity of the Xylariaceae, PD was calculated 1,000 times for subsets of 286 taxa (77 newly collected OTU plus a random selection of 209 previously named Xylariaceae taxa), which is equal to the total number of previously named Xylariaceae taxa in the tree. The observed PD of all previously named Xylariaceae taxa was compared to this distribution to generate a distribution-independent p-value (Supplemental Fig. 2).

2.8. Comparison of newly collected strains with known taxa

To assess the potential novelty of newly cultured isolates when the topology of the tree alone is inconclusive, we compared ITS rDNA sequences of taxa present in the Xylariaceae tree (when available) to ITS rDNA sequence data for 205 Xylariaceae taxa identified to species but not represented in the multi-locus dataset due to lack of sequences for non-ITS rDNA loci in NCBI (Supplemental Table 6). Sequences were clustered into OTU at 95%, 97%, 99% and 100% using ESPRIT and mothur following methods described above (see also U'Ren et al. 2012 for methods). These data were used to assess the number of cases in which a newly cultured taxon was found within the same 95% OTU as a named taxon (see Table 1; Fig. 2).

2.9. Examining host breadth, substrate diversity, and geographic distribution of Xylariaceae

We next identified taxa from previously published studies that are closely related to taxa in our final data set. Most Xylariaceae are not represented by multiple loci in GenBank; instead, they are represented (when present) by ITS rDNA sequences. We accessed this larger sampling to address questions with regard to host breadth, substrate diversity, and geographic distribution across the multi-locus phylogenetic framework developed here.

ITS rDNA sequences for newly cultured isolates from our continental surveys, as well as reference taxa, were queried against NCBI's nr database using an e-value cutoff of 1×10^{-3} . The output was filtered to remove self hits, accessions from U'Ren et al. (2010; 2012), and hits with percent identity <99%. If the same accession was a hit for multiple taxa, the hit with the highest bit score was selected and the duplicate removed. A small fraction of BLASTn hits representing sequences from uncultured isolates (i.e., clones or next-generation sequences) also were excluded ($n = 24$; 1.9% of filtered hits).

From the list of filtered accession numbers, the geographic origin, host lineage (e.g., Angiosperm), and substrate information (e.g., surface sterilized leaf) for each isolate were extracted from the respective metadata in GenBank (Supplemental Tables 7-8) and parsed using custom scripts. In the cases of missing metadata, information was gathered (when possible) from manuscripts in which the sequences were published. Information on geographic distribution, host, and substrate for reference taxa also was collected from published species descriptions and online resources (Supplemental Table 9).

To visualize metadata in a phylogenetic framework, information for each terminal taxon was classified into broadly defined categories (Fig. 2). Categories for provenance included (1) U.S., Canada; (2) Mexico, South/Central America, Caribbean; (3) Europe, western Russia; (4) Asia, eastern Russia; and (5) "Other" (e.g., Hawaii, Australia, New Zealand, Papua New Guinea, Africa, the Middle East, and Antarctica, grouped as "other" due to a paucity of metadata from those sites). Isolates from the Hawaiian Islands were included in "other" rather than U.S./Canada due to their geographical isolation from the continental U.S. Categories for host breadth included (1) angiosperm; (2) "gymnosperm" (i.e., Pinophyta and *Ginkgo biloba*); (3) spore-bearing vascular plant (i.e., lycophytes and ferns); (4) bryophyte (i.e., mosses and liverworts) and (5) lichen (LT). Substrate categories included (1) living plant leaves or non-woody stems (LP); (2) dead plant leaves in canopy (DP); (3) fallen plant leaves in leaf litter (FP); (4) wood/bark; (5) root; (6) seed; (7) soil; (8) insect-associated; and (9) fallen fruits/inflorescences (Fig. 2). Data for each isolate were added to the phylogenetic tree using a custom R script.

2.10. Macromorphological characterization of newly collected strains

Newly collected isolates representing each putative species were subcultured from water vouchers onto 2% MEA (20 g/L of malt extract [Amresco, Solon, OH, USA] and 15 g/L of agar [Fisher Scientific, Pittsburgh, PA, USA]) and grown at room temperature under ambient light conditions. To verify the identity of each isolate prior to macromorphological characterization, DNA was extracted from each culture using the RedExtract-N-Amp Plant Kit (Sigma-Aldrich) following a modified protocol. Under sterile conditions a small piece of mycelium was placed in a 1.5 ml tube with 100 µl of extraction buffer and 100 µl of 0.5 mm zirconium oxide beads (Next Advance, Averill Park, NY, USA). After bead-beating for 1 min, the mycelium was incubated for 10 min at 95°C, after which 100 µl of dilution solution was added and the solution was vortexed briefly. DNA was stored at -20°C until used for PCR. The ITS-partial LSU rDNA was amplified by PCR with the primer pair ITS1F/LR3 using REExtract-N-Amp PCR Ready Mix (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's recommendations and the PCR protocol described in Arnold et al. (2007). PCR products were visualized and sequenced as described above. Sequences were verified as 100% identical to expected sequences for each strain. After verification, a 5 mm wide plug of each culture was transferred to 2% oatmeal agar (OA) plates to induce sporulation (Ju et al., 2005). Cultures were grown under ambient light/dark condition at room temperature (ca. 21.5°C) for up to six months before colony morphology was photographed (Supplemental Fig. 3).

3. Results and Discussion

In addition to well-known saprotrophs, previous studies have revealed numerous endophytic Xylariaceae in biomes ranging from high latitudes to the tropics (e.g., Arnold et al., 2009; Brunner and Petrini, 1992; Carroll and Carroll, 1978; Davey et al., 2014; Davis et al., 2003; Del Olmo-Ruiz and Arnold 2014; Higgins et al., 2007; Okane et al., 2012; Osono 2006; Petrini and Petrini, 1985; Petrini et al., 1995). Although tropical Xylariaceae occurring in living and dead plants and lichens

are considered to be particularly diverse, our surveys captured many species of xylariaceous fungi in subtropical, temperate, and boreal hosts (U'Ren, 2011; U'Ren et al., 2012). Incorporating this large collection of plant- and lichen-associated strains from diverse sites in the continental United States into multi-locus phylogenetic analyses with previously described taxa significantly increases the known phylogenetic diversity of Xylariaceae and provides an enriched perspective on relationships proximate to, and within, this ecologically diverse family (Fig. 1, Fig. 2, Supplemental Fig. 1, Supplemental Fig. 2).

3.1. Phylogenetic delimitation of Xylariaceae within Xylariomycetidae

Most taxa currently recognized as Xylariaceae formed a single clade in our single-locus (phylogenies not shown) and multi-locus analyses (Supplemental Fig. 1). However, several taxa previously described as Xylariaceae (*Anthostomella torosa* AFTOL-ID 732, *Dicyma funiculosa*, and *D. pulvinata*; Ju et al., 1993; Kohlmeyer and Volkmann-Kohlmeyer, 2002; Peláez et al., 2008; Stchigel and Guarro, 1998; Udagawa et al., 1994) appeared outside of this family in single-locus and four-locus analyses (Supplemental Fig. 1). Although placement of these taxa is uncertain due to low support, both *Dicyma* and *Anthostomella* are known to be problematic with regard to phylogenetic placement (Peláez et al., 2008; also see Daranagama et al., 2015).

Xylariaceae and Diatrypaceae have been proposed previously as sister families due to shared morphological features (Parguey-Leduc, 1972; Schrantz, 1960; Rogers, 1979a). However, a recent analysis suggests Xylariaceae may be more closely related to Cainiaceae (Senanayake et al., 2015; see also Maharachchikumbura et al., 2015). In our phylogenetic analysis of the subclass Xylariomycetidae the Cainaceae (represented here by *Seynesia erumpens*, was more closely related to Xylariaceae than the Diatrypaceae (with moderate support, Supplemental Fig. 1). When members of Diatrypaceae were used as the outgroup in our analysis focusing on Xylariaceae, *Seynesia erumpens* was nested within the ingroup but outside known Xylariaceae (Fig. 1). *Seynesia* was classified previously in Amphisphaeriaceae (Eriksson and Hawksworth, 1991a; 1993), but was moved to Xylariaceae based on ascospore morphology (see Barr, 1990;

Eriksson and Hawksworth, 1991b; Hyde, 1995). A recent phylogenetic analysis based on ITS + LSU rDNA placed the genus in Cainiaceae (Senanayake et al., 2015). Our analysis is not in conflict with that placement of *Seynesia*, but uncertainty regarding its family-level placement precludes confident delimitation of the Xylariaceae and family-level placement of the endophyte clades E1 and E2 (Fig. 1, Fig. 2). In future work, multi-locus data for members of the Cainiaceae, Lopadostomaceae, and Coniocessiaceae will be important to clarify the precise delimitation of Xylariaceae.

