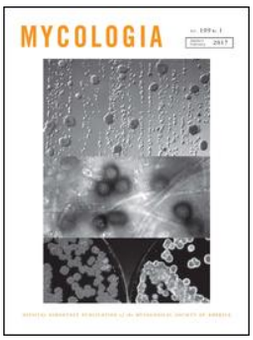

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Survey of corticioid fungi in North American pinaceous forests reveals hyperdiversity, underpopulated sequence databases, and species that are potentially ectomycorrhizal

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ABSTRACT

The corticioid fungi are commonly encountered, highly diverse, ecologically important, and understudied. We collected specimens in 60 pine and spruce forests across North America to survey corticioid fungal frequency and distribution and to compile an internal transcribed spacer (ITS) database for the group. Sanger sequences from the ITS region of vouchered specimens were compared with sequences on GenBank and UNITE, and with high-throughput sequence data from soil and roots taken at the same sites. Out of 425 high-quality Sanger sequences from vouchered specimens, we recovered 223 distinct operational taxonomic units (OTUs), the majority of which could not be assigned to species by matching to the BLAST database. Corticioid fungi were found to be hyperdiverse, as supported by the observations that nearly two-thirds of our OTUs were represented by single collections and species estimator curves showed steep slopes with no plateaus. We estimate that 14.8–24.7% of our voucher-based OTUs are likely to be ectomycorrhizal (EM). Corticioid fungi recovered from the soil formed a different community assemblage, with EM taxa accounting for 40.5–58.6% of OTUs. We compared basidioma sequences with EM root tips from our data, GenBank, or UNITE, and with this approach, we reiterate existing speculations that *Trechispora stellulata* is EM. We found that corticioid fungi have a significant distance-decay pattern, adding to the literature supporting fungi as having geographically structured communities. This study provides a first view of the diversity of this important group across North American pine forests, but much of the biology and taxonomy of these diverse, important, and widespread fungi remains unknown.

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INTRODUCTION

Despite major advances in fungal ecology due to the advent of molecular techniques (Peay et al. 2008), very little progress has been made in understanding the basic autecology of corticioid fungi, a nonmonophyletic group of Basidiomycota having a flat, crust-like morphology. Their inconspicuous basidiomata and the limited number of people trained to identify these fungi are the major reasons for the lack of studies. This is unfortunate because corticioid fungi are widespread and are known to fill multiple guilds as pathogens, saprotrophs, and ectomycorrhizal symbionts.

There is a wealth of information supporting the view that mushrooms, false truffles, truffles, and various cup-forming fungi are ectomycorrhizal (hereafter EM). In contrast, corticioid fungi were traditionally thought to be saprotrophic due to their association with dead plant material and consequently were ignored as potential EM associates (Larsen 1968, 1974). Although some early work showed that particular corticioid fungi were EM (Zak and Larsen 1978), it was a surprise when molecular ecology studies revealed them to be major components of EM communities (Kõljalg et al. 2000). In particular the Atheliales, a group entirely composed of corticioid species, are now widely accepted as dominant EM symbionts in many forest ecosystems. Erland (1995) found that *Tylospora* spp. were the most common EM fungi in a

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Swedish spruce forest, and a survey in Czech Republic spruce forests showed that they colonized nearly 70% of the spruce root tips in disturbed sites (Peter et al. 2008). In a North Carolina pine forest with elevated CO_2 levels, *Tylospora* spp. were also the most commonly recorded EM taxa, constituting over 30% of those recovered from soil ingrowth bags (Parrent and Vilgalys 2007). Species of *Piloderma*, another genus in the Atheliales, are common in EM community studies (Tedersoo et al. 2008; Lilleskov et al. 2011; Glassman et al. 2015) and have recently been shown to be the most transcriptionally active EM fungi in some pine forests (Liao et al. 2014).

The current view of fungal EM and saprotrophic communities is increasingly driven by developments in the field of molecular ecology (Peay et al. 2008; Lindahl et al. 2013). Recently, next-generation DNA sequence analyses of colonized roots and soil have been used to determine the identity and frequency of EM fungal species because this approach allows for much greater sample sizes and geographic coverage (Talbot et al. 2014; Tedersoo et al. 2014). However, a high percentage of these environmental sequences often fail to match identified fungal sequences present in the public databases and yet are well matched to other environmental sequences from previous studies. This means that environmental sequences likely represent fungi that are common enough to be resampled, but no information on their trophic status can be inferred. We speculated that many of these unmatched sequences may be corticioid fungi, and this idea motivated our study.

