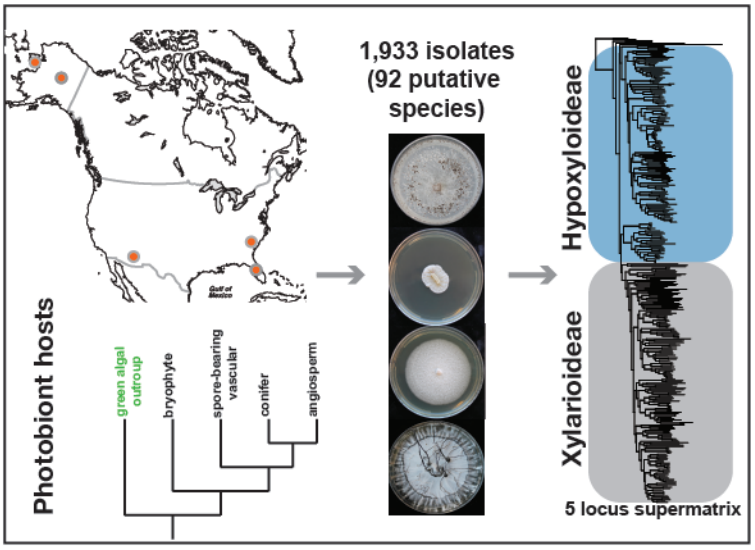
*Graphical Abstract (for review)



***Highlights (for review)**

- Endophytes illuminate Xylariaceae circumscription and phylogenetic structure.
- Endophytes occur in lineages previously not known for endophytism.
- Boreal and temperate lichens and non-flowering plants commonly host Xylariaceae.
- Many have endophytic and saprotrophic life stages and are widespread generalists.

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

Jana M. U'Ren [a],* Jolanta Miadlikowska [b], Naupaka B. Zimmerman [a], François Lutzoni [b], Jason E. Stajich[c], and A. Elizabeth Arnold [a,d]

[a] University of Arizona, School of Plant Sciences, 1140 E. South Campus Dr., Forbes 303, Tucson, AZ 85721, USA
[b] Duke University, Department of Biology, Durham, NC 27708-0338, USA
[c] University of California-Riverside, Department of Plant Pathology and Microbiology and Institute for Integrated Genome Biology, 900 University Ave., Riverside, CA 92521, USA
[d] University of Arizona, Department of Ecology and Evolutionary Biology, 1041 E. Lowell St., BioSciences West 310, Tucson, AZ 85721, USA

*** Corresponding author:** University of Arizona, School of Plant Sciences, 1140 E. South Campus Dr., Forbes 303, Tucson, AZ 85721, USA. Telephone: +1 520 626 1035; Fax: +1 520 621 7186; Email: juren@email.arizona.edu

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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 North America, 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 hypoxyloid 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 have both endophytic and saprotrophic life stages. 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 Rodriquez 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

105 and influencing host responses to abiotic stressors such as drought (Arnold et al., 2003; Arnold
106 and Engelbrecht, 2007; Costa Pinto et al., 2000; Estrada et al., 2013; Mejía et al., 2008). They
107 are increasingly recognized as a major source of novel metabolic products for use in medicine,
108 agriculture, and industry (Ding et al., 2009; Deyrup et al., 2007; Fan et al., 2014; Jiménez-
109 Romero et al., 2008; Paranagama et al., 2007; Staniek et al., 2008; Strobel et al., 1997; Strobel
110 and Long, 1998; Wu et al., 2011) and as an important but under-studied aspect of plant and
111 lichen biology.
112 Studies over the last four decades have revealed an extremely high richness of
113 xylariaceous endophytes (Xylariales, Sordariomycetes, Pezizomycotina) (e.g., Petrini and Petrini,
114 1985; Rogers, 2000). They inhabit the living tissues of phylogenetically diverse plants, including
115 conifers, angiosperms, ferns, lycophytes, and bryophytes in a diverse range of biogeographic
116 provinces (e.g., Arnold et al., 2009; Brunner and Petrini, 1992; Carroll and Carroll, 1978; Davey et
117 al., 2014; Davis et al., 2003; Del Olmo-Ruiz and Arnold, 2014; Higgins et al., 2007; Okane et al.,
118 2012; Petrini and Petrini, 1985; Petrini et al., 1995). They also occur frequently in taxonomically
119 diverse lichens encompassing diverse growth forms (e.g., foliose, fruticose, crustose) and
120 substrates (e.g., terricolous, saxicolous, epiphytic) (Arnold et al., 2009; Petrini and Petrini, 1985;
121 Suryanarayanan et al., 2005; Wu et al., 2011). Some xylariaceous endophytes persist in leaf
122 litter, reflecting abilities to decompose lignocellulose and the capacity of some species to directly
123 infect decaying leaves, potentially circumventing the need for an endophytic life stage (Osono,
124 2002; 2005; 2006).
125 Many endophytic Xylariaceae remain sterile in culture or reproduce only asexually on
126 standard media (Stadler et al., 2013), precluding identification based on teleomorphic characters
127 such as stromata features and ascospore number (see Petrini and Petrini, 1985; Rogers, 1979a;
128 2000; Rogers et al., 2002). Anamorphic cultures can be classified based on conidiophore
129 branching and the nature of conidiogenous cell proliferation (Ju and Rogers, 1996), as well as
130 cultural characteristics such a growth rates and color (Petrini and Petrini, 1985). However,
131 anamorphic characters alone often lack sufficient information for species-level identification
132 (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

161 systematics (e.g., Dennis, 1957; Hawksworth and Whalley, 1985; Ju et al., 1993; Ju and Rogers,
162 1996; Ju et al., 1998; Læssøe et al., 1989; Miller, 1961; Möller, 1901; Pouzar, 1985a; 1985b;
163 Rogers, 1981; Rogers and Ju, 1996; Rogers et al., 1997a; 1997b) is increasingly complemented
164 by chemotaxonomic (e.g., Fournier et al., 2010; Læssøe et al., 2010; 2013; Stadler and Fournier,
165 2006; Stadler et al., 2008; 2010) and molecular approaches (e.g., Bills et al., 2012; Hsieh et al.,
166 2005; 2010; Jaklitsch et al., 2014; Peršoh et al., 2009; Whalley, 1996). Several genera have been
167 updated or monographed recently (e.g., Daranagama et al., 2015; Rogers et al., 2002; Peršoh et
168 al., 2009; Stadler et al., 2014) and NCBI contains nucleotide data for >3,000 isolates representing
169 ca. 442 recognized species (as of August 2015). Phylogenetic analyses support monophyly of the
170 family as presently circumscribed (Tang et al., 2009), but numerous studies suggest that many
171 recognized genera and species may not be monophyletic (see Daranagama et al., 2015; Hsieh et
172 al., 2005; 2010; Pažoutová et al., 2010b), and many genera require taxonomic revisions (Stadler
173 et al., 2013). Integrating previously unknown strains into a robust phylogenetic framework for the
174 Xylariaceae can provide insight into the taxonomic circumscription at the family and infrafamilial
175 levels, illustrate previously unknown connections between anamorphic and teleomorphic species,
176 and inform evolutionary relationships, host ranges, distributions, major ecological modes, and
177 diversity of major clades, previously known taxa, newly found strains, and the family as a whole.
178 The goal of this study was to address the impact of a large collection of endophytic and
179 saprotrophic fungi on the circumscription of the Xylariaceae and infrafamilial taxa. Our work takes
180 advantage of 1,933 newly cultured isolates representing 92 putative species, which were
181 collected from living photosynthetic tissues of angiosperms, conifers, ferns, lycophytes,
182 bryophytes, and lichens, as well as senescent and decomposing leaves of selected woody plants
183 in five biomes across North America (U'Ren et al., 2010; 2012). Here, we place these strains in a
184 multi-locus phylogenetic framework in conjunction with additional sequencing of type specimens
185 from culture collections and previously published sequence data from described species. We then
186 address the following questions: (1) Does the inclusion of newly cultured isolates alter current
187 phylogenetic hypotheses regarding the delimitation of Xylariaceae and the relationships and
188 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 North America (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

217 non-living plant tissues (i.e., DP and/or FP) are referred to as saprotrophs. Each isolate is
218 maintained as an axenic voucher in sterile water at the Robert L. Gilbertson Mycological
219 Herbarium at the University of Arizona (ARIZ) (Supplemental Table 1).
220
221 *2.2. Sequencing the ITS-partial LSU rDNA of field-collected strains*
222
223 Overall, the field surveys described above generated 6,784 cultures, which were screened by
224 DNA sequencing for preliminary taxonomic placement. Methods for DNA extraction, PCR
225 amplification, DNA sequencing and sequence editing followed U'Ren et al. (2010). Briefly, DNA
226 was extracted from each isolate using phenol:chloroform:IAA (Arnold and Lutzoni, 2007). The
227 nuclear ribosomal internal transcribed spacers and 5.8S (i.e., ITS rDNA) were amplified by PCR
228 with ca. 500 bp of the adjacent nuclear ribosomal large subunit (LSU rDNA) as a single fragment
229 using primers ITS1F/LR3 or ITS5/LR3 (Gardes and Bruns, 1993; Vilgalys and Hester, 1990;
230 White et al., 1990). Amplicons were sequenced bidirectionally with the above primers using
231 Applied Biosystems BigDye® Terminator v3.1 cycle sequencing kits (Applied Biosystems 3730*xl*
232 DNA Analyzer; Foster City, CA, USA) at the University of Arizona Genetics Core. The software
233 applications *phred* and *phrap* (Ewing and Green, 1998; Ewing et al., 1998) were used to call
234 bases and assemble contigs with automation provided by the ChromaSeq package in Mesquite
235 (Maddison and Maddison, 2011; http://mesquiteproject.org). Base calls were verified by visual
236 inspection of chromatograms in Sequencher v. 4.5 (Gene Codes, Ann Arbor, MI).
237 Sequences were assembled into groups by first generating a distance matrix in ESPRIT
238 (Sun et al., 2009) based on pairwise Needleman-Wunsch alignments for all sequence pairs with
239 *k*-mer distances less than 0.5, followed by clustering using the furthest neighbor algorithm in
240 mothur (Schloss et al., 2009). Groups were defined by 100%, 99%, and 95% sequence similarity
241 as a proxy for delimiting genotypes (100%, 99%) and putative species (95%) following U'Ren et
242 al. (2009) and Liggenstoffer et al. (2010). A single representative sequence for each 95%
243 similarity group (hereafter, operational taxonomic unit, OTU) was queried against the curated ITS
244 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 µl reaction contained a final concentration of 5 ng of genomic DNA, 0.6 µM 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). All sequences generated from cultures have been deposited in GenBank under accession numbers XXXXX-XXXXX.

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 for these CBS isolates have been deposited in GenBank under accession numbers XXXXX-XXXXX (Supplemental Table 2).

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 back-translated to obtain nucleotide alignments and 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 ≥70% bootstrap value) based on one locus were strongly supported as non-monophyletic (i.e., ≥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 hypoxyloid 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,

385 but which appeared outside of the monophyletic Xylariaceae in these analyses, also were
386 removed (i.e., *Anthostomella torosa*, *Dicyma funiculosa*, *D. pulvinata*, and 13 newly isolated OTU
387 tentatively identified as Xylariaceae based on BLASTn; Supplemental Tables 1-2; Table 1).
388 Because analyses were performed prior to the reclassification of *Seynesia* in the Cainaceae (see
389 Senanayake et al., 2015), these analyses included *Seynesia erupens* as well as two endophyte
390 OTU (clades E1-E2) that are potentially outside Xylariaceae. As described below, their resulting
391 placement is not in conflict with recent studies (see Senanayake et al., 2015).
392
393 *2.7. Phylogenetic analyses of Xylariaceae using a cumulative supermatrix approach*
394
395 When taxon sampling was narrowed to focus on putative Xylariaceae, a total of 79 putative
396 species defined at 95% ITS-partial LSU rDNA sequence similarity from our collections
397 (representing 1,815 isolates total) remained in the analysis. The family-level focus decreased the
398 prevalence of ambiguous regions in the *RPB2* and LSU rDNA alignments, and alignments were
399 adjusted to gain additional phylogenetically informative characters (Supplemental Table 3). For
400 each individual locus, ML analyses and assessment of topological incongruence were performed
401 as described above. No significant conflict was detected between single locus tree topologies
402 with and without recoded data, such that all recoded data were kept in the concatenated dataset.
403 However, conflict among different loci resulted in the removal of seven taxa (FL0975, NC1612,
404 *Nemania diffusa* AT-113, *N. aenea* JF02118, *N. serpens* AT-114, *N. chestersii* JF04024, and
405 *Xylaria* sp. XT09003). For four additional taxa, single sequences that were in conflict with other
406 loci were removed (*RPB2* FL0933; β-tubulin FL0016, FL0804, and *Xylaria escharoidea* 658;
407 Supplemental Table 4). Conflicting sequences potentially represent alternative copies of β-tubulin
408 (see Keeling et al., 2000; Landvik et al., 2001) or contaminants. After removing conflicting
409 sequences single-locus analyses were repeated to assess congruence.
410 Following the assessment of congruence, the single-locus alignments were concatenated
411 into a single supermatrix for subsequent ML analysis. The supermatrix contained 77 putative
412 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 (XXXXX).

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). The R code is available at XXXX.

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.