More generally, the placement of many taxa within Xylariomycetidae was challenging due to low support values at many deep internodes. This speaks to a general challenge in the systematics and taxonomy of the subclass and its member families (e.g., Senanayake et al., 2015), leading us to examine the contributions of the loci included in phylogenetic analyses. The final Xylariomycetidae dataset consisted of four loci (ITS rDNA, LSU rDNA, *RPB2*, and β -tubulin and contained a total of 4,700 characters (Supplemental Table 3). In agreement with previous studies of other Ascomycota (e.g., Miadlikowska et al., 2014), alignments of protein coding genes yielded a greater proportion of alignable nucleotides than did the ribosomal genes (Supplemental Table 3). Although the alignment of LSU rDNA was slightly longer than that for *RPB2* (1,272 bp vs. 999 bp, respectively), 97% of nucleotides were unambiguously aligned for *RPB2*, whereas nearly half (46%) of nucleotides in the LSU rDNA alignment could not be aligned unequivocally due largely to the presence of introns (Supplemental Table 3). For the β -tubulin alignment, only exons could be aligned with confidence, but exons contain limited variation (618 distinct alignment patterns). Thus, introns for β -tubulin were recoded using PICS-Ord (Lücking et al., 2011), yielding an alignment with 1,664 distinct alignment patterns. No significant conflict was observed between β -tubulin gene trees with and without the recoded characters, and the percent of nodes with bootstrap support $\geq 70\%$ increased from 38.8% to 60.9%. A similar strategy was used to recode ITS1 rDNA and ITS2 rDNA, as these hypervariable regions could not be aligned reliably across such distantly related taxa (see Bruns, 2001). Inclusion of recoded ITS rDNA increased the number of alignment patterns for this region from 58 to 305, and the number of well-supported nodes increased from 1.5% to 25.4% in the single locus tree. Accordingly,

581 additional sequences of phylogenetically informative loci from extended taxon sampling will be
582 necessary to clarify the ordinal- and family-level relationships within the Xylariomycetidae.

583
584 *3.2. Placement of several newly cultured isolates outside Xylariaceae illustrates conflicts with*
585 *BLASTn results*

586
587 Overall, 13 of 92 putative species from our surveys were tentatively identified as Xylariaceae
588 based on BLASTn hits for ITS-partial LSU rDNA, but were placed outside of the Xylariaceae in
589 our four-locus analysis (Table 1, Supplemental Fig. 1). These 13 OTU represent endophytes
590 (nine OTU) and saprotrophs (four OTU) and comprise 118 isolates overall (Table 1;
591 Supplemental Table 1). They were placed in clades containing species from four different families
592 of Xylariomycetidae or in clades containing no closely related reference taxa; however, their
593 phylogenetic placements are inconclusive due to low bootstrap support and insufficient taxon
594 sampling (Supplemental Fig. 1).

595 Discrepancies between BLASTn and phylogenetic analyses could occur because of (1) a
596 lack of closely related isolates in GenBank (especially problematic when analyses were restricted
597 only to named sequences), (2) high sequence similarity to species previously thought to be in the
598 Xylariaceae but of uncertain placement under current systematics frameworks (i.e., top BLASTn
599 hit to *Creosphaeria sassafras*; see also Supplemental Fig. 1), or (3) misidentified sequences in
600 GenBank (Peršoh et al., 2009; Vilgalys, 2003). Examination of BLAST results illustrates that
601 seven of the 13 OTU had top BLASTn hits to *Anthostomella conorum* CBS 119333 (EU552099).
602 The remaining five OTU had top BLASTn hits to unknown fungi with the closest named taxa in
603 the Xylariaceae. These results reiterate that assigning taxonomy (even at the family level) for
604 unknown isolates based solely on the top ITS BLASTn hit can be problematic (see also Stadler et
605 al., 2013; Gazis et al., 2012; U'Ren et al., 2009; but see Kõljalg et al., 2013 regarding the UNITE
606 ITS rDNA database).

607
608 *3.3. Subfamily structure within Xylariaceae*

609

610 We used five loci (ITS rDNA, LSU rDNA, *RPB2*, β -tubulin, and α -actin) to examine the
611 relationships of 298 taxa classified as Xylariaceae (>200 species) from 24 genera representing
612 two recognized subfamilies (Hypoxyloideae and Xylarioideae; Supplemental Table 1), in
613 conjunction with 79 potentially novel species representing 1,815 isolates of endophytic and
614 saprotrophic Xylariaceae from sites across the continental United States (Table 1; Fig. 1, Fig. 2).
615 Two putative species from our surveys were removed from final analyses due to conflict among
616 loci, resulting in the inclusion of 77 putative species (OTU) from our collections.

617 Genera within the Xylariaceae generally are organized into the subfamilies based on
618 geniculosporium- or nodulisporium-like conidiophores in the anamorphic state (Ju and Rogers,
619 1996). However, the family currently contains numerous genera of uncertain placement due to
620 anamorphs that differ from those above (e.g., libertella-like conidiophores) or have unknown
621 conidial states (Ju and Rogers, 1996; Stadler et al., 2013). Previous analyses of the Xylariaceae
622 focused only on select taxa (e.g., Peršoh et al., 2009; Tang et al., 2009), were based on single
623 loci (e.g., ITS rDNA; Peláez et al., 2008; Sánchez-Ballesteros et al., 2000), or were restricted to
624 one subfamily (Hypoxyloideae, Hsieh et al., 2005; Xylarioideae, Hsieh et al., 2010), such that the
625 phylogenetic relationships of the two subfamilies and the placement of numerous genera *incertae*
626 *sedis* could not be addressed.

627 Although the clades containing currently recognized Hypoxyloideae and Xylarioideae
628 (sensu Stadler et al., 2013) were highly supported in our analyses, the two subfamilies were not
629 recovered here as sister clades (Fig. 1). Instead, our analyses suggest that the Xylariaceae may
630 be divided into three main lineages: (1) *Hypoxylon*, *Daldinia*, *Annulohypoxylon*, and closely
631 related genera; (2) a clade comprised of *Durotheca* and *Biscogniauxia* (*Biscogniauxia*, *Camillea*,
632 and *Obolarina*); and (3) Xylarioideae and related taxa. However, additional data is needed to
633 confirm these relationships with high support values. Our analysis places four endophyte-only
634 clades (E4-E7) as a grade preceding the origin of the Xylarioideae as currently delimited (see 3.5,
635 below; see also Fig. 1). These clades contained 12 of 77 putative species of Xylariaceae from our
636 collections (Fig. 1). Overall, early divergences of endophyte clades associated with each

subfamily and the Xylariaceae as a whole suggest an early origin of endophytic and/or endolichenic fungi associated with diverse photosynthetic partners.

3.4. Phylogenetic perspectives on Hypoxyloideae

Overall, 28 of 77 putative species from our collection (i.e., 26 endophytic and two saprotrophic OTU) were placed within the Hypoxyloideae in our multi-locus analyses, or in affiliation with *Whalleya*, here treated as within Hypoxyloideae (see below). These strains were placed in all genera of Xylariaceae included in the analysis with the exception of *Durotheca* and *Obolarina*.

Results of our analyses are largely congruent with the study of Hypoxyloideae by Ju et al. (2007). However, in our topology *Whalleya microplaca* was placed in a clade outside of species currently classified as Hypoxyloideae, whereas Ju et al. (2007) suggested a close relationship of *Whalleya* with species of *Biscogniauxia* and *Theissenia* (renamed *Durotheca*; see Læssøe et al., 2013). Developmentally, *Whalleya microplaca* resembles *Biscogniauxia* (i.e., the presence of bipartite stromata), but stromata and anamorphs are morphologically similar to diatrypaceous fungi (e.g., anamorphic isolates of *Whalleya* have libertella-like conidiogeneous structures; Rogers et al., 1997b; Stadler et al., 2014; see also Glawe and Rogers, 1986). Our analyses placed three endophyte OTU with *W. microplaca* (clade E3, Fig. 1) and placed E3 as sister to the known Hypoxyloideae, with strong support from two bootstrap analyses. Pending morphological examination, we suggest that the Hypoxyloideae may be expanded to include clade E3 (Fig. 1).

Within Hypoxyloideae, Hsieh et al. (2005) recognized three major clades based on ML and Bayesian analyses of β -tubulin and α -actin genes: the *Biscogniauxia* clade, the *Annulohypoxylon* clade, and the *Hypoxylon/Daldinia* clade. Our analyses recovered a well-supported clade containing all of the *Hypoxylon*, *Daldinia*, and *Annulohypoxylon* taxa, as well as an isolate of *Rostrophypoxylon terebratum* (see Fournier et al., 2010 for a description of the genus and its paraphyly) (Fig. 1). Within this clade, *Daldinia* and *Annulohypoxylon* form well supported clades in our analyses (Fig. 1). However, species of *Hypoxylon* were found in multiple clades and

relationships among the previously defined subclades H1, H2, and H3 (sensu Hsieh et al., 2005) were not confirmed (Fig. 1).

Additionally, the position of *Biscogniauxia* as sister to the clade containing *Hypoxylon*, *Annulohypoxylon*, and *Daldinia* (see Hsieh et al., 2005; see also Læssøe et al., 2013) was not recovered here. Our topology suggests that *Biscogniauxia* and *Durotheca* (see Læssøe et al., 2013 for genus description) are sister to one another, but the relationship is not well supported and the placement of this two-genus clade relative to other genera in the Hypoxylidae is uncertain in our analysis. A sister relationship between these genera would agree with results of high performance liquid chromatography (HPLC) illustrating that the chemical profiles of *Biscogniauxia* and *Durotheca* are closer to each other than to *Hypoxylon* and *Daldinia* (Læssøe et al., 2013). In turn, the highly supported *Biscogniauxia* clade contained isolates of *Camillea tinctor* and *Obolarina dryophila* (see Pažoutová et al., 2010b for discussion of *Biscogniauxia* paraphyly).

3.5. Phylogenetic perspectives on Xylariidae

Overall, 34 of 77 putative species from our collection (i.e., 32 endophytic and two saprotrophic OTU) were placed within the Xylariidae as currently circumscribed. Although endophyte OTU were found within all major lineages of the subfamily, they were most abundant in the “NR” and “HY” clades (sensu Hsieh et al., 2010; Figs. 1, and 2D).