To contribute to the knowledge of EM corticioid fungi in North American pinaceous forests, we collected and sequenced specimens from 60 *Pinus*- and *Picea*-dominated forests across the continent. Soil, EM roots, and pine seedling bioassays from a subset of these sites were previously studied with next-generation sequencing (Talbot et al. 2013, 2014; Glassman et al. 2015), rendering a wealth of molecular data suitable for investigating the prevalence of these fungi. Our goals were the following: (i) to obtain an initial view of the distribution and diversity of corticioid fungi, particularly those in the Atheliales, in North American pinaceous forests; (ii) to help fill gaps in the sequence database of corticioid fungi; and (iii) to identify additional taxa that are likely to be EM symbionts.

MATERIALS AND METHODS

Study sites and collection.—Corticioid basidiomata were collected from 60 pine and spruce forest sites across 15 North American states and provinces during the 2012 and 2013 summer months (Fig. 1A). Sites were typically monospecific stands of different species in the Pinaceae family that were selected in order to study the community structure of EM fungi on a local and the continental scale (Talbot et al. 2014) (Table 1). At each site, basidiomata were collected at

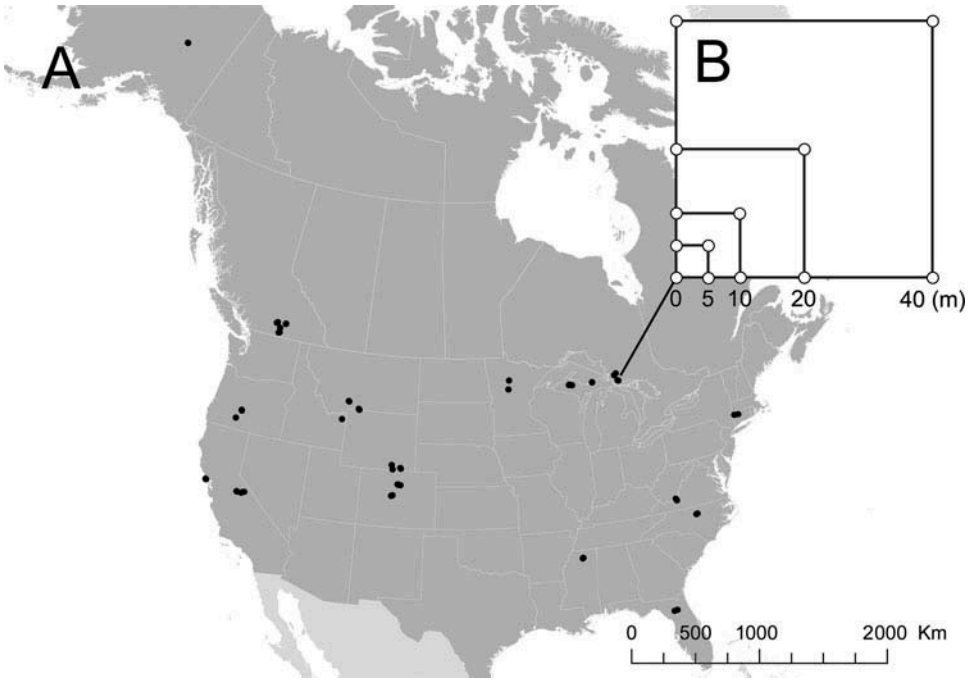

Figure 1. A. Distribution map of sample sites across North American pine forests (n = 60 sites). B. At each site sampling followed a 40 × 40 m^2 nested square design where every corner designated a collection point (n = 13 samples).

Table 1. Locations, samples types, and sequence numbers.