468 ITS rDNA sequences for newly cultured isolates from our continental surveys, as well as
469 reference taxa, were queried against NCBI's nr database using an e-value cutoff of 1 x e^{-3}. The
470 output was filtered to remove self hits, accessions from U'Ren et al. (2010; 2012), and hits with
471 percent identity <99%. If the same accession was a hit for multiple taxa, the hit with the highest
472 bit score was selected and the duplicate removed. A small fraction of BLASTn hits representing
473 sequences from uncultured isolates (i.e., clones or next-generation sequences) also were
474 excluded (n = 24; 1.9% of filtered hits).
475 From the list of filtered accession numbers, the geographic origin, host lineage (e.g.,
476 Angiosperm), and substrate information (e.g., surface sterilized leaf) for each isolate were
477 extracted from the respective metadata in GenBank and parsed using custom scripts (XXXX)
478 (Supplemental Tables 7-8). In the cases of missing metadata, information was gathered (when
479 possible) from manuscripts in which the sequences were published. Information on geographic
480 distribution, host, and substrate for reference taxa also was collected from published species
481 descriptions and online resources (Supplemental Table 9).
482 To visualize metadata in a phylogenetic framework, information for each terminal taxon
483 was classified into broadly defined categories (Fig. 2). Categories for provenance included (1)
484 U.S., Canada; (2) Mexico, South/Central America, Caribbean; (3) Europe, Russia; (4) Asia; and
485 (5) "Other" (e.g., Hawaii, Australia, New Zealand, Papua New Guinea, Africa, the Middle East,
486 and Antarctica, grouped as "other" due to a paucity of metadata from those sites). Isolates from
487 the Hawaiian Islands were included in "other" rather than U.S./Canada due to their geographical
488 isolation from the continental U.S. Categories for host breadth included (1) angiosperm; (2)
489 "gymnosperm" (i.e., Pinophyta and *Ginkgo biloba*); (3) spore-bearing vascular plant (i.e.,
490 lycophytes and ferns); (4) bryophyte (i.e., mosses and liverworts) and (5) lichen (LT). Substrate
491 categories included (1) living plant leaves or non-woody stems (LP); (2) dead plant leaves in
492 canopy (DP); (3) fallen plant leaves in leaf litter (FP); (4) wood/bark; (5) root; (6) seed; (7) soil; (8)
493 insect-associated; and (9) fallen fruits/inflorescences (Fig. 2). Data for each isolate were added to
494 the phylogenetic tree using a custom R script (XXXX).
495

496 *2.10. Macromorphological characterization of newly collected strains*

497

498 Newly collected isolates representing each putative species were subcultured from water
499 vouchers onto 2% MEA (20 g/L of malt extract [Amresco, Solon, OH, USA] and 15 g/L of agar
500 [Fisher Scientific, Pittsburgh, PA, USA]) and grown at room temperature under ambient light
501 conditions. To verify the identity of each isolate prior to macromorphological characterization,
502 DNA was extracted from each culture using the RedExtract-N-Amp Plant Kit (Sigma-Aldrich)
503 following a modified protocol. Under sterile conditions a small piece of mycelium was placed in a
504 1.5 ml tube with 100 µl of extraction buffer and 100 µl of 0.5 mm zirconium oxide beads (Next
505 Advance, Averill Park, NY, USA). After bead-beating for 1 min, the mycelium was incubated for
506 10 min at 95°C, after which 100 µl of dilution solution was added and the solution was vortexed
507 briefly. DNA was stored at -20°C until used for PCR. The ITS-partial LSU rDNA was amplified by
508 PCR with the primer pair ITS1F/LR3 using REDExtract-N-Amp PCR Ready Mix (Sigma-Aldrich,
509 St. Louis, MO, USA) following the manufacturer's recommendations and the PCR protocol
510 described in Arnold et al. (2007). PCR products were visualized and sequenced as described
511 above. Sequences were verified as 100% identical to expected sequences for each strain. After
512 verification, a 5 mm wide plug of each culture was transferred to 2% oatmeal agar (OA) plates to
513 induce sporulation (Ju et al., 2005). Cultures were grown under ambient light/dark condition at
514 room temperature (ca. 21.5°C) for up to six months before colony morphology was photographed
515 (Supplemental Fig. 3).

516

517 **3. Results and Discussion**

518

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

524 are considered to be particularly diverse, our surveys captured many species of xylariaceous
525 fungi in subtropical, temperate, and boreal hosts (U'Ren, 2011; U'Ren et al., 2012). Incorporating
526 this large collection of plant- and lichen-associated strains from diverse sites in North America
527 into multi-locus phylogenetic analyses with previously described taxa significantly increases the
528 known phylogenetic diversity of Xylariaceae and provides an enriched perspective on
529 relationships proximate to, and within, this ecologically diverse family (Fig. 1, Fig. 2,
530 Supplemental Fig. 1, Supplemental Fig. 2).
531
532 *3.1. Phylogenetic delimitation of Xylariaceae within Xylariomycetidae*
533
534 Most taxa currently recognized as Xylariaceae formed a single clade in our single-locus
535 (phylogenies not shown) and multi-locus analyses (Supplemental Fig. 1). However, several taxa
536 previously described as Xylariaceae (*Anthostomella torosa* AFTOL-ID 732, *Dicyma funiculosa*,
537 and *D. pulvinata*; Ju et al., 1993; Kohlmeyer and Volkmann-Kohlmeyer, 2002; Peláez et al., 2008;
538 Stchigel and Guarro, 1998; Udagawa et al., 1994) appeared outside of this family in single-locus
539 and four-locus analyses (Supplemental Fig. 1). Athough placement of these taxa is uncertain due
540 to low support, both *Dicyma* and *Anthostomella* are known to be problematic with regard to
541 phylogenetic placement (Peláez et al., 2008; also see Daranagama et al., 2015).
542 Xylariaceae and Diatrypaceae have been proposed previously as sister families due to
543 shared morphological features (Parguey-Leduc, 1972; Schrantz, 1960; Rogers, 1979a). However,
544 a recent analysis suggests Xylariaceae may be more closely related to Cainiaceae (Senanayake
545 et al., 2015; see also Maharachchikumbura et al., 2015). In our phylogenetic analysis of the
546 subclass Xylariomycetidae the Cainaceae (represented here by *Seynesia erumpens*, was more
547 closely related to Xylariaceae than the Diatrypaceae (with moderate support, Supplemental Fig.
548 1). When members of Diatrypaceae were used as the outgroup in our analysis focusing on
549 Xylariaceae, *Seynesia erumpens* was nested within the ingroup but outside known Xylariaceae
550 (Fig. 1). *Seynesia* was classified previously in Amphisphaeriaceae (Eriksson and Hawksworth,
551 1991a; 1993), but was moved to Xylariaceae based on ascospore morphology (see Barr, 1990;

552 Eriksson and Hawksworth, 1991b; Hyde, 1995). A recent phylogenetic analysis based on ITS +
553 LSU rDNA placed the genus in Cainiaceae (Senanayake et al., 2015). Our analysis is not in
554 conflict with that placement of *Seynesia,* but uncertainty regarding its family-level placement
555 precludes confident delimitation of the Xylariaceae and family-level placement of the endophyte
556 clades E1 and E2 (Fig. 1, Fig. 2). In future work, multi-locus data for members of the Cainiaceae,
557 Lopadostomaceae, and Coniocessiaceae will be important to clarify the precise delimitation of
558 Xylariaceae.
559 More generally, the placement of many taxa within Xylariomycetidae was challenging due
560 to low support values at many deep internodes. This speaks to a general challenge in the
561 systematics and taxonomy of the subclass and its member families (e.g., Senanayake et al.,
562 2015), leading us to examine the contributions of the loci included in phylogenetic analyses. The
563 final Xylariomycetidae dataset consisted of four loci (ITS rDNA, LSU rDNA, *RPB2*, and β-tubulin
564 and contained a total of 4,700 characters (Supplemental Table 3). In agreement with previous
565 studies of other Ascomycota (e.g., Miadlikowska et al., 2014), alignments of protein coding genes
566 yielded a greater proportion of alignable nucleotides than did the ribosomal genes (Supplemental
567 Table 3). Although the alignment of LSU rDNA was slightly longer than that for *RPB2* (1,272 bp
568 vs. 999 bp, respectively), 97% of nucleotides were unambiguously aligned for *RPB2*, whereas
569 nearly half (46%) of nucleotides in the LSU rDNA alignment could not be aligned unequivocally
570 due largely to the presence of introns (Supplemental Table 3). For the β-tubulin alignment, only
571 exons could be aligned with confidence, but exons contain limited variation (618 distinct
572 alignment patterns). Thus, introns for β-tubulin were recoded using PICS-Ord (Lücking et al.,
573 2011), yielding an alignment with 1,664 distinct alignment patterns. No significant conflict was
574 observed between β-tubulin gene trees with and without the recoded characters, and the percent
575 of nodes with bootstrap support ≥70% increased from 38.8% to 60.9%. A similar strategy was
576 used to recode ITS1 rDNA and ITS2 rDNA, as these hypervariable regions could not be aligned
577 reliably across such distantly related taxa (see Bruns, 2001). Inclusion of recoded ITS rDNA
578 increased the number of alignment patterns for this region from 58 to 305, and the number of
579 well-supported nodes increased from 1.5% to 25.4% in the single locus tree. Accordingly,

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

3.2. Placement of several newly cultured isolates outside Xylariaceae illustrates conflicts with BLASTn results

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

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

3.3. Subfamily structure within Xylariaceae

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

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

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

636 subfamily and the Xylariaceae as a whole suggest an early origin of endophytic and/or
637 endolichenic fungi associated with diverse photosynthetic partners.
638
639 *3.4. Phylogenetic perspectives on Hypoxyloideae*
640
641 Overall, 28 of 77 putative species from our collection (i.e., 26 endophytic and two saprotrophic
642 OTU) were placed within the Hypoxyloideae in our multi-locus analyses, or in affiliation with
643 *Whalleya*, here treated as within Hypoxyloideae (see below). These strains were placed in all
644 genera of Xylariaceae included in the analysis with the exception of *Durotheca* and *Obolarina*.
645 Results of our analyses are largely congruent with the study of Hypoxyloideae by Ju et al.
646 (2007). However, in our topology *Whalleya microplaca* was placed in a clade outside of species
647 currently classified as Hypoxyloideae, whereas Ju et al. (2007) suggested a close relationship of
648 *Whalleya* with species of *Biscogniauxia* and *Theissenia* (renamed *Durotheca*; see Læssøe et al.,
649 2013). Developmentally, *Whalleya microplaca* resembles *Biscogniauxia* (i.e., the presence of
650 bipartite stromata), but stromata and anamorphs are morphologically similar to diatrypaceous
651 fungi (e.g., anamorphic isolates of *Whalleya* have libertella-like conidiogeneous structures;
652 Rogers et al., 1997b; Stadler et al., 2014; see also Glawe and Rogers, 1986). Our analyses
653 placed three endophyte OTU with *W. microplaca* (clade E3, Fig. 1) and placed E3 as sister to the
654 known Hypoxyloideae, with strong support from two bootstrap analyses. Pending morphological
655 examination, we suggest that the Hypoxyloideae may be expanded to include clade E3 (Fig. 1).
656 Within Hypoxyloideae, Hsieh et al. (2005) recognized three major clades based on ML
657 and Bayesian analyses of β-tubulin and α-actin genes: the *Biscogniauxia* clade, the
658 *Annulohypoxylon* clade, and the *Hypoxylon*/*Daldinia* clade. Our analyses recovered a well-
659 supported clade containing all of the *Hypoxylon, Daldinia*, and *Annulohypoxylon* taxa, as well as
660 an isolate of *Rostrohypoxylon terebratum* (see Fournier et al., 2010 for a description of the genus
661 and its paraphyly) (Fig. 1). Within this clade, *Daldinia* and *Annulohypoxylon* form well supported
662 clades in our analyses (Fig. 1). However, species of *Hypoxylon* were found in multiple clades and

663 relationships among the previously defined subclades H1, H2, and H3 (sensu Hsieh et al., 2005)
664 were not confirmed (Fig. 1).
665 Additionally, the position of *Biscogniauxia* as sister to the clade containing *Hypoxylon*,
666 *Annulohypoxylon*, and *Daldinia* (see Hsieh et al., 2005; see also Læssøe et al., 2013) was not
667 recovered here. Our topology suggests that *Biscogniauxia* and *Durotheca* (see Læssøe et al.,
668 2013 for genus description) are sister to one another, but the relationship is not well supported
669 and the placement of this two-genus clade relative to other genera in the Hypoxyloideae is
670 uncertain in our analysis. A sister relationship between these genera would agree with results of
671 high performance liquid chromatography (HPLC) illustrating that the chemical profiles of
672 *Biscogniauxia* and *Durotheca* are closer to each other than to *Hypoxylon* and *Daldinia* (Læssøe
673 et al., 2013). In turn, the highly supported *Biscogniauxia* clade contained isolates of *Camillea*
674 *tinctor* and *Obolarina dryophila* (see Pažoutová et al., 2010b for discussion of *Biscogniauxia*
675 paraphyly).
676
677 3.5. *Phylogenetic perspectives on Xylarioideae*
678
679 Overall, 34 of 77 putative species from our collection (i.e., 32 endophytic and two saprotrophic
680 OTU) were placed within the Xylarioideae as currently circumscribed. Although endophyte OTU
681 were found within all major lineages of the subfamily, they were most abundant in the “NR” and
682 “HY” clades (sensu Hsieh et al., 2010; Figs. 1, and 2D).
683 Hsieh et al. (2010) grouped members of the Xylarioideae into four major clades: (1) the
684 clade containing *Xylaria* spp. associated with termite nests (i.e., subgenus *Pseudoxylaria*; TE); (2)
685 the clade containing *Xylaria hypoxylon* and closely related species (HY); (3) the clade containing
686 *Nemania*, *Rosellinia*, *Entoleuca*, and *Euepixylon* (NR); and (4) the clade containing *Xylaria*
687 *polymorpha* and closely related species (PO). Our analyses strongly support the monophyly of
688 these clades and relationships among them, with the exception of the node encompassing all four
689 clades, which was recovered in all four analyses but never with support values ≥ 70% (Fig. 1).
690 Overall, relationships among terminal branches were highly similar to those reported for