Hsieh et al. (2010) grouped members of the Xylariidae into four major clades: (1) the clade containing *Xylaria* spp. associated with termite nests (i.e., subgenus *Pseudoxylaria*; TE); (2) the clade containing *Xylaria hypoxylon* and closely related species (HY); (3) the clade containing *Nemania*, *Rosellinia*, *Entoleuca*, and *Euepixylon* (NR); and (4) the clade containing *Xylaria polymorpha* and closely related species (PO). Our analyses strongly support the monophyly of these clades and relationships among them, with the exception of the node encompassing all four clades, which was recovered in all four analyses but never with support values $\geq 70\%$ (Fig. 1). Overall, relationships among terminal branches were highly similar to those reported for

described species, and our analyses confirm the early divergence of the *Poronia-Podosordaria* clade within the Xylarioideae (see Hsieh et al., 2010).

Our results further suggest a potential expansion of the current circumscription of Xylarioideae. Clades E4-E7, which subtend the currently recognized subfamily, include 12 endophyte OTU as well as an *Anthostomella* sp. from Puerto Rico (Fig. 1, Fig. 2C). The phylogenetic placement of two of these three lineages is well supported in our analyses. Two OTU have highly similar ITS rDNA sequences to *Anthostomella* spp. that were not represented in the tree due to lack of multi-locus data (NC1622 with *Anthostomella sepebilis* strain F-160, 797; AZ1047 with *Anthostomella pinea* CBS 128205) (Table 1, Fig. 2C). However, FL1105, which is placed among isolates with hits to *Anthostomella*, has >95% ITS rDNA sequence identity to *Muscodor vitigenus* in NCBI (Table 1, Fig. 2C). The genus *Muscodor* initially was proposed to include an endophytic fungus that produces antimicrobial volatiles (Worapong et al., 2001), but its taxonomic placement in the Xylariaceae was uncertain (see Stadler et al., 2013). Placement of FL1105 in clade E5 and the monophyly of this clade is highly supported in our Xylariaceae tree (Figs. 1, 2C). Overall, our results suggest that *Anthostomella* is polyphyletic based on the positions of AK1471, FL1651, AZ1047, and NC1622, which are placed in different clades despite high sequence similarity to previously described species of *Anthostomella* (Table 1; Fig. 1; also see Lu et al., 2000; Daranagama et al., 2015). Based on the strong phylogenetic support reported here (Fig. 1, Fig. 2C) and pending morphological examination, we suggest that the Xylarioideae may be expanded to include clades E5-E7.

3.6. Limitations of current data sets

As noted in previous studies, ribosomal genes alone do not provide enough phylogenetic information to confidently resolve infrafamilial relationships in Xylariomycetidae (Duong et al., 2004) or the evolutionary history among genera of Xylariaceae (Peláez et al., 2008; Tang et al., 2009). In these circumstances, protein-coding genes such as *RPB2* can provide additional, phylogenetic signal that result in more resolved phylogenies of Xylariaceae with higher statistical

support for clades (Tang et al., 2007; see also Reeb et al., 2004). Other protein-coding genes, such as β -tubulin and α -actin, also have been used to reconstruct evolutionary relationships for *Hypoxylon* and closely related genera of Xylariaceae (Hsieh et al., 2005). However, in the present study we found that the protein coding genes β -tubulin and *RPB2* were insufficient to resolve infrafamilial relationships in the Xylariomycetidae even when combined with ribosomal loci (ITS rDNA, 5.8S rDNA, LSU rDNA). Analyses of the Xylariaceae using five loci (β -tubulin, α -actin, *RPB2*, ITS plus 5.8S rDNA, and LSU rDNA) also failed to provide high statistical support for many clades within the family.

ITS1 and ITS2 rDNA and introns of β -tubulin and α -actin were too divergent to be aligned unambiguously at the ordinal or familial level. However, we used a non-alignment based method (PICS-ord; Lücking et al., 2011) to extract phylogenetic information from these regions that were excluded from the alignments subjected to phylogenetic searches. This method recovered a substantial amount phylogenetic signal and greatly reduced phylogenetic uncertainty.

Overall, our results indicate that resolving evolutionary relationships in the Xylariaceae and Xylariomycetidae with high confidence will require sequence data from additional protein-coding genes that can be aligned unambiguously at broad taxonomic levels. Data from the gene for RNA polymerase II largest subunit (*RPB1*), the gene for the minichromosome maintenance complex component 7 (*MCM7*) (Chen et al., 2015; Miadlikowska et al., 2014), and/or additional new molecular markers derived from the AFToL 2 project (aftol.org) are likely to be useful. Our results also argue for increased taxon sampling of both endophytes and morphologically delimited species not currently represented in public sequence databases.

3.7. Phylogenetic perspectives on the potential novelty of newly collected strains

Putatively novel species of Xylariaceae isolated from plants and lichens across the continental United States were frequently placed in previously described, major clades of Xylariaceae in both subfamilies, including clades containing well-known plant pathogens (i.e., *Biscogniauxia*, *Kretzschmaria*), saprotrophs (i.e., *Xylaria*), and clades thought to be specific to insects or animal

748 dung (i.e., *Pseudoxylaria* clade and *Poronia* + *Podosordaria* clade, respectively; see Krug et al.,
749 2004; Hsieh et al., 2010; Rogers et al., 2005; Visser et al., 2009; Figs. 1 and 2C).

750 Although we recovered endophyte-only clades distinct from known or previously
751 sequenced isolates (e.g., E1, 2, 4, 5, 7, 8, and 9), each of which included one or more putative
752 species, numerous OTU were resolved as closely related to previously described taxa. For
753 example, nine endophyte and saprotroph OTU were placed within a well-supported monophyletic
754 clade with multiple representatives of representative species (Fig. 1, Fig. 2; FL1408 within the
755 *Daldinia eschscholzii* clade; AK1016, AZ0526, AK0128, AK0995 within the *D. loculata* clade;
756 FL1170 within the *Hypoxylon rubiginosum* clade; AZ0703 within the *Biscogniauxia mediterranea*
757 clade; AK0226 within the *Nemania serpens* clade; and NC1011 within the *Xylaria cubensis*
758 clade).

759 However, in cases where endophytic fungi are sister to a single representative of a
760 described species, the topology of the tree alone does not help us determine whether unknown
761 isolates represent the asexual or anamorphic life stage of a described teleomorphic species or a
762 potentially novel closely related species. To address this uncertainty we analyzed the similarity of
763 our isolates to described species in the tree using ITS rDNA clustering. Despite the fact that
764 undescribed isolates are often found in the same clade or are sister to described species, only 14
765 of 77 Xylariaceae OTU isolated in our surveys (i.e., 18.2%) were part of the same 95% OTU as a
766 described species present in the tree (Fig. 2).

767 We further analyzed the ITS rDNA similarity of endophytic and saprotrophic fungi with
768 205 named Xylariaceae taxa ($n = 85$ species) represented in GenBank by ITS rDNA sequences,
769 but not by β -tubulin, α -actin, *RPB2*, or LSU rDNA (and thus not included in the phylogenetic
770 analyses; Supplemental Table 6). This information, presented within a phylogenetic framework,
771 accesses a large pool of publicly available sequences that exceeds the taxonomic representation
772 available based on other loci or multiple loci. Based on ITS rDNA OTU clustering at 95%
773 similarity, 10 additional OTU found in the multi-locus analysis are highly similar to named
774 sequences in NCBI (see Table 1). Two additional OTU also are similar to sequences in NCBI
775 based on ITS rDNA clustering, but were removed from the concatenated supermatrix due to

conflict (Table 1). Combining ITS rDNA similarity in a phylogenetic framework identified several GenBank sequences with taxonomic names in apparent conflict with tree topology (e.g., *Xylaria mellissii* F-048,697 found within *Nemania* + *Rosellinia* clade, Fig. 2D; also see above for discussion of *Anthostomella* spp). Only five of the 12 taxa whose ITS rDNA sequences match our isolates are vouchered in easily accessed culture collections (e.g., Centraalbureau voor Schimmelcultures [CBS], American Type Culture Collection [ATTC], Agricultural Research Service Culture Collection [NRRL]) (Table 1; Supplemental Table 6), thus precluding additional morphological and molecular characterization.

Overall, 44 of 79 (55.7%) xylariaceous species collected in our surveys (representing 42 endophyte and two saprotroph OTU) appear to lack closely related, described species (Table 1; Fig. 2). Of these apparently novel OTU, 34 also lacked closely related BLASTn hits ($\geq 99\%$ ID) to other unnamed fungi in GenBank (Fig. 2). Importantly, these results are based only on the species diversity represented in public databases, and thus do not assess novelty with respect to those species of Xylariaceae known only from their teleomorphs or from drawings, or otherwise not represented in public databases (discussed by Stadler et al., 2013).