Location[a]	Dominant tree[b]	Year	No. of sites	Collection No.	Sequence no.[c]	Singleton no./total OTUs[d]
AK[sr]	PICg	2012	2	43	21	12/15
BC	Pc, Pp PICe, PICe/Al	2013	7	125	61	28/38
CA-1[sr]	Pa, Pc, Pmon, Pp	2012	6	72	41	22/30
CA-2[sr]	Pmur	2012	3	40	30	21/25
CA-3	Pp, Pmur., and others	2011–2013			23	18/20
CO	Pc, Pf, Pp PICe/Al	2013	7	68	25	19/22
CT	Ps	2012	2	23	15	15/15
FL	Pt, Pel	2012	2	32	12	10/11
MI[s]	Pb, Ps, PICg, PICm/Ab	2013	6	47	27	17/20
MN[s]	Pb, Pb/Pr	2012	2	30	14	10/12
MS[s]	Pt, Pec	2012	2	27	13	10/11
MT	Pa, Pc, PICe/Al	2013	5	73	44	22/31
NC[s,r]	Pt	2012	2	38	23	18/19
ON	Pb, Ps, PICg, PICm/Ab	2013	4	49	27	13/18
OR	Pc, Pp	2012	4	45	32	16/23
VA	Ps	2013	2	18	10	8/9
WY	Pc, Pp, PICe	2013	4	27	9	9/9
Total			60	730	425	140/223

[a]AK: Alaska, USA; BC: British Colombia; CA-1: California, USA, Sierra Nevada plots; CA-2: California, USA, Point Reyes National Seashore plots; CA-3: California, USA, outside plots, Point Reyes, Yosemite, and Santa Cruz area; CO: Colorado, USA; CT: Connecticut, USA; FL: Florida, USA; MI: northern Michigan, USA; MN: Minnesota, USA; MS: Mississippi, USA; MT: Montana; NC: North Carolina, USA; ON: Ontaria, Canada; OR: Oregon, USA; VA: Virginia, USA; WY: Wyoming, USA.
[b]Pa: *Pinus albicaulis*; Pb: *P. banksiana*; Pc: *P. contorta*; Pec: *P. echinata*; Pel: *P. elliottii*; Pf: *P. flexilis*; Pmur: *P. muricata*; Pmon: *P. monticola*; Pp: *P. ponderosa*; Pr: *P. resinosa*; Pt: *P. taeda*; PS: *P. strobus*; PICe: *Picea engelmannii*; PICg: Picea glauca; PICm: *Picea mariana*; Al: *Abies lasiocarpa*; Ab: *A. balsamifera*.
[c]Number of sequences derived from vouchered collections. Next-generation sequences from soil, roots, or bioassays are not listed.
[d]Number of s derived from sequenced collections that are singletons (unique)/total s derived from collections.
Superscript s and r indicate locations having sites with spore bioassay and EM roots sequenced, respectively. Sites sampled in 2012 have associated 454 soil sequences, and those from 2013 have Illumina sequences. Soil sequences were not collected from CA-3 sites.

13 points within a 40 × 40 m^2 nested square (Fig. 1B). If no basidioma was present at the sample point, the search was expanded a few meters until the nearest one was found, and if none was still found within a few meters, the sample point was skipped. When fruiting was abundant, two collections per sampling point were made and additional samples were collected from the general area. In efforts to sample EM corticoid fungi, we tried to target specimens in the Atheliales, which usually have a sparse, yet smooth and continuous hymenium. Collectors examined well-decayed wood and litter, selecting the first light-colored corticioid basidioma with a cobweb-like appearance. When fungi with a light-colored and thin morphology could not be found, we also sampled corticioid fungi of various morphologies and resupinate polypores. Finally, we augmented our collection with an additional 23 samples that were collected along the California coast and in the Yosemite region of the Sierra Nevada mountains. Each specimen was photographed, saved for DNA extraction, and dried as a voucher to be deposited in the University of California (UC) Herbarium (UC2022802–UC2023243).

DNA extraction, PCR, and sequencing of basidiomata.—DNA was extracted using the Sigma Extract-N-Amp kit (Sigma Aldrich, St. Louis, Missouri, USA). The basidiomata were often covered in other material that can hinder the ability to obtain high-quality, uncontaminated sequences. To circumvent this problem, we picked very small tissue samples from each specimen under a dissecting microscope using sterile insect pins and placed each into 10 μL of extraction solution. After heating to 80 C for 15 min, 30 μL of dilution solution was added. Typically, 1 μL of final diluted extract was used in a 25-μL reaction to amplify the internal transcribed spacer (ITS) region using the standard fungal primers ITS-1F and ITS-4 (White et al. 1990; Gardes and Bruns 1993). The Basidiomycete-specific primer ITS-4B (Gardes and Bruns 1993) was occasionally used instead of ITS-4 to exclude Ascomycete contaminants. Polymerase chain reaction (PCR) products yielding a single band during gel electrophoresis were purified using ExoSAP-IT (Sigma Aldrich) and cycle-sequenced in both directions with the BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, California, USA). Sequences were read with the ABI 3730 DNA analyzer (Applied Biosystems). Bidirectional sequences were edited using Sequencher (GeneCodes, Ann Arbor, Michigan, USA) and Geneious Pro v5.4.6 (BioMatters, Auckland, New Zealand) and aligned to form contiguous sequences. These were deposited in GenBank under accession numbers KP814141–KP814565.