691 described species, and our analyses confirm the early divergence of the *Poronia-Podosordaria*
692 clade within the Xylarioideae (see Hsieh et al., 2010).
693 Our results further suggest a potential expansion of the current circumscription of
694 Xylarioideae. Clades E4-E7, which subtend the currently recognized subfamily, include 12
695 endophyte OTU as well as an *Anthostomella* sp. from Puerto Rico (Fig. 1, Fig. 2C). The
696 phylogenetic placement of two of these three lineages is well supported in our analyses. Two
697 OTU have highly similar ITS rDNA sequences to *Anthostomella* spp. that were not represented in
698 the tree due to lack of multi-locus data (NC1622 with *Anthostomella sepelibilis* strain F-160, 797;
699 AZ1047 with *Anthostomella pinea* CBS 128205) (Table 1, Fig. 2C). However, FL1105, which is
700 placed among isolates with hits to *Anthostomella*, has >95% ITS rDNA sequence identity to
701 *Muscodor vitigenus* in NCBI (Table 1, Fig. 2C). The genus *Muscodor* initially was proposed to
702 include an endophytic fungus that produces antimicrobial volatiles (Worapong et al., 2001), but its
703 taxonomic placement in the Xylariaceae was uncertain (see Stadler et al., 2013). Placement of
704 FL1105 in clade E5 and the monophyly of this clade is highly supported in our Xylariaceae tree
705 (Figs. 1, 2C). Overall, our results suggest that *Anthostomella* is polyphyletic based on the
706 positions of AK1471, FL1651, AZ1047, and NC1622, which are placed in different clades despite
707 high sequence similararity to previously described species of *Anthostomella* (Table 1; Fig. 1; also
708 see Lu et al., 2000; Daranagama et al., 2015). Based on the strong phylogenetic support reported
709 here (Fig. 1, Fig. 2C) and pending morphological examination, we suggest that the Xylarioideae
710 may be expanded to include clades E5-E7.
711
712 *3.6. Limitations of current data sets*
713
714 As noted in previous studies, ribosomal genes alone do not provide enough phylogenetic
715 information to confidently resolve infrafamilial relationships in Xylariomycetidae (Duong et al.,
716 2004) or the evolutionary history among genera of Xylariaceae (Peláez et al., 2008; Tang et al.,
717 2009). In these circumstances, protein-coding genes such as *RPB2* can provide additional,
718 phylogenetic signal that result in more resolved phylogenies of Xylariaceae with higher statistical

719 support for clades (Tang et al., 2007; see also Reeb et al., 2004). Other protein-coding genes,
720 such as β-tubulin and α-actin, also have been used to reconstruct evolutionary relationships for
721 *Hypoxylon* and closely related genera of Xylariaceae (Hsieh et al., 2005). However, in the present
722 study we found that the protein coding genes β-tubulin and *RPB2* were insufficient to resolve
723 infrafamilial relationships in the Xylariomycetidae even when combined with ribosomal loci (ITS
724 rDNA, 5.8S rDNA, LSU rDNA). Analyses of the Xylariaceae using five loci (β-tubulin, α-actin,
725 *RPB2*, ITS plus 5.8S rDNA, and LSU rDNA) also failed to provide high statistical support for many
726 clades within the family.
727 ITS1 and ITS2 rDNA and introns of β-tubulin and α-actin were too divergent to be aligned
728 unambiguously at the ordinal or familial level. However, we used a non-alignment based method
729 (PICS-ord; Lücking et al., 2011) to extract phylogenetic information from these regions that were
730 excluded from the alignments subjected to phylogenetic searches. This method recovered a
731 substantial amount phylogenetic signal and greatly reduced phylogenetic uncertainty.
732 Overall, our results indicate that resolving evolutionary relationships in the Xylariaceae
733 and Xylariomycetidae with high confidence will require sequence data from additional protein-
734 coding genes that can be aligned unambiguously at broad taxonomic levels. Data from the gene
735 for RNA polymerase II largest subunit (*RPB1*), the gene for the minichromosome maintenance
736 complex component 7 (*MCM7*) (Chen et al., 2015; Miadlikowska et al., 2014), and/or additional
737 new molecular markers derived from the AFToL 2 project (aftol.org) are likely to be useful. Our
738 results also argue for increased taxon sampling of both endophytes and morphologically delimited
739 species not currently represented in public sequence databases.
740
741 *3.7. Phylogenetic perspectives on the potential novelty of newly collected strains*
742
743 Putatively novel species of Xylariaceae isolated from plants and lichens across North America
744 were frequently placed in previously described, major clades of Xylariaceae in both subfamilies,
745 including clades containing well-known plant pathogens (i.e., *Biscogniauxia*, *Kretzschmaria*),
746 saprotrophs (i.e., *Xylaria*), and clades thought to be specific to insects or animal dung (i.e.,

747 *Pseudoxylaria* clade and *Poronia + Podosordaria* clade, respectively; see Krug et al., 2004; Hsieh
748 et al., 2010; Rogers et al., 2005; Visser et al., 2009; Figs. 1 and 2C).
749 Although we recovered endophyte-only clades distinct from known or previously
750 sequenced isolates (e.g., E1, 2, 4, 5, 7, 8, and 9), each of which included one ore more putative
751 species, numerous OTU were resolved as closely related to previously described taxa. For
752 example, nine endophyte and saprotroph OTU were placed within a well-supported monophyletic
753 clade with multiple representatives of representative species (Fig. 1, Fig. 2; FL1408 within the
754 *Daldinia eschscholzii* clade; AK1016, AZ0526, AK0128, AK0995 within the *D. loculata* clade;
755 FL1170 within the *Hypoxylon rubiginosum* clade; AZ0703 within the *Biscogniauxia mediterranea*
756 clade; AK0226 within the *Nemania serpens* clade; and NC1011 within the *Xylaria cubensis*
757 clade).
758 However, in cases where endophytic fungi are sister to a single representative of a
759 described species, the topology of the tree alone does not help us determine whether unknown
760 isolates represent the asexual or anamorphic life stage of a described teleomorphic species or a
761 potentially novel closely related species. To address this uncertainty we analyzed the similarity of
762 our isolates to described species in the tree using ITS rDNA clustering. Despite the fact that
763 undescribed isolates are often found in the same clade or are sister to described species, only 14
764 of 77 Xylariaceae OTU isolated in our surveys (i.e., 18.2%) were part of the same 95% OTU as a
765 described species present in the tree (Fig. 2).
766 We further analyzed the ITS rDNA similarity of endophytic and saprotrophic fungi with
767 205 named Xylariaceae taxa (n = 85 species) represented in GenBank by ITS rDNA sequences,
768 but not by β-tubulin, α-actin, *RPB2*, or LSU rDNA (and thus not included in the phylogenetic
769 analyses; Supplemental Table 6). This information, presented within a phylogenetic framework,
770 accesses a large pool of publicly available sequences that exceeds the taxonomic representation
771 available based on other loci or multiple loci. Based on ITS rDNA OTU clustering at 95%
772 similarity, 10 additional OTU found in the multi-locus analysis are highly similar to named
773 sequences in NCBI (see Table 1). Two additional OTU also are similar to sequences in NCBI
774 based on ITS rDNA clustering, but were removed from the concatenated supermatrix due to

775 conflict (Table 1). Combining ITS rDNA similarity in a phylogenetic framework identified several
776 GenBank sequences with taxonomic names in apparent conflict with tree topology (e.g., *Xylaria*
777 *mellissii* F-048,697 found within *Nemania* + *Rosellinia* clade, Fig. 2D; also see above for
778 discussion of *Anthostomella* spp). Only five of the 12 taxa whose ITS rDNA sequences match our
779 isolates are vouchered in easily accessed culture collections (e.g., Centraalbureau voor
780 Schimmelcultures [CBS], American Type Culture Collection [ATTC], Agricultural Research
781 Service Culture Collection [NRRL]) (Table 1; Supplemental Table 6), thus precluding additional
782 morphological and molecular characterization.
783 Overall, 44 of 79 (55.7%) xylariaceous species collected in our surveys (representing 42
784 endophyte and two saprotroph OTU) appear to lack closely related, described species (Table 1;
785 Fig. 2). Of these apparently novel OTU, 34 also lacked closely related BLASTn hits (≥99% ID) to
786 other unnamed fungi in GenBank (Fig. 2). Importantly, these results are based only on the
787 species diversity represented in public databases, and thus do not assess novelty with respect to
788 those species of Xylariaceae known only from their teleomorphs or from drawings, or otherwise
789 not represented in public databases (discussed by Stadler et al., 2013).
790 Such conclusions are dependent on a defined level of ITS rDNA sequence similarity;
791 however, the degree of intraspecific ITS rDNA variability differs among taxonomic groups (see
792 Nilsson et al., 2008). For five *Xylaria* spp., U'Ren et al. (2009) reported low intraspecific variation
793 in ITS rDNA (1.43% ± 2.94%), and variation between sister taxa averaged 4.18% ± 2.18%.
794 However, Stadler et al. (2013) noted that different morphological species can have identical ITS
795 rDNA sequences (e.g., *Daldinia concentrica* and *D. steglichii*). Across both subfamilies, we found
796 12 cases where different morphological species shared the same 95% OTU designation (e.g.,
797 *Xylaria plebeja*, *X. luteostromata* var. *macrospora*, and *X. intracolorata* designated OTU 265; Fig.
798 2). This appears more common for species of Xylarioideae than Hypoxyloideae (Fig. 2).
799 Accordingly, endophyte isolates putatively identified as previously described species may in fact
800 represent novel species. Indeed, we found cases where a comparison of the culture morphology
801 on oatmeal agar for described species (when available) differed from our observations for
802 endophytic isolates. For example, *Hypoxylon 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*, *Hypoxylon 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 gibbisporus* 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,

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

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

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

887 the limited range of their host (e.g., *Pseudoxylaria* on Macrotermitinae termite nests; see above),
888 dispersal limitation, climate, or habitat restrictions (see Whalley, 1985), and/or missing metadata
889 (Supplemental Tables 6-7). Additional sampling from diverse locations and hosts, using both
890 culturing and culture-free next generation sequencing, will help clarify the biogeographic patterns
891 of xylariaceous fungi.
892
893 *3.9. Contributions of endophytes to understanding the ecology of described species:* Daldinia
894 loculata
895
896 Our analyses revealed a monophyletic clade containing five endophytic OTU interspersed with
897 two isolates of *Daldinia loculata* (Fig. 2B). Ascomata of *D. loculata* usually are recovered from
898 burnt or damaged Betulaceae in temperate, boreal, and montane forests of the Northern
899 Hemisphere, although the species also has been observed on wood of Salicaceae, Fagaceae,
900 and Rosaceae (Stadler et al., 2014). It has been reported previously as a foliar endophyte of non-
901 angiosperm hosts (see Pažoutová et al., 2010a). In our surveys, members of the *D. loculata*
902 clade were frequently isolated as endophytes of diverse plants and lichens in boreal and
903 subarctic Alaska. For example, in Eagle Summit, Alaska, we isolated *D. loculata* from
904 asymptomatic, living photosynthetic tissue of evergreen angiosperms, conifers, lycophytes, and
905 bryophytes and long-lived thalli of nine lichen species representing different growth forms,
906 substrates, and photobionts. ITS rDNA analyses suggest that closely related isolates occur in
907 Nome, Alaska, as well as Arizona, Florida, Jamaica, Europe, and New Zealand in a range of
908 biomes from subarctic tundra to tropical high-elevation forest (Fig. 2; Supplemental Tables 1, 6-
909 7). Although previously thought to be rare or absent from subtropical and tropical forests, these
910 data illustrate that the species has a wider geographic and host range than reported previously.
911 Additionally, our data may help illuminate aspects of the lifecycle of *D. loculata*.
912 Previously, the species was proposed to inhabit asymptomatic, living wood of *Betula* until fire kills
913 the host (Guidot et al., 2003; Johannesson et al., 2001a; 2001b; see also Rayner and Boddy,
914 1988). The fungus then grows rapidly within host tissue, forming conidia beneath the bark that are

915 dispersed by insects among burnt trees. When conidia of different mating types interact, the
916 sexual cycle is initiated, producing ascospores that are wind-dispersed to infect the unburned
917 wood of young saplings, where the fungus presumably is endophytic in woody tissues of the host
918 until another fire begins the cycle again (Guidot et al., 2003). Our data illustrate that *D. loculata*
919 was abundant in the photosynthetic tissues of living lichens and evergreen plants in Eagle
920 Summit (Alaska), as well as present in lower abundances in senescent leaves still attached in the
921 canopy and fallen leaves in leaf litter from the previous year) (i.e., DL, FP; Table 1; Supplemental
922 Table 1). However, we did not detect *D. loculata* in newly flushed leaves of *Salix* and *Betula*,
923 which suggests that leaves of these hosts may be colonized from airborne inoculum each
924 season. Whether inoculum is from wind-dispersed ascospores or asexual conidia produced on
925 senescent evergreen plants or lichens in the same site remains to be elucidated, but it implies
926 that the fungus is reproducing during intervals between fires (ca. 50-150 years in this area;
927 Johannesson et al., 2000).
928
929 *3.10. Contributions of endophytes to understanding the ecology of described species:* Xylaria
930 cubensis
931
932 Ascomata of *Xylaria cubensis* are commonly encountered on decomposing angiosperm wood in
933 tropical, subtropical, and temperate forests across the globe, degrading both lignin and cellulose
934 thereby causing a physiological white rot (Rogers, 1984). However, closely related isolates are
935 frequently cultured in endophyte surveys, especially of tropical angiosperms, ferns, and
936 lycophytes (e.g., Fan et al., 2014; Fröhlich et al, 2000; Okane et al., 2008; 2012; Rodrigues,
937 1994; Rodrigues et al., 1995; Rodrigues and Samuels, 1990; 1999; Rodrigues and Petrini, 1997)
938 (Fig. 2E). Our results indicate that *X. cubensis* (represented by NC1011) is a frequent inhabitant
939 of temperate and subtropical lichens (Fig. 2E; Table 1; Supplemental Table 1). Of the 193 *X.*
940 *cubensis* isolates in a single 95% ITS rDNA OTU, 154 were cultured from lichens, whereas the
941 remaining were found from angiosperms, conifers, and bryophytes (Supplemental Table 1; Fig.
942 2E). Even at a finer scale there was no evidence for host specificity: numerous unique ITS rDNA