Such conclusions are dependent on a defined level of ITS rDNA sequence similarity; however, the degree of intraspecific ITS rDNA variability differs among taxonomic groups (see Nilsson et al., 2008). For five *Xylaria* spp., U'Ren et al. (2009) reported low intraspecific variation in ITS rDNA ($1.43\% \pm 2.94\%$), and variation between sister taxa averaged $4.18\% \pm 2.18\%$. However, Stadler et al. (2013) noted that different morphological species can have identical ITS rDNA sequences (e.g., *Daldinia concentrica* and *D. steglichii*). Across both subfamilies, we found 12 cases where different morphological species shared the same 95% OTU designation (e.g., *Xylaria plebeja*, *X. luteostromata* var. *macrospora*, and *X. intracolorata* designated OTU 265; Fig. 2). This appears more common for species of Xylarioideae than Hypoxyloideae (Fig. 2). Accordingly, endophyte isolates putatively identified as previously described species may in fact represent novel species. Indeed, we found cases where a comparison of the culture morphology on oatmeal agar for described species (when available) differed from our observations for endophytic isolates. For example, *Hypoxyylon submonticulosum* (Ju and Rogers, 1996) is

described as a fast-growing isolate with pale-mouse grey, cinnamon, to grayish sepia color, with sporulating regions scattered over the colony surface. However, an endophyte representing the same OTU (NC0708) remained as sterile, white mycelia after six months in culture on the same media (Supplemental Fig. 3).

Conversely, we found seven cases where isolates of the same putative species occurred in different 95% OTU (*Xylaria cubensis*, *X. curta*, *Hypoxyton dieckmannii*, *H. crocopeplum*, *H. fendleri*, *H. haematostroma*, and *Nemania serpens*). Assuming these isolates are correctly identified and represent a single species rather than a species complex (e.g., as for *Fusarium solani*; O'Donnell, 2000), we may overestimate the novelty of unknown fungi. For example, ITS-partial LSU rDNA clustering of isolates collected in our surveys identified multiple endophyte OTU at 95% sequence similarity (i.e., AK0995, AK0128, AZ0526, AK0222, and AK1016). However, representatives of these isolates were placed within a well-supported clade containing two isolates of *Daldinia loculata*, suggesting that these OTU are all *D. loculata* rather than multiple species (Fig. 2). Interestingly, these isolates represent different macromorphologies on 2% MEA and OA and will need additional morphological characterization (Supplemental Fig. 3). Thus, incorporating representative isolates into a multi-locus phylogenetic framework is only the first step in assessing the potential novelty of endophytic and saprotrophic fungi.

3.8. Metadata availability, host breadth, substrate diversity, and geographic distribution of Xylariaceae

Many Xylariaceae species have broad distributions in forests across both the Northern and Southern Hemispheres (e.g., *Daldinia* spp. Stadler et al., 2014), whereas other species appear to be restricted geographically (e.g., *Xylotumulus gibbispurus* endemic to Hawai'i; Rogers and Ju, 2012). However, distribution data based on ascomata are likely to be incomplete due to the fact that Xylariaceae species typically only form ascomata on a "preferred" host (e.g., *Obolarina dryophila* on *Quercus*; Pažoutová et al., 2010b), despite the potential to live asymptomatically on a wide diversity of plant species and substrates (e.g., Petrini and Petrini, 1985). Additionally,

species of Xylariaceae reported to have cosmopolitan distributions may potentially represent complexes of cryptic species revealed only by detailed morphological, chemical, or molecular analyses (e.g., *Daldinia eschscholzii*; see Stadler et al., 2004). Therefore, incorporating publicly available sequence data (many representing cryptic microfungi, including endophytes) coupled with records of ascomata can provide further information to estimate the geographic distribution, host breadth, and substrate diversity of xylariaceous species.

Our sampling of endophytes from diverse plant and lichen species in five sites across the continental United States emphasizes that lichen thalli are a very common habitat for Xylariaceae (see also Arnold et al., 2009). The interiors of apparently healthy lichen thalli yielded 1,259 isolates representing 67 putative species (84.8% of the 79 xylariaceous OTU from our surveys considered here; see Table 1). These fungi were isolated from 46 lichen species representing 10 major lineages of mycobionts (7 orders and 3 families *incertae sedis* in Lecanoromycetes) (Supplemental Table 1). Although the highly diverse nature of xylariaceous endophytes from angiosperms has been previously reported, especially in tropical forests (Bayman et al., 1998; Govinda Rajulu et al., 2013; Linnakoski et al., 2012; Okane et al., 2008; Whalley, 1996), our data illustrate that in temperate and boreal communities, Xylariaceae are especially diverse in conifers, lycophytes, and bryophytes (Supplemental Table 2; see also Davis et al., 2003).

Although these hosts harbored a high diversity of endophytes, we observed no clear phylogenetic pattern with regard to host phylogeny (Fig. 2). The vast majority of endophytic Xylariaceae appear to be host generalists: 91 of 124 terminal taxa (73.4%) capable of living endophytically (i.e., reported at least once from living leaves, lichen thalli, or asymptomatic inner bark, cortex, sapwood, or branches of a living host) occurred on more than one host lineage (e.g., angiosperm, “gymnosperm”, spore-bearing vascular plant, bryophyte, or lichen; Fig. 2). There was no apparent clade or species-level specificity with regard to lichen photobiont or mycobiont (e.g., OTU found in cyanolichens also occurred in lichens with green algal symbionts; Supplemental Table 1). At the community level, previous work suggested a unique connection among fungi occurring in lichens and bryophytes (U'Ren et al., 2010; 2012), but 77.8% of 27 OTU cultured in our surveys from both lichens and bryophytes also occurred in living tissues of

vascular plant hosts. Thus, non-xylariaceous taxa seem to account for the patterns observed by U'Ren et al. (2010; 2012).

The majority of terminal taxa in the tree with an endophytic life stage also were recovered from senescent leaves or decomposing leaves, wood, bark, fruits, or flowers (74.2%; $n = 92$ of 124 terminal taxa; Fig. 2), suggesting that for many species endophytism is only one stage of a complex lifecycle that can involve interactions with diverse host lineages. For example, early-diverging endophyte OTU (i.e., FL0915, FL2044, and FL0641) were cultured from living tissues of both lichens and angiosperms, as well as dead leaves of a conifer host (Table 1; Fig. 2). In contrast, only 33 of 241 (13.7%) terminal taxa not found as endophytes were collected from multiple host lineages and substrates, a pattern that may reflect greater ecological specialization. Indeed, a few more recently diverged clades appear to have evolved more specific host ranges (e.g., *Xylaria hypoxylon* aggregate on angiosperms; see also Læssøe and Lodge, 1994; Rogers, 1979b; Whalley, 1985). However, additional sampling of endophytes of tropical lichens and non-angiosperm lineages is needed to confirm the pattern. Additionally, inferences can be limited due to missing or incomplete metadata in public databases. For example, 32.8% and 46.7% of 1,264 filtered BLASTn hits lacked information for host lineage and substrate, respectively (Supplemental Tables 7-8), revealing a pressing need for standardized formats and requirements for metadata submission with sequences.

After incorporating both morphological records and GenBank metadata, we found that 155 of 365 terminal taxa (42.5%) were reported only in a single geographic region. Importantly, geographic regions were broadly defined, such that a taxon reported from a single region might still be geographically widespread within a region (e.g., *Xylaria tuberoidea* in southern Mexico, Venezuela, French Guiana, and Guyana). Over a third of terminal taxa ($n = 137$; 37.5%) were found in ≥ 3 regions (e.g., US/Canada, S. Mexico/C. and S. America/Caribbean, Europe, Asia, or "Other"; Fig. 2). When analyzed in a phylogenetic context, taxa with widespread geographic ranges were distributed throughout the Xylariaceae. However, *Obolarina*, *Durotheca*, *Pseudoxylaria*, *Podosordaria* and *Poronia*, and endophyte-only clades appear more geographically limited (Fig. 2). The apparent localization of certain taxa or clades may be due to

the limited range of their host (e.g., *Pseudoxylaria* on Macrotermitinae termite nests; see above), dispersal limitation, climate, or habitat restrictions (see Whalley, 1985), and/or missing metadata (Supplemental Tables 6-7). Additional sampling from diverse locations and hosts, using both culturing and culture-free next generation sequencing, will help clarify the biogeographic patterns of xylariaceous fungi.

3.9. Contributions of endophytes to understanding the ecology of described species: *Daldinia loculata*

Our analyses revealed a monophyletic clade containing five endophytic OTU interspersed with two isolates of *Daldinia loculata* (Fig. 2B). Ascomata of *D. loculata* usually are recovered from burnt or damaged Betulaceae in temperate, boreal, and montane forests of the Northern Hemisphere, although the species also has been observed on wood of Salicaceae, Fagaceae, and Rosaceae (Stadler et al., 2014). It has been reported previously as a foliar endophyte of non-angiosperm hosts (see Pažoutová et al., 2010a). In our surveys, members of the *D. loculata* clade were frequently isolated as endophytes of diverse plants and lichens in boreal and subarctic Alaska. For example, in Eagle Summit, Alaska, we isolated *D. loculata* from asymptomatic, living photosynthetic tissue of evergreen angiosperms, conifers, lycophytes, and bryophytes and long-lived thalli of nine lichen species representing different growth forms, substrates, and photobionts. ITS rDNA analyses suggest that closely related isolates occur in Nome, Alaska, as well as Arizona, Florida, Jamaica, Europe, and New Zealand in a range of biomes from subarctic tundra to tropical high-elevation forest (Fig. 2; Supplemental Tables 1, 6-7). Although previously thought to be rare or absent from subtropical and tropical forests, these data illustrate that the species has a wider geographic and host range than reported previously.