Taxonomic identification of basidiomata.—Basidioma sequences including ITS1-5.8s-ITS2 regions were grouped into operational taxonomic units (OTUs) using the UCLUST method (Edgar 2010) in QIIME (Caporaso et al. 2010). We compared similarity cutoffs of 95–99% and found a linear relationship between the

number of resulting OTUs and percent sequence identity in the 95–97% range, followed by an upward inflection in the 98–99% range. For that reason, we adopted the 97% cutoff as a conservative estimate of species. To reduce artifactual OTU inflation caused by the software, sets of OTUs that BLASTed to the same sequences were manually aligned and combined if the sequence similarity was greater than 97%.

Representative sequences were compared against the UNITE and NCBI GenBank databases using the massBLASTer search option on the UNITE interface (Kõljalg et al. 2013). We chose reference sequences to infer taxonomic identification by placing more confidence in UNITE voucher-based sequences and those derived from in-depth systematics studies.

A consensus was reached for each OTU identity, dependent upon the sequence similarity and taxonomic annotation of the BLAST match. Generally, we adopted the same species name of matches with a sequence similarity greater than 97% and the same genus name for matches that were more than 95% similar. Since ITS variation fluctuates across groups and many of our OTUs matched unresolved species complexes, we assessed the identity of OTUs on an individual basis and did not rely solely on a hard cutoff. Individual naming decisions are explained more completely in the comments following Supplementary Table 1. In some cases when sequence-based identification failed, we could assign names by morphologically identifying specimens using Bernicchia and Gorjón (2010). Positive identifications were limited by the difficult morphological taxonomy of these fungi, and the absence of distinctive cystidia, hyphae, basidia, and spores in some specimens. Corticioid taxonomy is very much a work in progress, resulting in many nonmonophyletic groups. In light of this, we assigned tentative names to some OTUs, adding sensu lato when they matched to a species that fell outside of the sensu stricto clade, or when it was morphologically identified to a group but the sequence lacked a close match. All names were updated to the current nomenclature in IndexFungorum (http://www.indexfungorum.org/). OTUs that matched well to non-corticioid Basidiomycota or Ascomycota were assumed to be contaminants or sterile mycelium and were excluded.

Analysis of taxonomic distribution and alpha diversity.—A ranked abundance curve was constructed for samples across all genera to understand the taxonomic distribution of our basidioma collection. Those that were best identified to a group higher than genus were considered unidentified. The curve also served to directly compare the diversity of aboveground and belowground corticioids. To assess species richness, we included all OTUs, treated them as if they were drawn from a common pool, and produced a rarefaction curve and a Chao2 species estimator curve (Chao 1987) using the program EstimateS (Colwell 2013).

Determination of EM Fungi.—Given that the taxonomic assignments were often imprecise and our knowledge of the nutritional modes of many taxa is still in a state of flux (Rinaldi et al. 2008), we adopted two approaches to estimate the proportion of EM corticioid fungi in our data set. Conservatively, OTUs were classified as EM only if they BLAST-matched to established EM taxa listed in Tedersoo et al. (2010). Less conservatively, we classified OTUs as possibly EM if they matched genera that include EM species, even if congeners have other nutritional modes (e.g., *Sistotrema, Sebacina)*. Additionally, we classified any OTU as potentially EM if it matched sequences from EM root tips in GenBank or EM root tips from our in-house EM spore bank bioassay (Glassman et al. 2015) and root database with at least a 95% similarity.