943 genotypes (based on 100% sequence similarity) were shared among different host species,
944 substrates (lichens, living, and dead plant tissues), as well as geographic locations (see also
945 Okane et al., 2012; Supplemental Table 2). The species was collected from 22 species of lichens
946 in Arizona, North Carolina, and Florida, representing five mycobiont orders (Lecanorales,
947 Teloschistales, Ostropales, Peltigerales, and Umbilicariales) and diverse substrates, growth
948 forms, and photobionts (e.g., various Trebouxiales and *Nostoc* species) (Supplemental Table 1).
949 All investigated lichen genera in North Carolina and Florida (>10 spp. per site) yielded *X.*
950 *cubensis* with the exception of the epiphytic crustose lichen *Herpothallon rubrocintum*, the only
951 representative from the class Arthoniomycetes. However, isolates of *X. cubensis* are reported to
952 have high morphological, genetic, and chemical diversity (Casella et al., 2013; Rodrigues et al.,
953 1993; Rodriguez et al., 1995; also see above for discussion of intraspecific ITS rDNA variation)
954 and additional studies are necessary to elucidate whether *X. cubensis*, as currently defined,
955 represents a complex of several species, each with potentially different host and substrate
956 preferences.
957
958 *3.11. Conclusions*
959
960 The goal of this study was to address the impact of a large collection of plant- and lichen-
961 associated strains on the circumscription and phylogenetic structure of the Xylariaceae, and to
962 evaluate their evolutionary history and ecology in a multi-locus phylogenetic context. Our results,
963 coupled with previous molecular phylogenetic studies, reiterate the need for taxonomic revision at
964 several levels within the Xylariaceae (Hsieh et al., 2005; 2010; Pažoutová et al., 2010b). Such
965 revisionary work is hindered by the sheer diversity of xylariaceous fungi, a shortage of trained
966 mycologists, the lack of reproductive structures in culture for many strains, biases in existing data,
967 the lack of molecular data from type specimens (especially non-ITS rDNA sequence data) and
968 the need for additional phylogenetic molecular markers (see Stadler et al., 2013; Stadler et al.,
969 2014 for discussion of revision; also see Daranagama et al., 2015; Senanayake et al., 2015).
970 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 North America 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 North America 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

1687 included in analyses by Hsieh et al. (2005) and Hsieh et al. (2010), respectively. Black font
1688 indicates isolates from studies listed in Supplemental Table 2 (with the exception of *Obolarina*
1689 and *Durotheca*, none of the genera listed in black has been described formally using both
1690 morphological and molecular data). Light blue strain- and species names indicate specimens
1691 sequenced for the present study. Representative isolates from our surveys are shown as a two-
1692 letter code (AZ, NC, FL, AK) followed by the isolate number, the host and substrate information
1693 for the 95% ITSrDNA-partial LSU OTU represented by that isolate (LT, lichen thallus; LP, living
1694 plant tissue; DP, dead leaves in the canopy; and FP, dead plant leaves in leaf litter), and the total
1695 number of isolates in the OTU (Table 1). Subfamily classifications (i.e., Hypoxyloideae or
1696 Xylarioideae) are based on Stadler et al. (2013). Genera currently unclassified at the subfamily
1697 level (i.e., *Whalleya* and *Anthostomella*; see Stadler et al., 2013), as well as endophyte clades
1698 E3-E7 are indicated on the tree as incertae subfamiliae. Two endophyte OTU (clades E1-E2) as
1699 well as *Seynesia erumpens* are labeled as incertae familiae reflecting the recent proposal of
1700 *Seynesia* as a member of the Cainaceae rather than Xylariaceae (see Senanayake et al., 2015).
1701 The blue star denotes *Hypoxylon argillaceum* CBS 527.63, a potentially misidentified isolate for
1702 which taxonomic revision may be warranted.
1703
1704 **Figure 2.** Phylogenetic integration of geographic and ecological metadata in the evolutionary
1705 context of the Xylariaceae (Fig. 1). Tree topology and clade definitions follow Fig. 1. Thickened
1706 branches indicate maximum likelihood bootstrap values ≥70% from all four bootstrap analyses of
1707 the cumulative supermatrix approach. An asterisk (*) denotes nodes with ≥70% support from at
1708 least one of the bootstrap analyses from the cumulative supermatrix approach (e.g., 5 + 4) (see
1709 Fig. 1). Major clades are shaded according to the ecological mode of the majority of taxa used in
1710 the multilocus analysis: green shading, endophyte clade (numbered E1-E9); grey, saprotroph;
1711 white, pathogen; brown, dung-associated; peach, termite-associated; and purple, equivocal.
1712 Within clades, terminals are labeled with colored fonts to indicate ecological mode of the terminal
1713 taxon based on review of the literature (see Supplemental Table 9 and legend). Within taxon
1714 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.

Supplemental Information

Supplemental Figure 1. Phylogenetic relationships among 517 putative members of the Xylariomycetidae (92 endophytic and saprotrophic OTU [representing 93 total isolates] collected in our surveys and 424 previously named taxa) inferred with maximum likelihood analysis of combined ITS rDNA, LSU rDNA, *RPB2*, and β-tubulin sequences. Three species of *Ophiostoma* (Ophiostomatales, Sordariomycetes) were used to root the tree based on Huhndorf et al. (2004) and the availability of multi-locus data (Supplemental Table 2). Isolates are color-coded based on

recently proposed family designations (see Senanayake et al., 2015). The main clade representing Xylariaceae plus *Graphostroma platystoma* (Graphostromataceae) and *Seynesia erumpens* (Cainaceae), as well as 79 endophyte and saptrotroph OTU from our surveys is collapsed for readability. See main text for discussion of *Graphostroma* and *Seynesia.* Several taxa previously classified as Xylariaceae (*Anthostomella torosa*, *Dicyma funiculosa*, and *D. pulvinata*) were found outside Xylariaceae. Support values are based on 1,000 maximum likelihood bootstrap replicates. Bootstrap values <50% are not shown.

Supplemental Figure 2. Probability density of phylogenetic diversity (PD; the sum of all the edge lengths in the subtree given by a subset of tips) calculated for 77 endophytic taxa and 1,000 random subsets of 209 previously named Xylariaceae taxa (for a total of 286 taxa, which matches the total number of named Xylariaceae taxa used in the phylogenetic analyses; mean PD ± SD = 29.5 ± 0.41). The dotted line represents the observed PD of all previously named Xylariaceae taxa without the OTU gathered in our surveys (PD = 27.91; P<0.001).

Supplemental Figure 3. Macromorphological characterization of 70 xylariaceous isolates (representing 69 OTU based on 95% ITS-partial LSU rDNA similarity) collected in our surveys from five North American sites. Cultures were grown on 2% malt extract agar (MEA) and 2% oatmeal agar (OA) under ambient light/dark condition at room temperature (ca. 21.5°C) for up to six months.

Supplemental Table 1. Geographic and host information for xylariaceous isolates collected in our surveys from five North American sites.

Supplemental Table 2. Reference taxa included in the present study.

Supplemental Table 3. Description of the single locus datasets used for analyses of the Xylariomycetidae and Xylariaceae.

Supplemental Table 4. Taxa with significant conflict (as defined in Materials and Methods) detected among single-locus phylogenies.

Supplemental Table 5. Characteristics of datasets used for the cumulative supermatrix analyses of the Xylariaceae.

Supplemental Table 6. Accession numbers for ITS rDNA sequences from 205 previously named Xylariaceae taxa not present in phylogenetic analyses due to lack of multi-locus data.

Supplemental Table 7. Metadata from ITS rDNA BLASTn hits with ≥99% identity to endophytic, endolichenic, and saprotrophic OTU collected in our surveys from five North American sites. Data reported as listed in NCBI.

Supplemental Table 8. Metadata from ITS rDNA BLASTn hits with ≥99% identity to previously named Xylariaceae taxa. Data reported as listed in NCBI.

Supplemental Table 9. Geographic, host, and substrate information for previously identified Xylariaceae taxa based on published monographs, species descriptions, or online resources.

Figure1

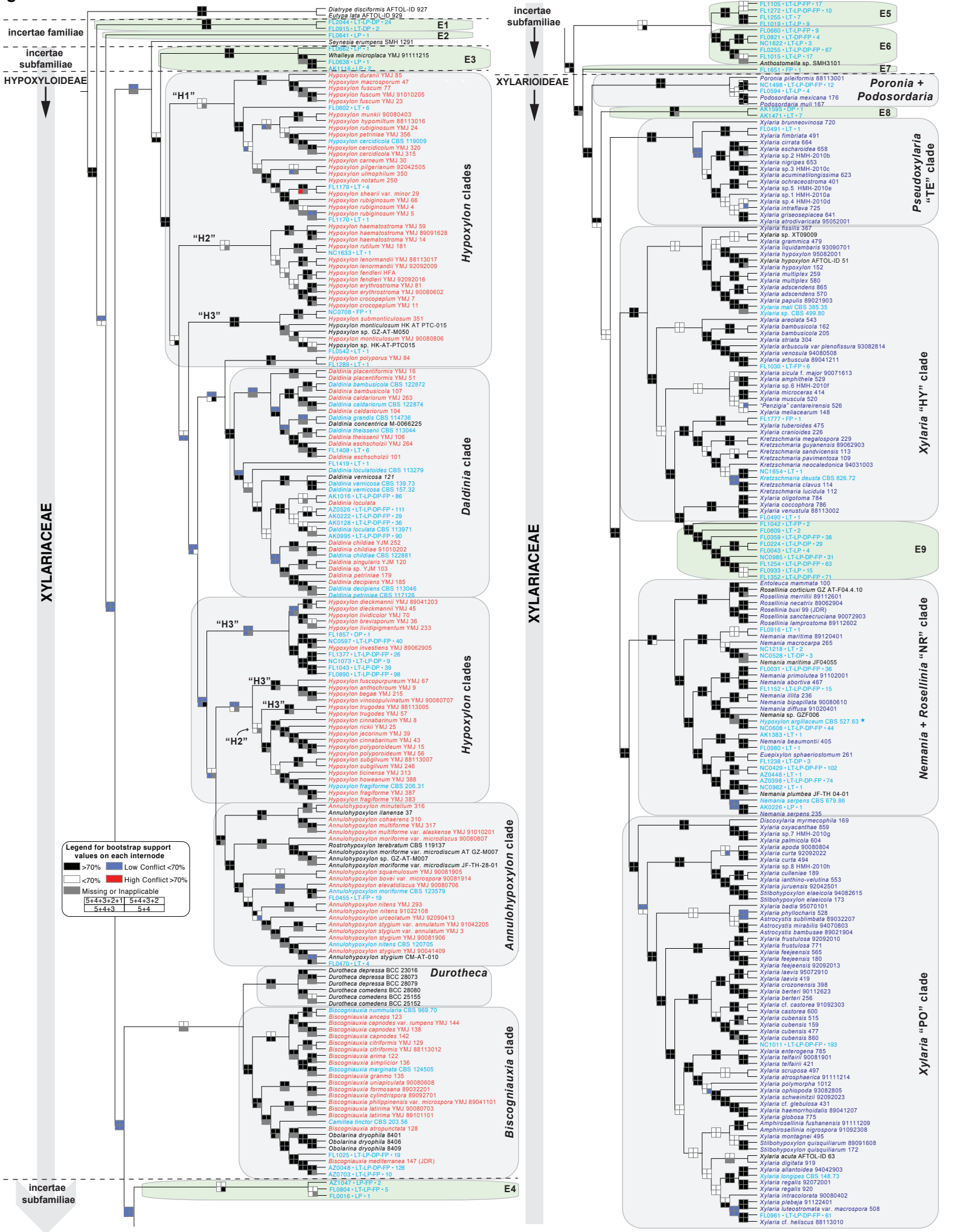
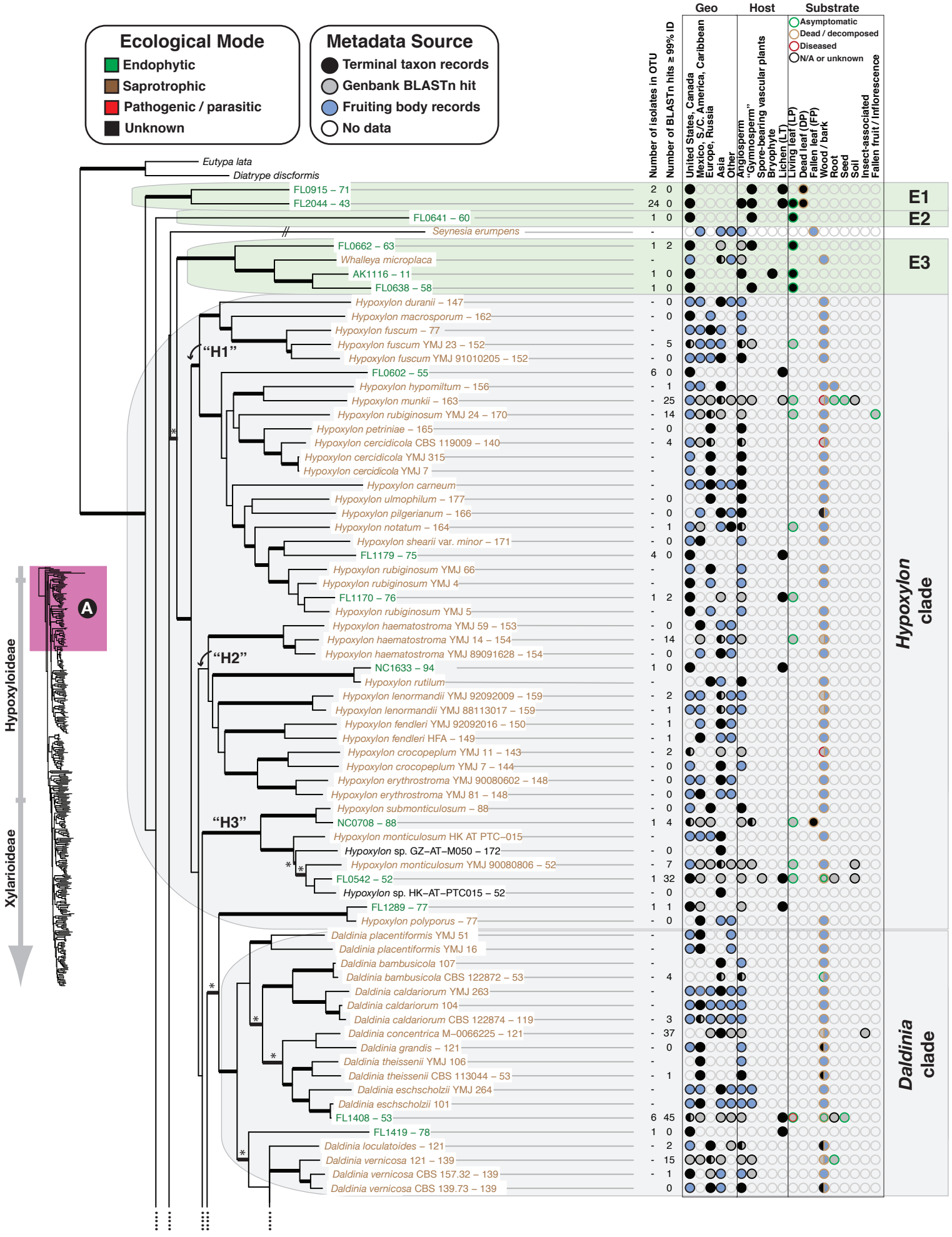
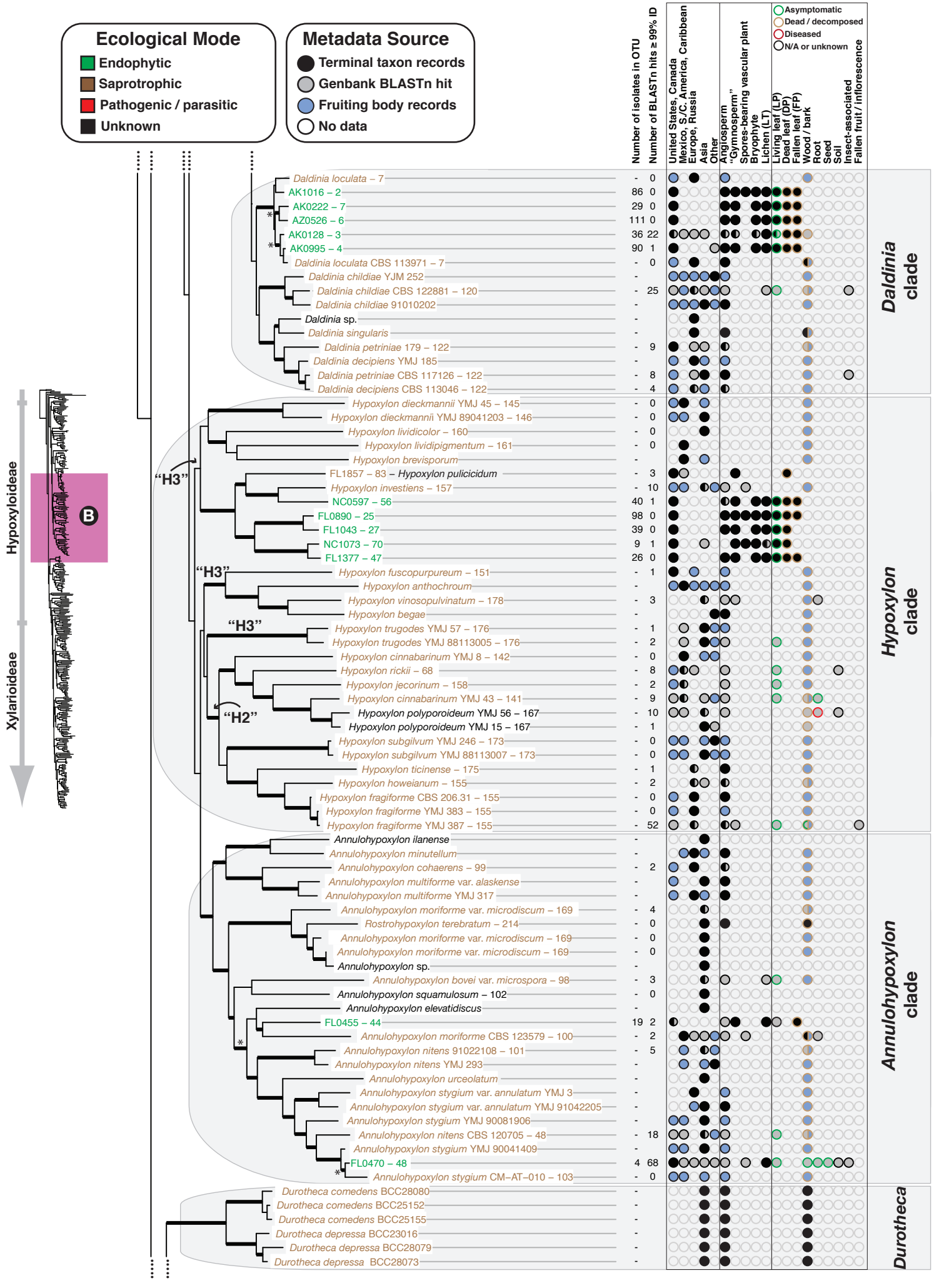