Additionally, our data may help illuminate aspects of the lifecycle of *D. loculata*. Previously, the species was proposed to inhabit asymptomatic, living wood of *Betula* until fire kills the host (Guidot et al., 2003; Johannesson et al., 2001a; 2001b; see also Rayner and Boddy, 1988). The fungus then grows rapidly within host tissue, forming conidia beneath the bark that are

dispersed by insects among burnt trees. When conidia of different mating types interact, the sexual cycle is initiated, producing ascospores that are wind-dispersed to infect the unburned wood of young saplings, where the fungus presumably is endophytic in woody tissues of the host until another fire begins the cycle again (Guidot et al., 2003). Our data illustrate that *D. loculata* was abundant in the photosynthetic tissues of living lichens and evergreen plants in Eagle Summit (Alaska), as well as present in lower abundances in senescent leaves still attached in the canopy and fallen leaves in leaf litter from the previous year) (i.e., DL, FP; Table 1; Supplemental Table 1). However, we did not detect *D. loculata* in newly flushed leaves of *Salix* and *Betula*, which suggests that leaves of these hosts may be colonized from airborne inoculum each season. Whether inoculum is from wind-dispersed ascospores or asexual conidia produced on senescent evergreen plants or lichens in the same site remains to be elucidated, but it implies that the fungus is reproducing during intervals between fires (ca. 50-150 years in this area; Johannesson et al., 2000).

3.10. Contributions of endophytes to understanding the ecology of described species: *Xylaria cubensis*

Ascomata of *Xylaria cubensis* are commonly encountered on decomposing angiosperm wood in tropical, subtropical, and temperate forests across the globe, degrading both lignin and cellulose thereby causing a physiological white rot (Rogers, 1984). However, closely related isolates are frequently cultured in endophyte surveys, especially of tropical angiosperms, ferns, and lycophytes (e.g., Fan et al., 2014; Fröhlich et al., 2000; Okane et al., 2008; 2012; Rodrigues, 1994; Rodrigues et al., 1995; Rodrigues and Samuels, 1990; 1999; Rodrigues and Petrini, 1997) (Fig. 2E). Our results indicate that *X. cubensis* (represented by NC1011) is a frequent inhabitant of temperate and subtropical lichens (Fig. 2E; Table 1; Supplemental Table 1). Of the 193 *X. cubensis* isolates in a single 95% ITS rDNA OTU, 154 were cultured from lichens, whereas the remaining were found from angiosperms, conifers, and bryophytes (Supplemental Table 1; Fig. 2E). Even at a finer scale there was no evidence for host specificity: numerous unique ITS rDNA

genotypes (based on 100% sequence similarity) were shared among different host species, substrates (lichens, living, and dead plant tissues), as well as geographic locations (see also Okane et al., 2012; Supplemental Table 2). The species was collected from 22 species of lichens in Arizona, North Carolina, and Florida, representing five mycobiont orders (Lecanorales, Teloschistales, Ostropales, Peltigerales, and Umbilicariales) and diverse substrates, growth forms, and photobionts (e.g., various Trebouxiales and *Nostoc* species) (Supplemental Table 1). All investigated lichen genera in North Carolina and Florida (>10 spp. per site) yielded *X. cubensis* with the exception of the epiphytic crustose lichen *Herpothallon rubrocintum*, the only representative from the class Arthoniomycetes. However, isolates of *X. cubensis* are reported to have high morphological, genetic, and chemical diversity (Casella et al., 2013; Rodrigues et al., 1993; Rodriguez et al., 1995; also see above for discussion of intraspecific ITS rDNA variation) and additional studies are necessary to elucidate whether *X. cubensis*, as currently defined, represents a complex of several species, each with potentially different host and substrate preferences.

3.11. Conclusions

The goal of this study was to address the impact of a large collection of plant- and lichen-associated strains on the circumscription and phylogenetic structure of the Xylariaceae, and to evaluate their evolutionary history and ecology in a multi-locus phylogenetic context. Our results, coupled with previous molecular phylogenetic studies, reiterate the need for taxonomic revision at several levels within the Xylariaceae (Hsieh et al., 2005; 2010; Pažoutová et al., 2010b). Such revisionary work is hindered by the sheer diversity of xylariaceous fungi, a shortage of trained mycologists, the lack of reproductive structures in culture for many strains, biases in existing data, the lack of molecular data from type specimens (especially non-ITS rDNA sequence data) and the need for additional phylogenetic molecular markers (see Stadler et al., 2013; Stadler et al., 2014 for discussion of revision; also see Daranagama et al., 2015; Senanayake et al., 2015). Given the current situation, it is premature to assign taxonomic names to the majority of our

endophytic and saprotrophic OTU. Importantly, representative cultures have been deposited in the Gilbertson Mycological Herbarium (ARIZ), where they are available on request for additional characterization. Additionally, this work detects potentially misidentified specimens and sequences in public databases, illuminates large gaps in available metadata for sequences deposited in NCBI, and identifies the need for novel methods to integrate ITS rDNA sequences into robust, multi-locus phylogenetic analyses.

More generally, our study expands current knowledge regarding the ecology, host use, and geographic distributions of well-known Xylariaceae. We found that the majority of xylariaceous endophytes obtained in large surveys in the continental United States can be classified in the hypoxyloid and xylaroid subfamilies, although numerous endophytes also were found outside of these lineages (as currently circumscribed). Most newly cultured strains appear to represent novel species rather than previously described species, but inferences are limited by the potential for previously known Xylariaceae to be absent from public databases. Representatives of the lineages associated with the origin of Xylariaceae were found in living, asymptomatic leaves of angiosperms, gymnosperms, and bryophytes, consistent with the purported origin of endophytism early in the evolution of the Pezizomycotina (Lutzoni et al., in review). Our data suggest that in general, temperate and boreal xylariaceous endophytes have both endophytic (in both plants and lichens) and saprotrophic life stages (i.e., many endophyte OTU also contained isolates found inside non-living leaves (consistent with observations by Osono, 2002; 2005; 2006). However, additional work is necessary to determine the saprotrophic capabilities of endophytic OTU presented here, and the genomic, transcriptomic, and metabolic base of endophytic, saprotrophic, and pathogenic modes in this compelling and diverse family.

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Figure Legends

Figure 1. Phylogenetic relationships among 365 Xylariaceae representatives, including 77 newly collected endophyte and saprotroph putative species (representing 78 isolates) from field surveys of plants and lichens in five sites across the continental United States and 287 previously named taxa. The tree was inferred by maximum likelihood based on a combined five-locus dataset (i.e., ITS rDNA, LSU rDNA, *RPB2*, α -actin, and β -tubulin sequences, described in the text as the 5 + 4 + 3 + 2 + 1 dataset). Two species of Diatrypaceae (*Diatrype disciformis* and *Eutypa lata*) were used to root the tree based on the results of a four-locus supermatrix analysis of the Xylariomycetidae (Supplemental Fig. 1) and the availability of multi-locus sequence data (Supplemental Table 2). The four-box grid at each internode indicates maximum likelihood bootstrap support values derived from our cumulative supermatrix approach (see legend and Materials and Methods). Major clades of Hypoxyloideae (*Hypoxylon* clades “H1”, “H2”, and “H3”, *Daldinia* clade, *Annulohypoxylon* clade, *Durotheca*, and *Biscogniauxia* clade) are defined according to Hsieh et al. (2005) and Læssøe et al. (2013). Major clades of Xylarioideae are defined according to Hsieh et al. (2010): subgenus *Pseudoxylaria* (*Pseudoxylaria* “TE” clade); *Xylaria hypoxylon* and closely related taxa (*Xylaria* “HY” clade); the clade containing

Nemania spp., *Rosellinia* spp. and *Entoleuca mammata* (*Nemania* + *Rosellinia* “NR” clade); and the clade containing *Xylaria polymorpha* and closely related taxa (*Xylaria* “PO” clade). Red and dark-blue fonts indicate taxa included in analyses by Hsieh et al. (2005) and Hsieh et al. (2010), respectively. Black font indicates isolates from studies listed in Supplemental Table 2 (with the exception of *Obolarina* and *Durotheca*, none of the genera listed in black has been described formally using both morphological and molecular data). Light blue strain- and species names indicate specimens sequenced for the present study. Representative isolates from our surveys are shown as a two-letter code (AZ, NC, FL, AK) followed by the isolate number, the host and substrate information for the 95% ITSrDNA-partial LSU OTU represented by that isolate (LT, lichen thallus; LP, living plant tissue; DP, dead leaves in the canopy; and FP, dead plant leaves in leaf litter), and the total number of isolates in the OTU (Table 1). Subfamily classifications (i.e., Hypoxyloideae or Xylarioideae) are based on Stadler et al. (2013). Genera currently unclassified at the subfamily level (i.e., *Whalleya* and *Anthostomella*; see Stadler et al., 2013), as well as endophyte clades E3-E7 are indicated on the tree as incertae subfamiliae. Two endophyte OTU (clades E1-E2) as well as *Seynesia erumpens* are labeled as incertae familiae reflecting the recent proposal of *Seynesia* as a member of the Cainaceae rather than Xylariaceae (see Senanayake et al., 2015). The blue star denotes *Hypoxylon argillaceum* CBS 527.63, a potentially misidentified isolate for which taxonomic revision may be warranted.