Connecting aboveground and belowground sequences.—We connected our specimen-based sequences to ITS sequences from soil cores, spore bioassays, and root samples for three reasons: (i) to understand the spatial patterns of corticoid fungi, (ii) to provide insight into the belowground activity of corticoid fungi, and (iii) to compare the taxonomic diversity of corticioid fungi aboveground and belowground. Soil was collected from the O and A horizons from the same collection sites, DNA was extracted, and the ITS region of rDNA was amplified. DNA from soil samples collected at sites visited in 2012 (n = 25 sites) was sequenced on the 454 platform as previously described and reported (Talbot et al. 2013, 2014), and that from sites visited in 2013 (n = 35) was processed via Illumina sequencing (unpublished data). Implemented on the UPARSE pipeline (Edgar 2013), these sequences were quality filtered, denoised, trimmed, and paired as in Smith and Peay (2014). Soil collected from 19 sites for EM spore bank bioassays, as reported by Glassman et al. (2015), and EM roots of resident trees from 9 sites were collected and DNA was extracted, the ITS region of rDNA was amplified, and amplicons were sequenced via 454 pyrosequencing as an unpublished part of the Talbot et al. (2014) study. Details regarding which sites are associated with the soil cores, EM spore bank bioassays, and root samples are provided in Table 1.

Before merging the 454 and Illumina data to complete the soil sequence data set, ITS1 was extracted from the 454 sequences so that sequences from both platforms were of comparable length. OTUs were then picked with the UPARSE method in USEARCH (Edgar 2010), using the standard settings of a 97% sequence similarity, de novo and reference-based chimera checking, and removal of singletons. OTUs were taxonomically assigned with the BLAST method in QIIME, utilizing reference data sets and id-to-taxonomy maps from fungal database UNITE (Kõljalg et al. 2005; http://unite.ut.ee/index.php). We generated a list of known corticioid genera and family- or ordinal-level corticioid-exclusive taxonomic groups from Bernicchia and Gordon (2010) and from two large-scale phylogenetic studies of corticioid fungi by Larsson et al. (2004) and Binder et al. (2005). Soil OTUs that matched names in this list were identified and extracted. In order to identify any soil OTUs that were not previously included due to omissions in the public databases, we repicked OTUs from the soil sequences using the ITS1 extracted region of our vouchered corticioid OTUs as a reference database, utilizing QIIME and a 97% identity cutoff. Augmented with any additional OTUs matching aboveground corticioid fungi, this soil sequence data set represented our belowground corticoid sequences.

The comprehensively sampled set of soil sequences afforded us the ability to analyze the relationship between community dissimilarity and physical distance of corticioid fungi. Sample sequence depth ranged tremendously; thus, we rarefied the samples to the 10th and 15th percentile sequence depths (1145 and 1663 sequences, respectively). Utilizing the beta_diversity.py command in QIIME, OTU tables were rarefied to both levels and three beta-diversity indices (the presence/absence-based Sorensen-Dice and Jaccard indices and the abundance-based Bray-Curtis index) were produced as matrices of pairwise comparisons between each sample site for both rarefication levels. Mantel tests, from the vegan package (Oksanen et al. 2012) in R version 3.0.1 (R Development Core Team 2013), compared these metrics with the physical distance matrix, produced from the geosphere package (Hijmans et al. 2012). Mantel correlograms from vegan were used to detect distance-decay pattern and to provide insight into the scale at which species turnover occurs.

To identify corticioid taxa that are potentially EM, we looked for voucher sequences that matched sequences from colonized root tips in the plots or colonized root tips from EM spore bioassays. We employed the same reference-based OTU picking procedure described above to connect ITS sequences from EM spore bioassays and EM root samples to our specimen-based sequences.

Finally, the soil sequence data set was used to compare the taxonomic diversity between aboveground and belowground communities. In deciding a common metric between soil and basidioma sequences, we chose presence/absence in soil core samples instead of read abundance, because the read abundance has a very weak relationship with quantity across different species (Amend et al. 2010; Nguyen et al. 2014).