incertae familiae
incertae subfamiliae
HYPOXYLOIDEAE
XYLARIACEAE
incertae subfamiliae
XYLARIOIDEAE
XYLARIACEAE
incertae subfamiliae
E1
E2
E3
E4
E5
E6
E7
E8
E9
"H1"
"H2"
"H3"
Hypoxylon clades
Daldinia clade
Annulohypoxylon clade
Durotheca
Biscogniauxia clade
Pseudoxylaria "TE" clade
Poronia + Podosordaria
Xylaria "HY" clade
Nemania + Rosellinia "NR" clade
Xylaria "PO" clade
Legend for bootstrap support values on each internode
>70%
Low Conflict <70%
<70%
High Conflict >70%
Missing or Inapplicable
5+4+3+2+1
5+4+3+2
5+4+3
5+4

Figure2


Ecological Mode
Endophytic
Saprotrophic
Pathogenic / parasitic
Unknown
Metadata Source
Terminal taxon records
Genbank BLASTn hit
Fruiting body records
No data
Geo
Host
Substrate
Asymptomatic
Dead / decomposed
Diseased
N/A or unknown
Number of isolates in OTU
Number of BLASTn hits ≥ 99% ID
United States, Canada
Mexico, S./C. America, Caribbean
Europe, Russia
Asia
Other
Angiosperm
"Gymnosperm"
Spore-bearing vascular plants
Bryophyte
Lichen (LT)
Living leaf (LP)
Dead leaf (DP)
Fallen leaf (FP)
Wood / bark
Root
Seed
Soil
Insect-associated
Fallen fruit / Inflorescence
Eutypa lata
Diatrype discformis
FL0915 – 71 2 0
FL2044 – 43 24 0
FL0641 – 60 1 0
Seynesia erumpens -
FL0662 – 63 1 2
Whalleya microplaca -
AK1116 – 11 1 0
FL0638 – 58 1 0
E1
E2
E3
Hypoxylon duranii – 147 - 0
Hypoxylon macrosporum – 162 - 0
Hypoxylon fuscum – 77 -
Hypoxylon fuscum YMJ 23 – 152 - 5
Hypoxylon fuscum YMJ 91010205 – 152 - 0
"H1"
FL0602 – 55 6 0
Hypoxylon hypomiltum – 156 - 1
Hypoxylon munkii – 163 - 25
Hypoxylon rubiginosum YMJ 24 – 170 - 14
Hypoxylon petriniae – 165 - 0
Hypoxylon cercidicola CBS 119009 – 140 - 4
Hypoxylon cercidicola YMJ 315 -
Hypoxylon cercidicola YMJ 7 -
Hypoxylon carneum -
Hypoxylon ulmophilum – 177 - 0
Hypoxylon pilgerianum – 166 - 0
Hypoxylon notatum – 164 - 1
Hypoxylon shearii var. minor – 171 - 0
FL1179 – 75 4 0
Hypoxylon rubiginosum YMJ 66 -
Hypoxylon rubiginosum YMJ 4 -
FL1170 – 76 1 2
Hypoxylon rubiginosum YMJ 5 -
Hypoxylon haematostroma YMJ 59 – 153 - 0
Hypoxylon haematostroma YMJ 14 – 154 - 14
Hypoxylon haematostroma YMJ 89091628 – 154 - 0
"H2"
NC1633 – 94 1 0
Hypoxylon rutilum -
Hypoxylon lenormandii YMJ 92092009 – 159 - 2
Hypoxylon lenormandii YMJ 88113017 – 159 - 1
Hypoxylon fendleri YMJ 92092016 – 150 - 1
Hypoxylon fendleri HFA – 149 - 1
Hypoxylon crocopeplum YMJ 11 – 143 - 2
Hypoxylon crocopeplum YMJ 7 – 144 - 0
Hypoxylon erythrostroma YMJ 90080602 – 148 - 0
Hypoxylon erythrostroma YMJ 81 – 148 - 0
Hypoxylon submonticulosum – 88 - 0
"H3"
NC0708 – 88 1 4
Hypoxylon monticulosum HK AT PTC–015 -
Hypoxylon sp. GZ–AT–M050 – 172 - 0
Hypoxylon monticulosum YMJ 90080806 – 52 - 7
FL0542 – 52 1 32
Hypoxylon sp. HK–AT–PTC015 – 52 - 0
FL1289 – 77 1 1
Hypoxylon polyporus – 77 - 0
Hypoxylon clade
Daldinia placentiformis YMJ 51 -
Daldinia placentiformis YMJ 16 -
Daldinia bambusicola 107 -
Daldinia bambusicola CBS 122872 – 53 - 4
Daldinia caldariorum YMJ 263 -
Daldinia caldariorum 104 -
Daldinia caldariorum CBS 122874 – 119 - 3
Daldinia concentrica M–0066225 – 121 - 37
Daldinia grandis – 121 - 0
Daldinia theissenii YMJ 106 -
Daldinia theissenii CBS 113044 – 53 - 1
Daldinia eschscholzii YMJ 264 -
Daldinia eschscholzii 101 -
FL1408 – 53 6 45
FL1419 – 78 1 0
Daldinia loculatoides – 121 - 2
Daldinia vernicosa 121 – 139 - 15
Daldinia vernicosa CBS 157.32 – 139 - 1
Daldinia vernicosa CBS 139.73 – 139 0
Daldinia clade
A
Hypoxyloideae
Xylarioideae