Figure 2. Phylogenetic integration of geographic and ecological metadata in the evolutionary context of the Xylariaceae (Fig. 1). Tree topology and clade definitions follow Fig. 1. Thickened branches indicate maximum likelihood bootstrap values $\geq 70\%$ from all four bootstrap analyses of the cumulative supermatrix approach. An asterisk (*) denotes nodes with $\geq 70\%$ support from at least one of the bootstrap analyses from the cumulative supermatrix approach (e.g., 5 + 4) (see Fig. 1). Major clades are shaded according to the ecological mode of the majority of taxa used in the multilocus analysis: green shading, endophyte clade (numbered E1-E9); grey, saprotroph; white, pathogen; brown, dung-associated; peach, termite-associated; and purple, equivocal. Within clades, terminals are labeled with colored fonts to indicate ecological mode of the terminal

taxon based on review of the literature (see Supplemental Table 9 and legend). Within taxon names, numbers after the dash indicate the 95% OTU designation based on clustering analysis of ITS rDNA sequences for all isolates (when available) included in the phylogenetic analyses (e.g., *Daldinia vernicosa* 121—139 belongs to OTU 139). In cases where an isolate recovered in our surveys was within the same 95% ITS rDNA group as a named taxon not present in the tree due to lack of multi-locus data, the species name follows the OTU designation (e.g., FL1857 – 83 – *Hypoxylon pulicicidum*; see also Table 1). Columns of numbers after taxon names indicate (1) the total number of isolates recovered in our surveys represented per 95% ITS rDNA group (see also Table 1) and (2) the number of BLASTn hits with ≥99% identity (isolates lacking numbers did not have ITS rDNA sequences in NCBI) (Supplemental Tables 7-8). The geographic locations of collection, photobiont host lineage, and substrate type are indicated for each terminal taxon. Solid black circles represent metadata from our field surveys (Supplemental Table 1; Table 1) or reference taxa (Supplemental Table 2). Solid grey circles represent metadata gathered from filtered BLASTn hits at ≥99% similarity (Supplemental Tables 6-7). Solid blue circles represent information associated with fruiting bodies, gathered by reviewing recent species monographs, species descriptions, and public databases (Supplemental Table 9). For substrate metadata, the color of outer circles indicates the condition of the host tissue at the time of collection (e.g., asymptomatic living tissue (green), diseased living tissue (red), dead or decomposed tissue (brown), N/A or unknown (black); see substrate legend). The “Other” category for geographic location denotes isolates from Australia, New Zealand, Papua New Guinea, Africa, the Middle East, and Antarctica, which were rarely represented across the dataset.

Figure1

Ecological Mode

- Endophytic
- Saprotrophic
- Pathogenic / parasitic
- Unknown

Metadata Source

- Terminal taxon records
- Genbank BLASTn hit
- Fruiting body records
- No data

Geo

- United States, Canada
- Mexico, S/C. America, Caribbean
- Europe, western Russia
- Asia, eastern Russia
- Other

Host

- Angiosperm
- Gymnosperm
- Spore-bearing vascular plant
- Bryophyte
- Lichen (LT)
- Living leaf (LP)
- Dead leaf (DP)
- Fallen leaf (FP)
- Wood / bark
- Root
- Seed
- Soil
- Insect-associated
- Fallen fruit / inflorescence

Substrate

- Asymptomatic
- Dead / decomposed
- Diseased
- N/A or unknown

Number of isolates in OTU

Number of BLASTn hits ≥ 99% ID

E1

E2

E3

Hypoxylon clade

Daldinia clade

A

The figure displays a phylogenetic tree of Daldinia and Hypoxylon species. The tree is rooted with Eutypa lata and Diatrype disciformis. The tree is divided into three main clades: E1, E2, and E3. The Hypoxylon clade is further divided into three sub-clades: H1, H2, and H3. The Daldinia clade is also shown. Each species is associated with a number of isolates in OTU and a number of BLASTn hits ≥ 99% ID. The tree is annotated with ecological mode (Endophytic, Saprotrophic, Pathogenic / parasitic, Unknown) and metadata source (Terminal taxon records, Genbank BLASTn hit, Fruiting body records, No data). The tree is also annotated with geographical location (United States, Canada; Mexico, S/C. America, Caribbean; Europe, western Russia; Asia, eastern Russia; Other) and host (Angiosperm, Gymnosperm, Spore-bearing vascular plant, Bryophyte, Lichen (LT), Living leaf (LP), Dead leaf (DP), Fallen leaf (FP), Wood / bark, Root, Seed, Soil, Insect-associated, Fallen fruit / inflorescence). The tree is also annotated with substrate (Asymptomatic, Dead / decomposed, Diseased, N/A or unknown).

Table 1

Table 1. Host species, OTU designation (95% ITS-partial LSU rDNA) and number of representatives, geographic information, and GenBank accession numbers for endophytic, endolichenic, and saprotrophic fungi collected in our surveys of five sites in the continental United States. GenBank accession numbers in bold represent new sequences generated as part of this study.

Representative Isolate	Origin*	Host species	Tissue type**	OTU (95, 100%)	Total # isolates in 95% OTU	# Isolates per tissue type (LT, LP, DP, FP)**	# Isolates per site (AZC, NCH, FLA, AK)***	Putative taxonomic classification ^c	GenBank accession numbers			
									ITS rDNA + partial LSU rDNA	<i>RPB2</i>	β -tubulin	α -actin
AK1199	AKN	<i>Equisetum arvense</i>	LP	10, 14	6	0, 4, 0, 2	0, 2, 0, 4	Xylariomycetidae incertae sedis	JQ759542	KU684294	KU684114	KU684027
AZ0196	AZC	<i>Quercus rugosa</i>	DP	16, 25	69	0, 14, 35, 20	42, 18, 9, 0	Xylariomycetidae incertae sedis	HM122945	KU684295	KU684117	NA
AZ0339	AZC	<i>Quercus rugosa</i>	FP	19, 28	13	0, 0, 1, 12	2, 7, 4, 0	Xylariomycetidae incertae sedis	HM123082	KU684296	KU684118	NA
FL0674	FLA	<i>Pinus elliotii</i>	LP	33, 55	6	0, 3, 2, 1	0, 0, 6, 0	Xylariomycetidae incertae sedis	JQ760367	KU684297	KU684152	NA
FL0456	FLA	<i>Cladonia didyma</i>	LT	46, 86	1	1, 0, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760182	KU684298	NA	NA
FL0650	FLA	<i>Pinus elliotii</i>	LP	61, 248	2	0, 2, 0, 0	0, 0, 2, 0	Xylariomycetidae incertae sedis	JQ760354	KU684299	KU684149	NA
FL0677	FLA	<i>Pinus elliotii</i>	LP	64, 123	1	0, 1, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760370	KU684300	NA	NA
FL0850	FLA	<i>Herpothallon rubrocinctum</i>	LT	69, 133	1	1, 0, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760489	NA	NA	NA
FL1681	FLA	<i>Pinus clausa</i>	FP	80, 185	1	0, 0, 0, 1	0, 0, 1, 0	Xylariomycetidae incertae sedis	KU683871	KU684301	KU684182	NA
FL1780	FLA	<i>Quercus inopina</i>	FP	82, 188	1	0, 0, 0, 1	0, 0, 1, 0	Xylariomycetidae incertae sedis	KU683905	KU684302	NA	NA
FL2152	FLA	<i>Pinus clausa</i>	DP	85, 191	2	0, 0, 2, 0	0, 0, 2, 0	Xylariomycetidae incertae sedis	KU683958	KU684303	KU684186	NA
NC0532	NCH	<i>Hypnum</i> sp.	LP	87, 205	14	3, 11, 0, 0	0, 14, 0, 0	Xylariomycetidae incertae sedis	JQ761527	KU684304	NA	NA
NC1491	NCH	<i>Pseudevernia consocians</i>	LT	92, 236	1	1, 0, 0, 0	0, 1, 0, 0	Xylariomycetidae incertae sedis	JQ761899	KU684305	KU684201	NA
FL2044	FLA	<i>Serenoa repens</i>	DP	43, 95	24	17, 1, 6, 0	0, 0, 24, 0	Xylariomycetidae incertae sedis	KU683944	KU684265	KU684185	KU684086
FL0915	FLA	<i>Cladonia subradiata</i>	LT	71, 137	2	1, 0, 1, 0	0, 0, 2, 0	Xylariomycetidae sp. nov.	JQ760548	KU684238	KU684156	NA
FL0641	FLA	<i>Pinus elliotii</i>	LP	60, 120	1	0, 1, 0, 0	0, 0, 1, 0	Xylariomycetidae sp. nov.	JQ760347	KU684232	NA	KU684058
FL0662	FLA	<i>Pinus elliotii</i>	LP	63, 122	1	0, 1, 0, 0	0, 0, 1, 0	Xylariaceae sp. nov.	JQ760360	KU684234	KU684151	KU684060
FL0638	FLA	<i>Pinus elliotii</i>	LP	58, 118	1	0, 1, 0, 0	0, 0, 1, 0	Xylariaceae sp. nov.	JQ760345	KU684231	NA	KU684057
AK1116	AKN	<i>Cassiope tetragona</i>	LP	11, 15	2	0, 2, 0, 0	0, 0, 0, 2	Xylariaceae sp. nov.	JQ759464	KU684211	KU684113	NA
FL0602	FLA	<i>Cladonia evansii</i>	LT	55, 113	6	6, 0, 0, 0	0, 0, 6, 0	<i>Hypoxylon</i> sp. nov.	JQ760314	KU684229	KU684147	KU684055
FL1179	FLA	<i>Pyxine eschweileri</i>	LT	75, 161	4	4, 0, 0, 0	0, 0, 4, 0	<i>Hypoxylon</i> sp. nov.	JQ760795	KU684253	KU684171	KU684075
FL1170	FLA	<i>Parmotrema rampoddense</i>	LT	76, 159	1	1, 0, 0, 0	0, 0, 1, 0	<i>Hypoxylon rubiginosum</i>	JQ760786	KU684252	KU684170	KU684074
NC1633	NCH	<i>Diploschistes scruposus</i>	LT	94, 242	1	1, 0, 0, 0	0, 1, 0, 0	<i>Hypoxylon</i> sp. nov.	JQ761992	NA	NA	KU684104
NC0708	NCH	<i>Pinus strobus</i>	FP	88, 214	1	0, 0, 0, 1	0, 1, 0, 0	<i>Hypoxylon submonticulosum</i>	KU683969	KU684270	KU684195	KU684095
FL0542	FLA	<i>Cladonia leporina</i>	LT	52, 104	1	1, 0, 0, 0	0, 0, 1, 0	<i>Hypoxylon monticulosum</i>	JQ760257	KU684227	KU684145	KU684053
FL1289	FLA	<i>Cladonia leporina</i>	LT	77, 169	1	1, 0, 0, 0	0, 0, 1, 0	<i>Hypoxylon polyporus</i>	JQ760904	KU684258	KU684176	KU684080
FL1408	FLA	<i>Parmotrema tinctorum</i>	LT	53, 111	6	6, 0, 0, 0	0, 0, 6, 0	<i>Daldinia eschscholzii</i>	JQ761025	KU684261	KU684179	NA
FL1419	FLA	<i>Parmotrema tinctorum</i>	LT	78, 179	1	1, 0, 0, 0	0, 0, 1, 0	<i>Daldinia</i> sp. nov.	JQ761035	KU684262	KU684180	KU684083
AK1016	AKE	<i>Arctoparmelia separata</i>	LT	2, 2	86	53, 25, 2, 6	0, 0, 0, 86	<i>Daldinia loculata</i>	JQ759383	KU684210	KU684112	KU684026