RESULTS

Taxonomic identification of ITS sequences.—In total, we made 757 collections of corticoid basidiomata. The number of collections per 40 × 40 m^2 plot ranged from 3 to 32 with a mean of 14.6 per plot. From these 757 vouchers, we obtained 498 sequences, which represents a success rate of 66% for sequence acquisition. A preliminary BLAST analysis identified 63 sequences that matched non-corticioid taxa. These were dropped from subsequent analyses, leaving 425 high-quality bidirectional sequences. OTUs were picked using a 97% similarity threshold, followed by individually aligning and inspecting putative OTUs that BLASTed to the same sequence. The latter resulted in merging 38 erroneously split groups, resulting in a total of 223 OTUs. Representative sequences of each OTU were compared against UNITE and GenBank databases for taxonomic assignments (Supplementary Table 1).

A total of 61.9% of OTUs matched to a species-annotated sequence at 97% identity or higher. Of the OTUs with poor matches, we morphologically identified 36 OTUs to at least the generic level, leaving only 8 OTUs identified to either class or order. Among this set of morphologically identified specimens, *Tubulicrinis* was the dominant genus with 9 OTUs, followed by *Botryobasidium* with 5 OTUs. Many of these OTUs that we morphologically identified as *Tubululicrinis* or *Botryobasidium* had BLAST matches to the correct genus, but with a similarity that was often below 90%. Although we arrived at a name for the majority of samples, identification by DNA barcoding and morphology is imperfect and we indicate those that should be interpreted cautiously, either by cf., aff., or s.l. We examined the taxonomic distribution of our samples by genus (Fig. 2). The 68 genera recovered spanned all 12 major Agaricomycete (previously considered Homobasidiomycete) clades established by Binder et al. (2005). The ranked abundance curve shows a handful of dominant genera, followed by a long tail of rare taxa (Fig. 2). The nine most dominant genera accounted for over 50% of identified samples. In order of decreasing abundance, these were *Tubulicrinis, Piloderma,*