Ecological Mode
Endophytic
Saprotrophic
Pathogenic / parasitic
Unknown
Metadata Source
Terminal taxon records
Genbank BLASTn hit
Fruiting body records
No data
Number of isolates in OTU
Number of BLASTn hits ≥ 99% ID
United States, Canada
Mexico, S./C. America, Caribbean
Europe, Russia
Asia
Other
Angiosperm
"Gymnosperm"
Spores-bearing vascular plant
Bryophyte
Lichen (LT)
Asymptomatic
Dead / decomposed
Diseased
N/A or unknown
Living leaf (LP)
Dead leaf (DP)
Fallen leaf (FP)
Wood / bark
Root
Seed
Soil
Insect-associated
Fallen fruit / inflorescence
Hypoxyloideae
Xylarioideae
B
"H3"
"H3"
"H3"
"H2"
Daldinia clade
Hypoxylon clade
Annulohypoxylon clade
Durotheca
Daldinia loculata – 7 - 0
AK1016 – 2 86 0
AK0222 – 7 29 0
AZ0526 – 6 111 0
AK0128 – 3 36 22
AK0995 – 4 90 1
Daldinia loculata CBS 113971 – 7 - 0
Daldinia childiae YJM 252 -
Daldinia childiae CBS 122881 – 120 - 25
Daldinia childiae 91010202 -
Daldinia sp. -
Daldinia singularis -
Daldinia petriniae 179 – 122 - 9
Daldinia decipiens YMJ 185 -
Daldinia petriniae CBS 117126 – 122 - 8
Daldinia decipiens CBS 113046 – 122 - 4
Hypoxylon dieckmannii YMJ 45 – 145 - 0
Hypoxylon dieckmannii YMJ 89041203 – 146 - 0
Hypoxylon lividicolor – 160 - 0
Hypoxylon lividipigmentum – 161 - 0
Hypoxylon brevisporum -
FL1857 – 83 – Hypoxylon pulicicidum - 3
Hypoxylon investiens – 157 - 10
NC0597 – 56 40 1
FL0890 – 25 98 0
FL1043 – 27 39 0
NC1073 – 70 9 1
FL1377 – 47 26 0
Hypoxylon fuscopurpureum – 151 - 1
Hypoxylon anthochroum -
Hypoxylon vinosopulvinatum – 178 - 3
Hypoxylon begae -
Hypoxylon trugodes YMJ 57 – 176 - 1
Hypoxylon trugodes YMJ 88113005 – 176 - 2
Hypoxylon cinnabarinum YMJ 8 – 142 - 0
Hypoxylon rickii – 68 - 8
Hypoxylon jecorinum – 158 - 2
Hypoxylon cinnabarinum YMJ 43 – 141 - 9
Hypoxylon polyporoideum YMJ 56 – 167 - 10
Hypoxylon polyporoideum YMJ 15 – 167 - 1
Hypoxylon subgilvum YMJ 246 – 173 - 0
Hypoxylon subgilvum YMJ 88113007 – 173 - 0
Hypoxylon ticinense – 175 - 1
Hypoxylon howeianum – 155 - 2
Hypoxylon fragiforme CBS 206.31 – 155 - 0
Hypoxylon fragiforme YMJ 383 – 155 - 0
Hypoxylon fragiforme YMJ 387 – 155 - 52
Annulohypoxylon ilanense -
Annulohypoxylon minutellum -
Annulohypoxylon cohaerens – 99 - 2
Annulohypoxylon multiforme var. alaskense -
Annulohypoxylon multiforme YMJ 317 -
Annulohypoxylon moriforme var. microdiscum – 169 - 4
Rostrohypoxylon terebratum – 214 - 0
Annulohypoxylon moriforme var. microdiscum – 169 - 0
Annulohypoxylon moriforme var. microdiscum – 169 - 0
Annulohypoxylon sp. -
Annulohypoxylon bovei var. microspora – 98 - 3
Annulohypoxylon squamulosum – 102 - 0
Annulohypoxylon elevatidiscus -
FL0455 – 44 19 2
Annulohypoxylon moriforme CBS 123579 – 100 - 2
Annulohypoxylon nitens 91022108 – 101 - 5
Annulohypoxylon nitens YMJ 293 -
Annulohypoxylon urceolatum -
Annulohypoxylon stygium var. annulatum YMJ 3 -
Annulohypoxylon stygium var. annulatum YMJ 91042205 -
Annulohypoxylon stygium YMJ 90081906 -
Annulohypoxylon nitens CBS 120705 – 48 - 18
Annulohypoxylon stygium YMJ 90041409 -
FL0470 – 48 4 68
Annulohypoxylon stygium CM–AT–010 – 103 - 0
Durotheca comedens BCC28080 -
Durotheca comedens BCC25152 -
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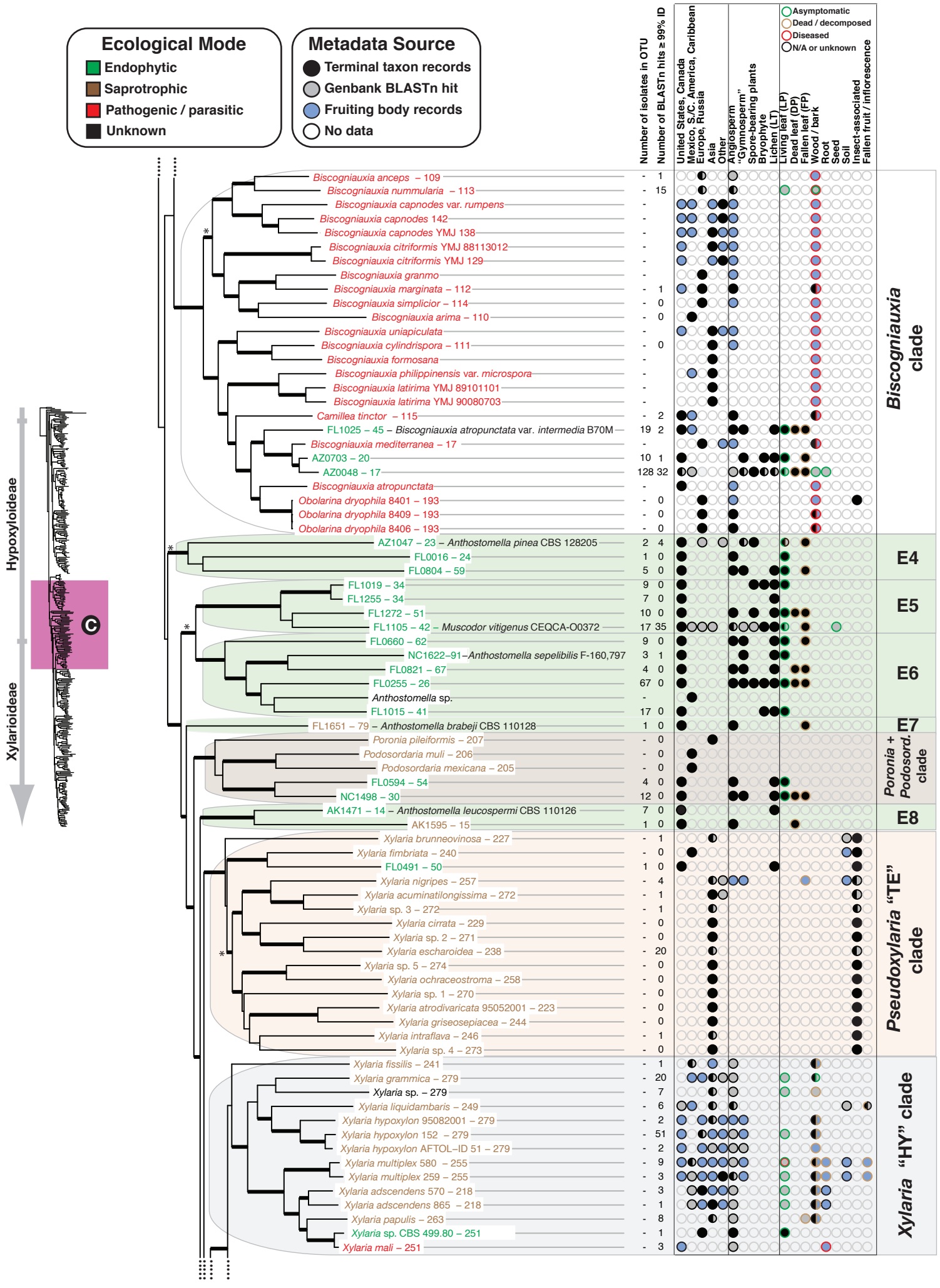
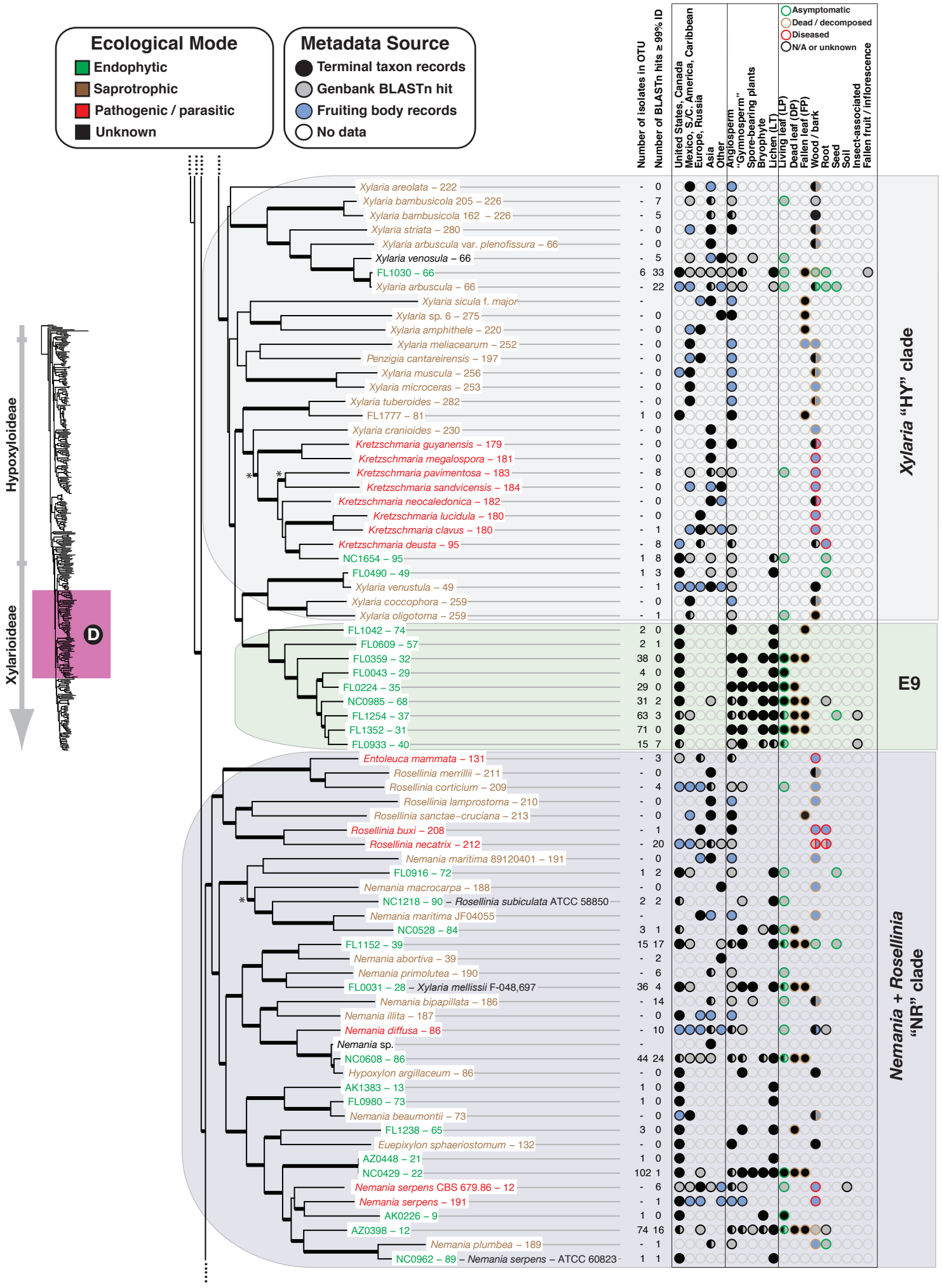
Durotheca depressa BCC23016 -
Durotheca depressa BCC28079 -
Durotheca depressa BCC28073 -