AZ0526	AZC	<i>Physcia caesia</i>	LT	6, 7	111	48, 38, 11, 14	1, 0, 0, 110	<i>Daldinia loculata</i>	HM123248	KU684216	KU684121	KU684033
AK0222	AKE	<i>Pleurozium schreberi</i>	LP	7, 8	29	16, 11, 1, 1	0, 0, 5, 24	<i>Daldinia loculata</i>	JQ758703	KU684207	KU684109	KU684024
AK0128	AKE	<i>Pleurozium schreberi</i>	LP	3, 3	36	17, 11, 2, 6	0, 0, 0, 36	<i>Daldinia loculata</i>	JQ758621	KU684206	KU684108	KU684023
AK0995	AKE	<i>Flavocetraria cucullata</i>	LT	4, 4	90	47, 37, 1, 5	0, 0, 0, 90	<i>Daldinia loculata</i>	JQ759362	KU684209	KU684111	KU684025
FL1857	FLA	<i>Pinus elliottii</i>	DP	83, 189	1	0, 0, 1, 0	0, 0, 1, 0	<i>Hypoxylon pulicicidum</i> (matches JX183075)	KU683912	KU684264	KU684184	KU684085
NC0597	NCH	<i>Pseudevernia consocians</i>	LT	56, 114	40	33, 5, 1, 1	0, 36, 4, 0	<i>Hypoxylon</i> sp. nov.	JQ761586	KU684268	KU684193	KU684093
FL1377	FLA	<i>Parmotrema tinctorum</i>	LT	47, 105	26	17, 4, 2, 3	0, 19, 7, 0	<i>Hypoxylon</i> sp. nov.	JQ760995	KU684260	KU684178	KU684082
NC1073	NCH	<i>Selaginella tortipila</i>	LP	70, 206	9	1, 6, 2, 0	0, 8, 1, 0	<i>Hypoxylon</i> sp. nov.	JQ761719	KU684274	KU684199	KU684099
FL1043	FLA	<i>Cladonia evansii</i>	LT	27, 46	39	26, 10, 3, 0	0, 0, 39, 0	<i>Hypoxylon</i> sp. nov.	JQ760666	KU684249	KU684167	KU684071
FL0890	FLA	<i>Pyxine eschweileri</i>	LT	25, 41	98	65, 15, 7, 11	0, 0, 98, 0	<i>Hypoxylon</i> sp. nov.	JQ760526	KU684237	KU684155	KU684061
FL0455	FLA	<i>Cladonia didyma</i>	LT	44, 246	19	18, 0, 0, 1	0, 0, 19, 0	<i>Annulohypoxylon</i> sp. nov.	JQ760181	KU684224	KU684141	KU684049
FL0470	FLA	<i>Cladonia didyma</i>	LT	48, 89	4	4, 0, 0, 0	0, 0, 4, 0	<i>Annulohypoxylon stygium</i>	JQ760192	KU684225	KU684142	KU684050
FL1025	FLA	<i>Cladonia evansii</i>	LT	45, 85	19	15, 2, 1, 1	0, 7, 12, 0	<i>Biscogniauxia atropunctata</i> var. <i>intermedia</i> (matches AJ390412)	JQ760650	KU684246	KU684164	KU684068
AZ0048	AZC	<i>Flavoparmelia praesignis</i>	LT	17, 23	128	109, 10, 5, 4	89, 30, 9, 0	<i>Biscogniauxia mediterranea</i>	HM122805	NA	NA	KU684030
AZ0703	AZC	<i>Pseudevernia intensa</i>	LT	20, 31	10	8, 1, 0, 1	2, 7, 1, 0	<i>Biscogniauxia mediterranea</i>	HM123416	KU684217	KU684122	KU684034
AZ1047	AZC	<i>Woodsia plummerae</i>	LP	23, 39	2	0, 1, 0, 1	2, 0, 0, 0	<i>Anthostomella pinea</i> (matches HQ599578)	HM123694	KU684218	NA	KU684035
FL0804	FLA	<i>Cladonia subtenius</i>	LT	59, 119	5	2, 1, 0, 2	0, 0, 5, 0	<i>Xylariaceae</i> sp. nov.	JQ760457	KU684235	KU684153	NA
FL0016	FLA	<i>Aristida stricta</i>	LP	24, 40	1	0, 1, 0, 0	0, 0, 1, 0	<i>Xylariaceae</i> sp. nov.	JQ759892	KU684219	KU684135	KU684044
FL1105	FLA	<i>Cladonia subtenius</i>	LT	42, 74	17	13, 1, 0, 3	0, 0, 17, 0	<i>Muscodor vitigenus</i> (matches KC771503)	JQ760728	KU684250	KU684168	KU684072
FL1272	FLA	<i>Usnea subscabrosa</i>	LT	51, 125	10	6, 1, 1, 2	0, 0, 10, 0	<i>Xylariaceae</i> sp. nov.	JQ760887	KU684257	KU684175	KU684079
FL1255 ^b	FLA	<i>Usnea subscabrosa</i>	LT	34, 83	7	7, 0, 0, 0	0, 0, 7, 0	<i>Xylariaceae</i> sp. nov.	JQ760870	KU684256	KU684174	KU684078
FL1019 ^b	FLA	<i>Parmotrema perforatum</i>	LT	34, 66	9	6, 3, 0, 0	0, 0, 9, 0	<i>Xylariaceae</i> sp. nov.	JQ760644	KU684245	KU684163	KU684067
FL0660	FLA	<i>Pinus elliottii</i>	LP	62, 121	9	2, 1, 0, 6	0, 0, 9, 0	<i>Xylariaceae</i> sp. nov.	JQ760358	KU684233	KU684150	KU684059
FL0821	FLA	<i>Cladonia subtenius</i>	LT	67, 129	4	1, 0, 1, 2	0, 0, 4, 0	<i>Xylariaceae</i> sp. nov.	JQ760469	KU684236	KU684154	NA
NC1622	NCH	<i>Tsuga canadensis</i>	LP	91, 241	3	1, 2, 0, 0	0, 3, 0, 0	<i>Anthostomella sepelibilis</i> (matches AY908990)	JQ761984	KU684277	NA	KU684103
FL0255	FLA	<i>Pinus elliottii</i>	LP	26, 43	67	36, 19, 2, 10	0, 0, 67, 0	<i>Xylariaceae</i> sp. nov.	JQ760030	NA	KU684139	NA
FL1015	FLA	<i>Parmotrema perforatum</i>	LT	41, 81	17	16, 1, 0, 0	0, 0, 17, 0	<i>Xylariaceae</i> sp. nov.	JQ760640	KU684244	KU684162	KU684066
FL1651	FLA	<i>Quercus inopina</i>	FP	79, 184	1	0, 0, 0, 1	0, 0, 1, 0	<i>Anthostomella brabeji</i> (matches EU552098) ^d	KU683867	NA	KU684181	NA
NC1498	NCH	<i>Xanthoparmelia conspersa</i>	LT	30, 237	12	7, 3, 1, 1	0, 4, 8, 0	<i>Xylariaceae</i> sp. nov.	JQ761906	KU684275	NA	KU684101
FL0594	FLA	<i>Cladonia evansii</i>	LT	54, 112	4	3, 1, 0, 0	0, 0, 4, 0	<i>Xylariaceae</i> sp. nov.	JQ760306	KU684228	KU684146	KU684054
AK1595 ^a	AKE	<i>Salix pulchra</i>	DP	15, 21	1	0, 0, 1, 0	0, 0, 0, 1	<i>Xylariaceae</i> sp. nov.	KU683770	KU684214	NA	NA
AK1471 ^a	AKN	<i>Peltigera aphthosa</i>	LT	14, 19	7	7, 0, 0, 0	2, 1, 2, 2	<i>Anthostomella leucospermi</i> (matches EU552100) ^d	JQ759768	KU684213	KU684116	KU684029