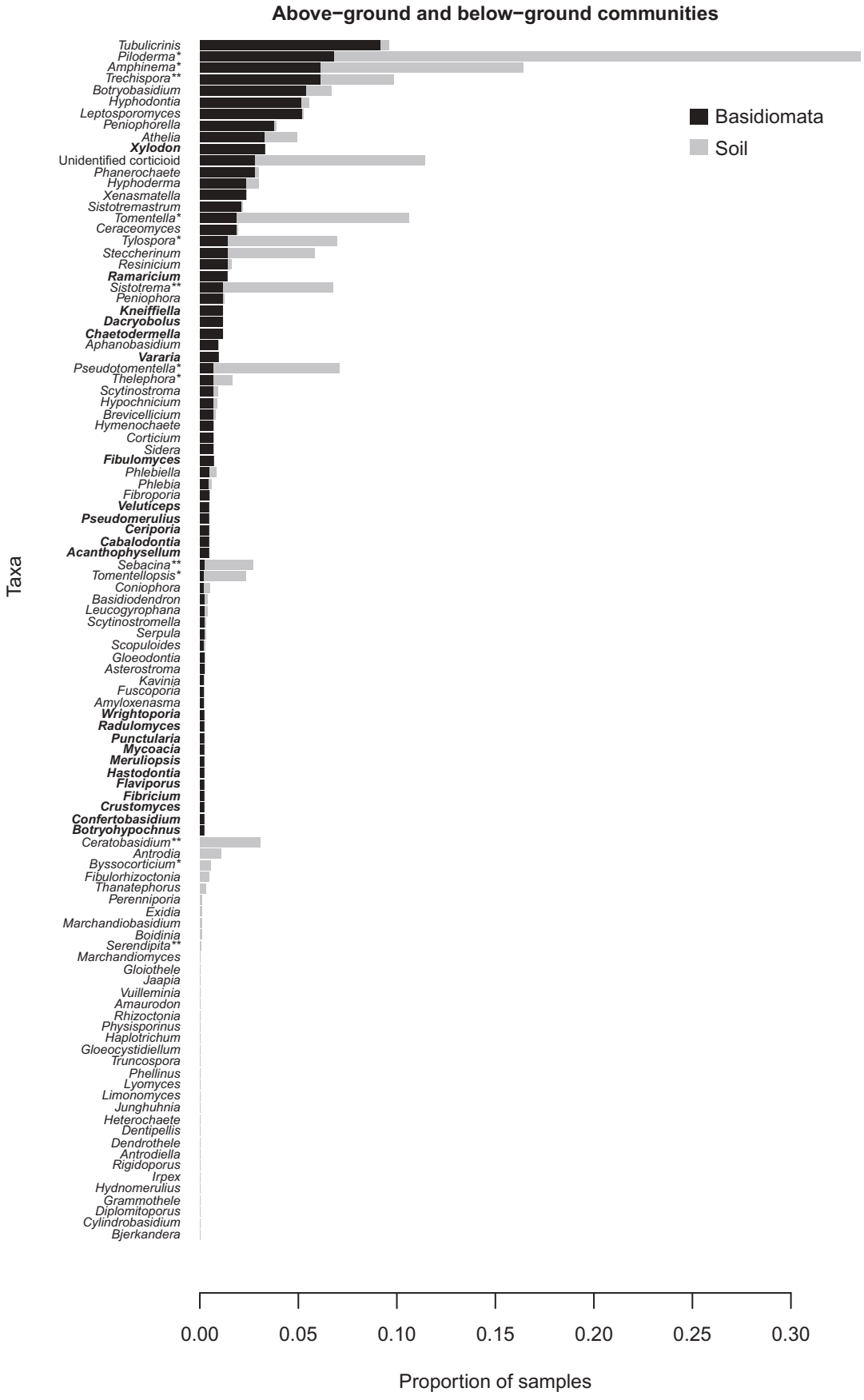

Figure 2. Ranked abundance curve of genera of the basidioma and soil communities. Basidioma (black) and soil (gray) communities display relative proportion of samples as stacked bars. Bolded taxa were found exclusively as basidiomata and EM or potentially EM taxa are indicated by * and **, respectively.