Ecological Mode
Endophytic
Saprotrophic
Pathogenic / parasitic
Unknown
Metadata Source
Terminal taxon records
Genbank BLASTn hit
Fruiting body records
No data
Asymptomatic
Dead / decomposed
Diseased
N/A or unknown
Number of isolates in OTU
Number of BLASTn hits ≥ 99% ID
United States, Canada
Mexico, S./C. America, Caribbean
Europe, Russia
Asia
Other
Angiosperm
"Gymnosperm"
Spore-bearing plants
Bryophyte
Lichen (LT)
Living leaf (LP)
Dead leaf (DP)
Fallen leaf (FP)
Wood / bark
Root
Seed
Soil
Insect-associated
Fallen fruit / inflorescence
Hypoxyloideae
Xylarioideae
C
Biscogniauxia anceps – 109 - 1
Biscogniauxia nummularia – 113 - 15
Biscogniauxia capnodes var. rumpens -
Biscogniauxia capnodes 142 -
Biscogniauxia capnodes YMJ 138 -
Biscogniauxia citriformis YMJ 88113012 -
Biscogniauxia citriformis YMJ 129 -
Biscogniauxia granmo -
Biscogniauxia marginata – 112 - 1
Biscogniauxia simplicior – 114 - 0
Biscogniauxia arima – 110 - 0
Biscogniauxia uniapiculata -
Biscogniauxia cylindrispora – 111 - 0
Biscogniauxia formosana -
Biscogniauxia philippinensis var. microspora -
Biscogniauxia latirima YMJ 89101101 -
Biscogniauxia latirima YMJ 90080703 -
Camillea tinctor – 115 - 2
FL1025 – 45 – Biscogniauxia atropunctata var. intermedia B70M 19 2
Biscogniauxia mediterranea – 17 -
AZ0703 – 20 10 1
AZ0048 – 17 128 32
Biscogniauxia atropunctata -
Obolarina dryophila 8401 – 193 - 0
Obolarina dryophila 8409 – 193 - 0
Obolarina dryophila 8406 – 193 - 0
Biscogniauxia clade
AZ1047 – 23 – Anthostomella pinea CBS 128205 2 4
FL0016 – 24 1 0
FL0804 – 59 5 0
E4
FL1019 – 34 9 0
FL1255 – 34 7 0
FL1272 – 51 10 0
FL1105 – 42 – Muscodor vitigenus CEQCA-O0372 17 35
E5
FL0660 – 62 9 0
NC1622–91–Anthostomella sepelibilis F-160,797 3 1
FL0821 – 67 4 0
FL0255 – 26 67 0
Anthostomella sp. -
FL1015 – 41 17 0
E6
FL1651 – 79 – Anthostomella brabeji CBS 110128 1 0
E7
Poronia pileiformis – 207 - 0
Podosordaria muli – 206 - 0
Podosordaria mexicana – 205 - 0
FL0594 – 54 4 0
NC1498 – 30 12 0
Poronia + Podosord. clade
AK1471 – 14 – Anthostomella leucospermi CBS 110126 7 0
AK1595 – 15 1 0
E8
Xylaria brunneovinosa – 227 - 1
Xylaria fimbriata – 240 - 0
FL0491 – 50 1 0
Xylaria nigripes – 257 - 4
Xylaria acuminatilongissima – 272 - 1
Xylaria sp. 3 – 272 - 1
Xylaria cirrata – 229 - 0
Xylaria sp. 2 – 271 - 0
Xylaria escharoidea – 238 - 20
Xylaria sp. 5 – 274 - 0
Xylaria ochraceostroma – 258 - 0
Xylaria sp. 1 – 270 - 0
Xylaria atrodivaricata 95052001 – 223 - 0
Xylaria griseosepiacea – 244 - 0
Xylaria intraflava – 246 - 1
Xylaria sp. 4 – 273 - 0
Pseudoxylaria "TE" clade
Xylaria fissilis – 241 - 1
Xylaria grammica – 279 - 20
Xylaria sp. – 279 - 7
Xylaria liquidambaris – 249 - 6
Xylaria hypoxylon 95082001 – 279 - 2
Xylaria hypoxylon 152 – 279 - 51
Xylaria hypoxylon AFTOL-ID 51 – 279 - 2
Xylaria multiplex 580 – 255 - 9
Xylaria multiplex 259 – 255 - 3
Xylaria adscendens 570 – 218 - 3
Xylaria adscendens 865 – 218 - 1
Xylaria papulis – 263 - 8
Xylaria sp. CBS 499.80 – 251 - 1
Xylaria mali – 251 - 3
Xylaria "HY" clade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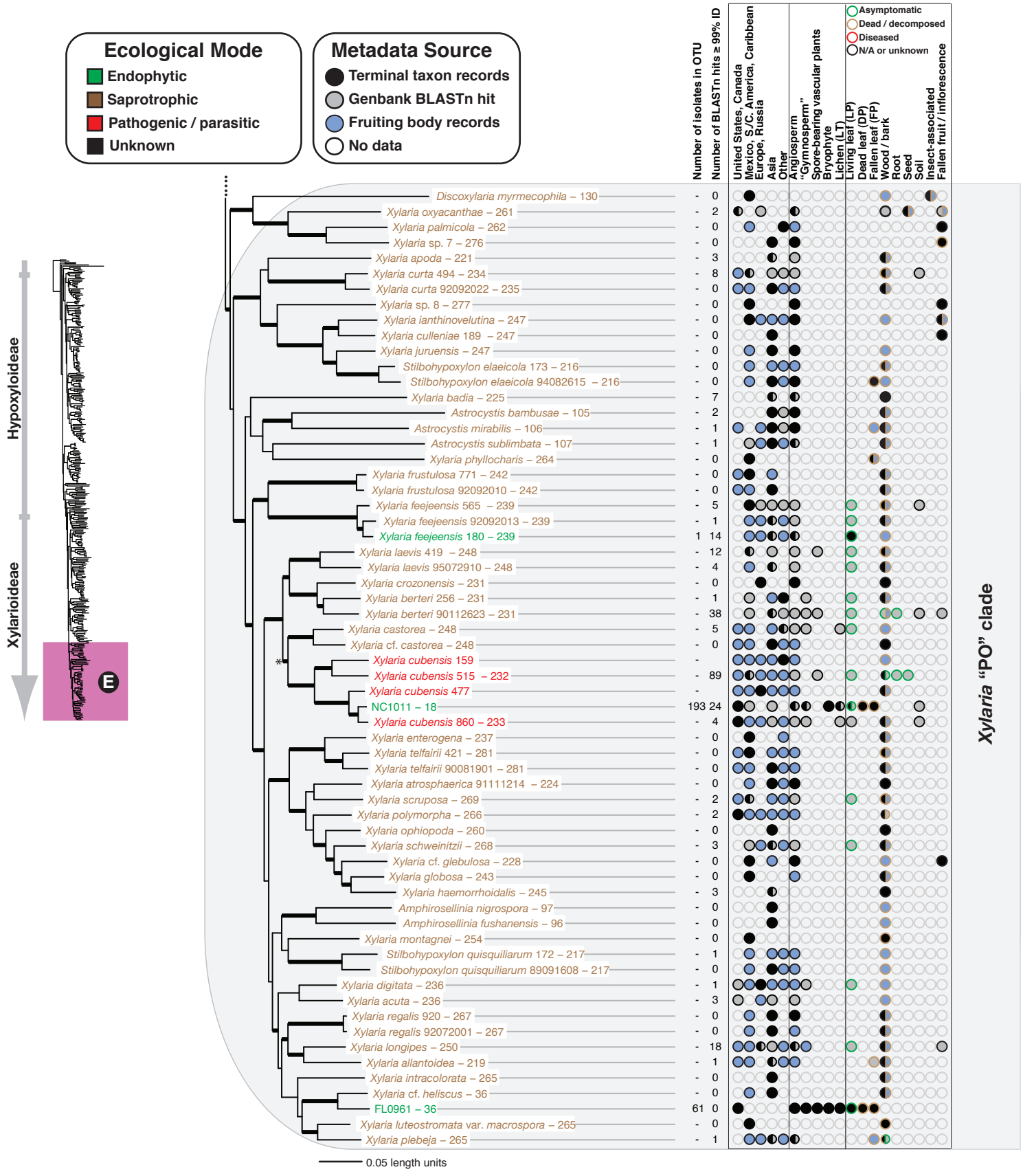
Living leaf (LP)
Dead leaf (DP)
Fallen leaf (FP)
Wood / bark
Root
Seed
Soil
Insect-associated
Fallen fruit / inflorescence
Xylaria areolata – 222 - 0
Xylaria bambusicola 205 – 226 - 7
Xylaria bambusicola 162 – 226 - 5
Xylaria striata – 280 - 0
Xylaria arbuscula var. plenofissura – 66 - 0
Xylaria venosula – 66 - 5
FL1030 – 66 6 33
Xylaria arbuscula – 66 - 22
Xylaria sicula f. major -
Xylaria sp. 6 – 275 - 0
Xylaria amphithele – 220 - 0
Xylaria meliacearum – 252 - 0
Penzigia cantareirensis – 197 - 0
Xylaria muscula – 256 - 0
Xylaria microceras – 253 - 0
Xylaria tuberoides – 282 - 0
FL1777 – 81 1 0
Xylaria cranioides – 230 - 0
Kretzschmaria guyanensis – 179 - 0
Kretzschmaria megalospora – 181 - 0
Kretzschmaria pavimentosa – 183 - 8
Kretzschmaria sandvicensis – 184 - 0
Kretzschmaria neocaledonica – 182 - 0
Kretzschmaria lucidula – 180 - 0
Kretzschmaria clavus – 180 - 1
Kretzschmaria deusta – 95 - 8
NC1654 – 95 1 8
FL0490 – 49 1 3
Xylaria venustula – 49 - 1
Xylaria coccophora – 259 - 0
Xylaria oligotoma – 259 - 1
FL1042 – 74 2 0
FL0609 – 57 2 1
FL0359 – 32 38 0
FL0043 – 29 4 0
FL0224 – 35 29 0
NC0985 – 68 31 2
FL1254 – 37 63 3
FL1352 – 31 71 0
FL0933 – 40 15 7
Entoleuca mammata – 131 - 3
Rosellinia merrillii – 211 - 0
Rosellinia corticium – 209 - 4
Rosellinia lamprostoma – 210 - 0
Rosellinia sanctae–cruciana – 213 - 0
Rosellinia buxi – 208 - 1
Rosellinia necatrix – 212 - 20
Nemania maritima 89120401 – 191 - 0
FL0916 – 72 1 2
Nemania macrocarpa – 188 - 0
NC1218 – 90 – Rosellinia subiculata ATCC 58850 2 2
Nemania maritima JF04055 -
NC0528 – 84 3 1
FL1152 – 39 15 17
Nemania abortiva – 39 - 2
Nemania primolutea – 190 - 6
FL0031 – 28 – Xylaria mellissii F-048,697 36 4
Nemania bipapillata – 186 - 14
Nemania illita – 187 - 0
Nemania diffusa – 86 - 10
Nemania sp. -
NC0608 – 86 44 24
Hypoxylon argillaceum – 86 - 0
AK1383 – 13 1 0
FL0980 – 73 1 0
Nemania beaumontii – 73 - 0
FL1238 – 65 3 0
Euepixylon sphaeriostomum – 132 - 0
AZ0448 – 21 1 0
NC0429 – 22 102 1
Nemania serpens CBS 679.86 – 12 - 6
Nemania serpens – 191 - 1
AK0226 – 9 1 0
AZ0398 – 12 74 16
Nemania plumbea – 189 - 1
NC0962 – 89 – Nemania serpens – ATCC 60823 1 1
Xylaria "HY" clade
E9
Nemania + Rosellinia "NR" clade
Hypoxyloideae
Xylarioideae
D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Soil
Insect-associated
Fallen fruit / inflorescence
Hypoxyloideae
Xylarioideae
E
Discoxylaria myrmecophila – 130 - 0
Xylaria oxyacanthae – 261 - 2
Xylaria palmicola – 262 - 0
Xylaria sp. 7 – 276 - 0
Xylaria apoda – 221 - 3
Xylaria curta 494 – 234 - 8
Xylaria curta 92092022 – 235 - 0
Xylaria sp. 8 – 277 - 0
Xylaria ianthinovelutina – 247 - 0
Xylaria culleniae 189 – 247 - 0
Xylaria juruensis – 247 - 0
Stilbohypoxylon elaeicola 173 – 216 - 0
Stilbohypoxylon elaeicola 94082615 – 216 - 0
Xylaria badia – 225 - 7
Astrocystis bambusae – 105 - 2
Astrocystis mirabilis – 106 - 1
Astrocystis sublimbata – 107 - 1
Xylaria phyllocharis – 264 - 0
Xylaria frustulosa 771 – 242 - 0
Xylaria frustulosa 92092010 – 242 - 0
Xylaria feejeensis 565 – 239 - 5
Xylaria feejeensis 92092013 – 239 - 1
Xylaria feejeensis 180 – 239 1 14
Xylaria laevis 419 – 248 - 12
Xylaria laevis 95072910 – 248 - 4
Xylaria crozonensis – 231 - 0
Xylaria berteri 256 – 231 - 1
Xylaria berteri 90112623 – 231 - 38
Xylaria castorea – 248 - 5
Xylaria cf. castorea – 248 - 0
Xylaria cubensis 159 -
Xylaria cubensis 515 – 232 - 89
Xylaria cubensis 477 -
NC1011 – 18 193 24
Xylaria cubensis 860 – 233 - 4
Xylaria enterogena – 237 - 0
Xylaria telfairii 421 – 281 - 0
Xylaria telfairii 90081901 – 281 - 0
Xylaria atrosphaerica 91111214 – 224 - 0
Xylaria scruposa – 269 - 2
Xylaria polymorpha – 266 - 2
Xylaria ophiopoda – 260 - 0
Xylaria schweinitzii – 268 - 3
Xylaria cf. glebulosa – 228 - 0
Xylaria globosa – 243 - 0
Xylaria haemorrhoidalis – 245 - 3
Amphirosellinia nigrospora – 97 - 0
Amphirosellinia fushanensis – 96 - 0
Xylaria montagnei – 254 - 0
Stilbohypoxylon quisquiliarum 172 – 217 - 1
Stilbohypoxylon quisquiliarum 89091608 – 217 - 0
Xylaria digitata – 236 - 1
Xylaria acuta – 236 - 3
Xylaria regalis 920 – 267 - 0
Xylaria regalis 92072001 – 267 - 0
Xylaria longipes – 250 - 18
Xylaria allantoidea – 219 - 1
Xylaria intracolorata – 265 - 0
Xylaria cf. heliscus – 36 - 0
FL0961 – 36 61 0
Xylaria luteostromata var. macrospora – 265 - 0
Xylaria plebeja – 265 - 1
0.05 length units
Xylaria "PO" clade

Table1

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 North American sites. GenBank accession numbers in bold represent new sequences generated as part of this study.

									GenBank accession numbers			
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]**	**ITS - partial LSU rDNA**	***RPB2***	**β-tubulin**	**α-actin**
AK1199	AKN	*Equisetum arvense*	LP	10, 14	6	0, 4, 0, 2	0, 2, 0, 4	Xylariomycetidae incertae sedis	JQ759542	**submit***	**submit***	**submit***
AZ0196	AZC	*Quercus rugosa*	DP	16, 25	69	0, 14, 35, 20	42, 18, 9, 0	Xylariomycetidae incertae sedis	HM122945	**submit***	**submit***	**submit***
AZ0339	AZC	*Quercus rugosa*	FP	19, 28	13	0, 0, 1, 12	2, 7, 4, 0	Xylariomycetidae incertae sedis	HM123082	**submit***	**submit***	**submit***
FL0674	FLA	*Pinus elliottii*	LP	33, 55	6	0, 3, 2, 1	0, 0, 6, 0	Xylariomycetidae incertae sedis	JQ760367	**submit***	**submit***	**submit***
FL0456	FLA	*Cladonia didyma*	LT	46, 86	1	1, 0, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760182	**submit***	**submit***	**submit***
FL0650	FLA	*Pinus elliottii*	LP	61, 248	2	0, 2, 0, 0	0, 0, 2, 0	Xylariomycetidae incertae sedis	JQ760354	**submit***	**submit***	**submit***
FL0677	FLA	*Pinus elliottii*	LP	64, 123	1	0, 1, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760370	**submit***	**submit***	NA
FL0850	FLA	*Herpothallon rubrocinctum*	LT	69, 133	1	1, 0, 0, 0	0, 0, 1, 0	Xylariomycetidae incertae sedis	JQ760489	**submit***	**submit***	NA
FL1681	FLA	*Pinus clausa*	FP	80, 185	1	0, 0, 0, 1	0, 0, 1, 0	Xylariomycetidae incertae sedis	submit*	**submit***	**submit***	NA
FL1780	FLA	*Quercus inopina*	FP	82, 188	1	0, 0, 0, 1	0, 0, 1, 0	Xylariomycetidae incertae sedis	submit*	**submit***	**submit***	**submit***
FL2152	FLA	*Pinus clausa*	DP	85, 191	2	0, 0, 2, 0	0, 0, 2, 0	Xylariomycetidae incertae sedis	submit*	**submit***	**submit***	**submit***
NC0532	NCH	*Hypnum* sp.	LP	87, 205	14	3, 11, 0, 0	0, 14, 0, 0	Xylariomycetidae incertae sedis	JQ761527	**submit***	NA	**submit***
NC1491	NCH	*Pseudevernia consocians*	LT	92, 236	1	1, 0, 0, 0	0, 1, 0, 0	Xylariomycetidae incertae sedis	JQ761899	**submit***	**submit***	**submit***
FL2044	FLA	*Serenoa repens*	DP	43, 95	24	17, 1, 6, 0	0, 0, 24, 0	Xylariomycetidae incertae sedis	submit*	**submit***	**submit***	**submit***
FL0915	FLA	*Cladonia subradiata*	LT	71, 137	2	1, 0, 1, 0	0, 0, 2, 0	Xylariomycetidae sp. nov.	JQ760548	**submit***	**submit***	NA
FL0641	FLA	*Pinus elliottii*	LP	60, 120	1	0, 1, 0, 0	0, 0, 1, 0	Xylariomycetidae sp. nov.	JQ760347	**submit***	NA	**submit***
FL0662	FLA	*Pinus elliottii*	LP	63, 122	1	0, 1, 0, 0	0, 0, 1, 0	Xylariaceae sp. nov.	JQ760360	**submit***	**submit***	**submit***
FL0638	FLA	*Pinus elliottii*	LP	58, 118	1	0, 1, 0, 0	0, 0, 1, 0	Xylariaceae sp. nov.	JQ760345	**submit***	NA	**submit***
AK1116	AKN	*Cassiope tetragona*	LP	11, 15	2	0, 2, 0, 0	0, 0, 0, 2	Xylariaceae sp. nov.	JQ759464	**submit***	**submit***	NA
FL0602	FLA	*Cladonia evansii*	LT	55, 113	6	6, 0, 0, 0	0, 0, 6, 0	*Hypoxylon* sp. nov.	JQ760314	**submit***	**submit***	**submit***
FL1179	FLA	*Pyxine eschweileri*	LT	75, 161	4	4, 0, 0, 0	0, 0, 4, 0	*Hypoxylon* sp. nov.	JQ760795	**submit***	**submit***	**submit***
FL1170	FLA	*Parmotrema rampoddense*	LT	76, 159	1	1, 0, 0, 0	0, 0, 1, 0	*Hypoxylon rubiginosum*	JQ760786	**submit***	**submit***	**submit***
NC1633	NCH	*Diploschistes scruposus*	LT	94, 242	1	1, 0, 0, 0	0, 1, 0, 0	*Hypoxylon* sp. nov.	JQ761992	NA	NA	**submit***
NC0708	NCH	*Pinus strobus*	FP	88, 214	1	0, 0, 0, 1	0, 1, 0, 0	*Hypoxylon submonticulosum*	submit*	**submit***	**submit***	**submit***
FL0542	FLA	*Cladonia leporina*	LT	52, 104	1	1, 0, 0, 0	0, 0, 1, 0	*Hypoxylon monticulosum*	JQ760257	**submit***	**submit***	**submit***
FL1289	FLA	*Cladonia leporina*	LT	77, 169	1	1, 0, 0, 0	0, 0, 1, 0	*Hypoxylon polyporus*	JQ760904	**submit***	**submit***	**submit***
FL1408	FLA	*Parmotrema tinctorum*	LT	53, 111	6	6, 0, 0, 0	0, 0, 6, 0	*Daldinia eschscholzii*	JQ761025	**submit***	**submit***	NA
FL1419	FLA	*Parmotrema tinctorum*	LT	78, 179	1	1, 0, 0, 0	0, 0, 1, 0	*Daldinia* sp. nov.	JQ761035	**submit***	**submit***	**submit***
AK1016	AKE	*Arctoparmelia separata*	LT	2, 2	86	53, 25, 2, 6	0, 0, 0, 86	*Daldinia loculata*	JQ759383	**submit***	**submit***	**submit***
AZ0526	AZC	*Physcia caesia*	LT	6, 7	111	48, 38, 11, 14	1, 0, 0, 110	*Daldinia loculata*	HM123248	**submit***	**submit***	**submit***
AK0222	AKE	*Pleurozium schreberi*	LP	7, 8	29	16, 11, 1, 1	0, 0, 5, 24	*Daldinia loculata*	JQ758703	**submit***	**submit***	**submit***
AK0128	AKE	*Pleurozium schreberi*	LP	3, 3	36	17, 11, 2, 6	0, 0, 0, 36	*Daldinia loculata*	JQ758621	**submit***	**submit***	**submit***