FL0491	FLA	<i>Cladonia didyma</i>	LT	50, 94	1	1, 0, 0, 0	0, 0, 1, 0	<i>Xylaria</i> sp. nov.	JQ760210	KU684226	KU684144	KU684052
FL1030	FLA	<i>Cladonia evansii</i>	LT	66, 146	6	5, 0, 0, 1	0, 0, 6, 0	<i>Xylaria arbuscula</i>	JQ760654	KU684247	KU684165	KU684069
FL1777	FLA	<i>Quercus inopina</i>	FP	81, 187	1	0, 0, 0, 1	0, 0, 1, 0	<i>Xylaria</i> sp. nov.	KU683903	KU684263	KU684183	KU684084
NC1654	NCH	<i>Flavoparmelia caperata</i>	LT	95, 244	1	1, 0, 0, 0	0, 1, 0, 0	<i>Kretzschmaria deusta</i>	JQ762008	KU684278	KU684203	KU684105
FL0490	FLA	<i>Cladonia didyma</i>	LT	49, 93	1	1, 0, 0, 0	0, 0, 1, 0	<i>Xylaria venustula</i>	JQ760209	NA	KU684143	KU684051
FL1042	FLA	<i>Cladonia evansii</i>	LT	74, 147	2	1, 0, 0, 1	0, 0, 2, 0	<i>Xylaria</i> sp. nov.	JQ760665	KU684248	KU684166	KU684070
FL0609	FLA	<i>Cladonia evansii</i>	LT	57, 116	2	2, 0, 0, 0	0, 0, 2, 0	<i>Xylaria</i> sp. nov.	JQ760320	KU684230	KU684148	KU684056
FL0359	FLA	<i>Pinus clausa</i>	LP	32, 53	38	28, 6, 2, 2	0, 9, 29, 0	<i>Xylaria</i> sp. nov.	JQ760113	KU684223	KU684140	KU684048
FL0224	FLA	<i>Pinus elliottii</i>	LP	35, 67	29	21, 7, 1, 0	0, 0, 29, 0	<i>Xylaria</i> sp. nov.	JQ760020	KU684222	KU684138	KU684047
FL0043	FLA	<i>Pinus elliottii</i>	LP	29, 50	4	3, 1, 0, 0	0, 0, 4, 0	<i>Xylaria</i> sp. nov.	JQ759911	KU684221	KU684137	KU684046
NC0985	NCH	<i>Flavoparmelia caperata</i>	LT	68, 222	31	19, 6, 4, 2	0, 15, 16, 0	<i>Xylaria</i> sp. nov.	JQ761635	KU684272	KU684197	KU684097
FL1254	FLA	<i>Usnea subscabrosa</i>	LT	37, 92	63	46, 7, 4, 6	0, 13, 50, 0	<i>Xylaria</i> sp. nov.	JQ760869	KU684255	KU684173	KU684077
FL0933	FLA	<i>Cladonia subradiata</i>	LT	40, 72	15	13, 2, 0, 0	0, 4, 11, 0	<i>Xylaria</i> sp. nov.	JQ760565	KU684240	KU684158	KU684063
FL1352	FLA	<i>Cladonia evansii</i>	LT	31, 103	71	55, 5, 6, 5	0, 13, 58, 0	<i>Xylaria</i> sp. nov.	JQ760970	KU684259	KU684177	KU684081
FL0916	FLA	<i>Cladonia subradiata</i>	LT	72, 138	1	1, 0, 0, 0	0, 0, 1, 0	<i>Nemania</i> sp. nov.	JQ760549	KU684239	KU684157	KU684062
NC1218	NCH	<i>Lasalia pensylvanica</i>	LT	90, 226	2	2, 0, 0, 0	0, 2, 0, 0	<i>Rosellinia subiculata</i> (matches AY909002) ^d	JQ761857	NA	KU684200	KU684100
NC0528	NCH	<i>Flavoparmelia caperata</i>	LT	84, 210	3	2, 0, 1, 0	0, 2, 1, 0	<i>Nemania</i> sp. nov.	JQ761524	KU684267	KU684192	KU684092
FL0031	FLA	<i>Pinus elliottii</i>	LP	28, 47	36	29, 4, 2, 1	0, 0, 36, 0	<i>Nemania</i> sp. (matches FJ175173)	JQ759904	KU684220	KU684136	KU684045
FL1152	FLA	<i>Parmotrema rampoddense</i>	LT	39, 71	15	10, 1, 2, 2	0, 0, 15, 0	<i>Nemania abortiva</i>	JQ760771	KU684251	KU684169	KU684073
NC0608	NCH	<i>Parmotrema reticulatum</i>	LT	86, 193	44	27, 6, 3, 8	0, 44, 0, 0	<i>Nemania diffusa</i>	JQ761596	KU684269	KU684194	KU684094
AK1383^a	AKN	<i>Hypogymnia physodes</i>	LT	13, 18	1	1, 0, 0, 0	0, 0, 0, 1	<i>Nemania</i> sp. nov.	JQ759694	KU684212	KU684115	KU684028
FL0980	FLA	<i>Usnea mutabilis</i>	LT	73, 142	1	1, 0, 0, 0	0, 0, 1, 0	<i>Nemania beaumontii</i>	JQ760608	KU684243	KU684161	KU684065
FL1238	FLA	<i>Usnea subscabrosa</i>	LT	65, 127	3	2, 0, 1, 0	0, 0, 3, 0	Xylariaceae sp. nov.	JQ760853	KU684254	KU684172	KU684076
NC0429^a	NCH	<i>Diploschistes scruposus</i>	LT	22, 199	102	67, 27, 3, 5	1, 33, 68, 0	<i>Nemania</i> sp. nov.	JQ761458	KU684266	KU684191	KU684091
AZ0448	AZC	<i>Punctelia hypoleucites</i>	LT	21, 32	1	1, 0, 0, 0	1, 0, 0, 0	<i>Nemania</i> sp. nov.	HM123176	NA	KU684120	KU684032
AZ0398^a	AZC	<i>Peltigera rufescens</i>	LT	12, 30	74	62, 6, 2, 4	35, 32, 2, 5	<i>Nemania serpens</i>	HM123137	KU684215	KU684119	KU684031
NC0962	NCH	<i>Sticta beauvoisii</i>	LT	89, 219	1	1, 0, 0, 0	0, 1, 0, 0	<i>Nemania serpens</i> (matches AF201703) ^d	JQ761617	KU684271	KU684196	KU684096
AK0226^a	AKE	<i>Pleurozium schreberi</i>	LP	9, 12	1	0, 1, 0, 0	0, 0, 0, 1	<i>Nemania serpens</i>	JQ758707	KU684208	KU684110	NA
NC1011	NCH	<i>Lecanora oreinoides</i>	LT	18, 27	193	154, 30, 4, 5	1, 142, 50, 0	<i>Xylaria cubensis</i>	JQ761659	KU684273	KU684198	KU684098
FL0961	FLA	<i>Usnea mutabilis</i>	LT	36, 99	61	46, 7, 3, 5	0, 23, 38, 0	<i>Xylaria</i> cf. <i>heliscus</i>	JQ760593	KU684241	KU684159	KU684064
FL0975†	FLA	<i>Usnea mutabilis</i>	LT	38, 69	36	33, 1, 0, 2	0, 5, 31, 0	<i>Muscodor yucatanensis</i> (matches FJ917287)	JQ760604	KU684242	KU684160	NA
NC1612†	NCH	<i>Lecanora oreinoides</i>	LT	93, 240	1	1, 0, 0, 0	0, 1, 0, 0	<i>Hypoxylon papillatum</i> (matches AF201710)	JQ761975	KU684276	KU684202	KU684102

* Abbreviations for sites correspond to U'Ren et al. (2012). Madrean Sky Island Archipelago of southeastern Arizona (AZC); the Appalachian Mountains of western North Carolina (NCH); subtropical scrub forest in Florida (FLA); Beringian tundra and boreal forest in the Seward Peninsula ecoregion of western Alaska (AKN); and inland, subalpine tundra in the Interior Highlands of eastern central Alaska (AKE).

** LT corresponds to lichen thallus; LP corresponds to living plant tissue; DP corresponds to dead leaves in the canopy; and FP corresponds to fallen plant leaves.

*** Isolates from AKN and AKF are pooled due to low isolation of endophytic fungi in these sites.

† Isolates were removed from the Xylariaceae multigene analyses due to topological conflict among single locus trees (Supplemental Table 4).

^a Although not *Xylaria* spp. based on phylogenetic analyses, isolates had top BLASTn hits to *Xylaria* sp. NRRL 40192 (EF157664), which was identified based solely on rDNA BLAST (Deyrup et al. 2007)

^b Information on total isolates, isolates per tissue type, and isolates per site is based on 99%

OTU.

^c Taxonomic classification is based on sister relationship and/or shared 95% ITS rDNA OTU to reference taxa in phylogenetic tree (Fig. 2). If reference species are not present in the tree due to lack of multilocus data, identification is based on only 95% ITS rDNA OTU similarity to sequences in GenBank (denoted by accession numbers following taxon names; Supplemental Table 6).

^d Based on our phylogeny (Fig. 2), this reference strain may be misidentified (see Supplemental Table 6).