AK0995	AKE	*Flavocetraria cucullata*	LT	4, 4	90	47, 37, 1, 5	0, 0, 0, 90	*Daldinia loculata*	JQ759362	**submit***	**submit***	**submit***
FL1857	FLA	*Pinus elliottii*	DP	83, 189	1	0, 0, 1, 0	0, 0, 1, 0	*Hypoxylon pulicicidum* (matches JX183075)	submit*	**submit***	**submit***	**submit***
NC0597	NCH	*Pseudevernia consocians*	LT	56, 114	40	33, 5, 1, 1	0, 36, 4, 0	Hypoxylon sp. nov.	JQ761586	**submit***	**submit***	**submit***
FL1377	FLA	*Parmotrema tinctorum*	LT	47, 105	26	17, 4, 2, 3	0, 19, 7, 0	Hypoxylon sp. nov.	JQ760995	**submit***	**submit***	**submit***
NC1073	NCH	*Selaginella tortipila*	LP	70, 206	9	1, 6, 2, 0	0, 8, 1, 0	Hypoxylon sp. nov.	JQ761719	**submit***	**submit***	**submit***
FL1043	FLA	*Cladonia evansii*	LT	27, 46	39	26, 10, 3, 0	0, 0, 39, 0	Hypoxylon sp. nov.	JQ760666	**submit***	**submit***	**submit***
FL0890	FLA	*Pyxine eschweileri*	LT	25, 41	98	65, 15, 7, 11	0, 0, 98, 0	Hypoxylon sp. nov.	JQ760526	**submit***	**submit***	**submit***
FL0455	FLA	*Cladonia didyma*	LT	44, 246	19	18, 0, 0, 1	0, 0, 19, 0	*Annulohypoxylon* sp. nov.	JQ760181	**submit***	**submit***	**submit***
FL0470	FLA	*Cladonia didyma*	LT	48, 89	4	4, 0, 0, 0	0, 0, 4, 0	*Annulohypoxylon stygium*	JQ760192	**submit***	**submit***	**submit***
FL1025	FLA	*Cladonia evansii*	LT	45, 85	19	15, 2, 1, 1	0, 7, 12, 0	*Biscogniauxia atropunctata* var. *intermedia* (matches AJ390412)	JQ760650	**submit***	**submit***	**submit***
AZ0048	AZC	*Flavoparmelia praesignis*	LT	17, 23	128	109, 10, 5, 4	89, 30, 9, 0	*Biscogniauxia mediterranea*	HM122805	NA	NA	**submit***
AZ0703	AZC	*Pseudevernia intensa*	LT	20, 31	10	8, 1, 0, 1	2, 7, 1, 0	*Biscogniauxia mediterranea*	HM123416	**submit***	**submit***	**submit***
AZ1047	AZC	*Woodsia plummerae*	LP	23, 39	2	0, 1, 0, 1	2, 0, 0, 0	*Anthostomella pinea* (matches HQ599578)	HM123694	**submit***	NA	**submit***
FL0804	FLA	*Cladonia subtenius*	LT	59, 119	5	2, 1, 0, 2	0, 0, 5, 0	Xylariaceae sp. nov.	JQ760457	**submit***	NA	**submit***
FL0016	FLA	*Aristida stricta*	LP	24, 40	1	0, 1, 0, 0	0, 0, 1, 0	Xylariaceae sp. nov.	JQ759892	**submit***	NA	**submit***
FL1105	FLA	*Cladonia subtenius*	LT	42, 74	17	13, 1, 0, 3	0, 0, 17, 0	*Muscodor vitigenus* (matches KC771503)	JQ760728	**submit***	**submit***	**submit***
FL1272	FLA	*Usnea subscabrosa*	LT	51, 125	10	6, 1, 1, 2	0, 0, 10, 0	Xylariaceae sp. nov.	JQ760887	**submit***	**submit***	**submit***
FL1255[b]	FLA	*Usnea subscabrosa*	LT	34, 83	7	7, 0, 0, 0	0, 0, 7, 0	Xylariaceae sp. nov.	JQ760870	**submit***	**submit***	**submit***
FL1019[b]	FLA	*Parmotrema perforatum*	LT	34, 66	9	6, 3, 0, 0	0, 0, 9, 0	Xylariaceae sp. nov.	JQ760644	**submit***	**submit***	**submit***
FL0660	FLA	*Pinus elliottii*	LP	62, 121	9	2, 1, 0, 6	0, 0, 9, 0	Xylariaceae sp. nov.	JQ760358	**submit***	**submit***	**submit***
FL0821	FLA	*Cladonia subtenius*	LT	67, 129	4	1, 0, 1, 2	0, 0, 4, 0	Xylariaceae sp. nov.	JQ760469	**submit***	**submit***	NA
NC1622	NCH	*Tsuga canadensis*	LP	91, 241	3	1, 2, 0, 0	0, 3, 0, 0	*Anthostomella sepelibilis* (matches AY908990)	JQ761984	**submit***	NA	**submit***
FL0255	FLA	*Pinus elliottii*	LP	26, 43	67	36, 19, 2, 10	0, 0, 67, 0	Xylariaceae sp. nov.	JQ760030	NA	**submit***	NA
FL1015	FLA	*Parmotrema perforatum*	LT	41, 81	17	16, 1, 0, 0	0, 0, 17, 0	Xylariaceae sp. nov.	JQ760640	**submit***	**submit***	**submit***
FL1651	FLA	*Quercus inopina*	FP	79, 184	1	0, 0, 0, 1	0, 0, 1, 0	*Anthostomella brabeji* (matches EU552098)[d]	submit*	**submit***	**submit***	NA
NC1498	NCH	*Xanthoparmelia conspersa*	LT	30, 237	12	7, 3, 1, 1	0, 4, 8, 0	Xylariaceae sp. nov.	JQ761906	**submit***	NA	**submit***
FL0594	FLA	*Cladonia evansii*	LT	54, 112	4	3, 1, 0, 0	0, 0, 4, 0	Xylariaceae sp. nov.	JQ760306	**submit***	**submit***	**submit***
AK1595[a]	AKE	*Salix pulchra*	DP	15, 21	1	0, 0, 1, 0	0, 0, 0, 1	Xylariaceae sp. nov.	JQ759870	**submit***	NA	NA
AK1471[a]	AKN	*Peltigera aphthosa*	LT	14, 19	7	7, 0, 0, 0	2, 1, 2, 2	*Anthostomella leucospermi* (matches EU552100)[d]	JQ759768	**submit***	**submit***	**submit***
FL0491	FLA	*Cladonia didyma*	LT	50, 94	1	1, 0, 0, 0	0, 0, 1, 0	*Xylaria* sp. nov.	JQ760210	**submit***	**submit***	**submit***
FL1030	FLA	*Cladonia evansii*	LT	66, 146	6	5, 0, 0, 1	0, 0, 6, 0	*Xylaria arbuscula*	JQ760654	**submit***	**submit***	**submit***
FL1777	FLA	*Quercus inopina*	FP	81, 187	1	0, 0, 0, 1	0, 0, 1, 0	*Xylaria sp. nov.*	submit*	**submit***	**submit***	**submit***
NC1654	NCH	*Flavoparmelia caperata*	LT	95, 244	1	1, 0, 0, 0	0, 1, 0, 0	*Kretzschmaria deusta*	JQ762008	**submit***	**submit***	**submit***
FL0490	FLA	*Cladonia didyma*	LT	49, 93	1	1, 0, 0, 0	0, 0, 1, 0	*Xylaria venustula*	JQ760209	NA	**submit***	**submit***
FL1042	FLA	*Cladonia evansii*	LT	74, 147	2	1, 0, 0, 1	0, 0, 2, 0	*Xylaria* sp. nov.	JQ760665	**submit***	**submit***	**submit***
FL0609	FLA	*Cladonia evansii*	LT	57, 116	2	2, 0, 0, 0	0, 0, 2, 0	*Xylaria* sp. nov.	JQ760320	**submit***	**submit***	**submit***

FL0359	FLA	*Pinus clausa*	LP	32, 53	38	28, 6, 2, 2	0, 9, 29, 0	*Xylaria* sp. nov.	JQ760113	**submit***	**submit***	**submit***
FL0224	FLA	*Pinus elliottii*	LP	35, 67	29	21, 7, 1, 0	0, 0, 29, 0	*Xylaria* sp. nov.	JQ760020	**submit***	**submit***	**submit***
FL0043	FLA	*Pinus elliottii*	LP	29, 50	4	3, 1, 0, 0	0, 0, 4, 0	*Xylaria* sp. nov.	JQ759911	**submit***	**submit***	**submit***
NC0985	NCH	*Flavoparmelia caperata*	LT	68, 222	31	19, 6, 4, 2	0, 15, 16, 0	*Xylaria* sp. nov.	JQ761635	**submit***	**submit***	**submit***
FL1254	FLA	*Usnea subscabrosa*	LT	37, 92	63	46, 7, 4, 6	0, 13, 50, 0	*Xylaria* sp. nov.	JQ760869	**submit***	**submit***	**submit***
FL0933	FLA	*Cladonia subradiata*	LT	40, 72	15	13, 2, 0, 0	0, 4, 11, 0	*Xylaria* sp. nov.	JQ760565	**submit***	**submit***	**submit***
FL1352	FLA	*Cladonia evansii*	LT	31, 103	71	55, 5, 6, 5	0, 13, 58, 0	*Xylaria* sp. nov.	JQ760970	**submit***	**submit***	**submit***
FL0916	FLA	*Cladonia subradiata*	LT	72, 138	1	1, 0, 0, 0	0, 0, 1, 0	*Nemania* sp. nov.	JQ760549	**submit***	**submit***	**submit***
NC1218	NCH	*Lasalia pensylvanica*	LT	90, 226	2	2, 0, 0, 0	0, 2, 0, 0	*Rosellinia subiculata* (matches AY909002)[d]	JQ761857	NA	**submit***	**submit***
NC0528	NCH	*Flavoparmelia caperata*	LT	84, 210	3	2, 0, 1, 0	0, 2, 1, 0	*Nemania* sp. nov.	JQ761524	**submit***	**submit***	**submit***
FL0031	FLA	*Pinus elliottii*	LP	28, 47	36	29, 4, 2, 1	0, 0, 36, 0	*Nemania* sp. (matches FJ175173)	JQ759904	**submit***	**submit***	**submit***
FL1152	FLA	*Parmotrema rampoddense*	LT	39, 71	15	10, 1, 2, 2	0, 0, 15, 0	*Nemania abortiva*	JQ760771	**submit***	**submit***	**submit***
NC0608	NCH	*Parmotrema reticulatum*	LT	86, 193	44	27, 6, 3, 8	0, 44, 0, 0	*Nemania diffusa*	JQ761596	**submit***	**submit***	**submit***
AK1383[a]	AKN	*Hypogymnia physodes*	LT	13, 18	1	1, 0, 0, 0	0, 0, 0, 1	*Nemania* sp. nov.	JQ759694	**submit***	**submit***	**submit***
FL0980	FLA	*Usnea mutabilis*	LT	73, 142	1	1, 0, 0, 0	0, 0, 1, 0	*Nemania beaumontii*	JQ760608	**submit***	**submit***	**submit***
FL1238	FLA	*Usnea subscabrosa*	LT	65, 127	3	2, 0, 1, 0	0, 0, 3, 0	Xylariaceae sp. nov.	JQ760853	**submit***	**submit***	**submit***
NC0429[a]	NCH	*Diploschistes scruposus*	LT	22, 199	102	67, 27, 3, 5	1, 33, 68, 0	Xylariaceae sp. nov.	JQ761458	**submit***	**submit***	**submit***
AZ0448	AZC	*Punctelia hypoleucites*	LT	21, 32	1	1, 0, 0, 0	1, 0, 0, 0	Xylariaceae sp. nov.	HM123176	**submit***	**submit***	**submit***
AZ0398[a]	AZC	*Peltigera rufescens*	LT	12, 30	74	62, 6, 2, 4	35, 32, 2, 5	*Nemania serpens*	HM123137	**submit***	**submit***	**submit***
NC0962	NCH	*Sticta beauvoisii*	LT	89, 219	1	1, 0, 0, 0	0, 1, 0, 0	*Nemania serpens* (matches AF201703)[d]	JQ761617	**submit***	**submit***	**submit***
AK0226[a]	AKE	*Pleurozium schreberi*	LP	9, 12	1	0, 1, 0, 0	0, 0, 0, 1	*Nemania serpens*	JQ758707	**submit***	**submit***	NA
NC1011	NCH	*Lecanora oreinoides*	LT	18, 27	193	154, 30, 4, 5	1, 142, 50, 0	*Xylaria cubensis*	JQ761659	**submit***	**submit***	**submit***
FL0961	FLA	*Usnea mutabilis*	LT	36, 99	61	46, 7, 3, 5	0, 23, 38, 0	*Xylaria* cf. *heliscus*	JQ760593	**submit***	**submit***	**submit***
FL0975†	FLA	*Usnea mutabilis*	LT	38, 69	36	33, 1, 0, 2	0, 5, 31, 0	*Muscodor yucatanensis* (matches FJ917287)	JQ760604	**submit***	**submit***	NA
NC1612†	NCH	*Lecanora oreinoides*	LT	93, 240	1	1, 0, 0, 0	0, 1, 0, 0	*Hypoxylon papillatum* (matches AF201710)	JQ761975	**submit***	**submit***	**submit***

* 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 mulitgene analyses due to toplogical 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 or

[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).

Supplementary Tables

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Supplementary Fig. 1

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