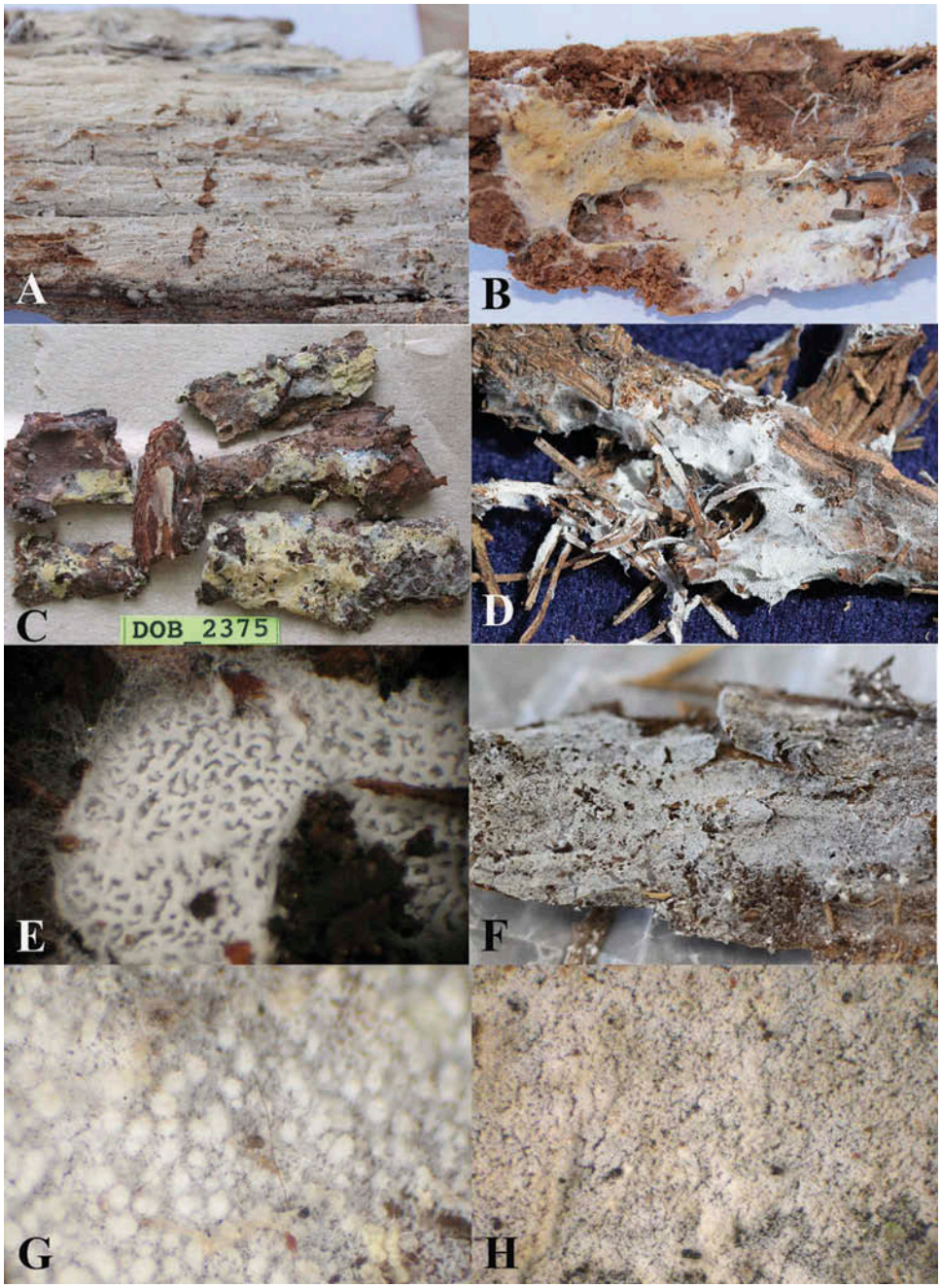

Figure 3. Examples of collected and sequenced basidiomata that resemble the athelioid morphology. (A) OTU 52 *Kneiffiella* sp.; (B) OTU 25 *Ceraceomyces* cf. *serpens*; (C) OTU 200 Atheliaceae; (D) OTU 253 *Trechispora* sp. 3; (E) OTU 27 *Sistotrema* sp.; (F) OTU 148 *Leptosporomyces* aff. *raunkiaeri*; (G) OTU 87 *Hyphodontia floccosa*; (H) OTU 153 *Botryobasidium* sp. 1. Dissecting scope views ~30× shown in E, G, and H.

Amphinema, Trechispora, Botryobasidium, Hyphodontia, Leptosporomyces, Peniophorella, and *Athelia*.

The Atheliales, in accordance with Larsson et al. (2004) and Binder et al. (2010), represented 17.0% of OTUs and 23.8% of samples and were composed of the following: *Amphinema* (6 OTUs, 26 samples), *Athelia* (7, 14), *Piloderma* (11, 29), *Leptosporomyces* (8, 22), *Tylospora* (4, 6), and unidentified Atheliales (2, 4). A total of 32.7% of OTUs and 33.4% of samples belonged to other genera that also have a light-colored and thin hymenium (Fig. 3) but are not in the Atheliales. This set included species in *Botryobasidium* (11 OTUs, 23 samples), *Ceraceomyces* (7, 8), *Hyphoderma* (7, 10), *Hyphodontia* (11, 22), *Sistotrema* (5, 5), *Sistotremastrum* (2, 9), *Trechispora* (12, 26), and *Tubulicrinus* (18, 39). In total, 49.7% of OTUs and 57.2% of the collections had the targeted morphology.

Fungi from soil. —The soil sequences contained a diverse corticioid assemblage that partially overlapped with aboveground fungi and was enriched in EM fungi (Fig. 2). In total, we found 938 OTUs representing 3,423,152 corticioid sequences in the soil cores. The overwhelming majority, 96.8%, were identified via UNITE reference data sets and id-to-taxonomy maps, whereas 3.2% matched only our voucher collections. Only 108 of the 223 (48.3%) vouchered OTUs were also found in the soil. This limited overlap is surprising because there were more than four times as many soil OTUs than voucher-based OTUs. Out of the 80 genera represented in the soil sequences, 35 were found exclusively in the soil samples. The taxonomic distribution of corticioids in the soil was heavily dominated by a small number of EM genera, with *Piloderma, Amphinema, Tomentella*, and *Pseudotomentella*, in ranked order of abundance, present in over 50% of the soil samples. Conversely, 23 genera were only found in the voucher collection (see bolded taxa, Fig. 2), and this set of taxa appears to be enriched in non-EM taxa.

Species richness.—Our basidioma collection was predominately composed of singletons, representing 62.8% of all OTUs (140/223 OTUs), and only eight of the OTUs were collected at five or more sites (Supplementary Table 1). Due to the high percentage of singletons, the species accumulation curve of basidioma collections was nonasymptotic, and the Chao 2 species estimator curve did not plateau (Fig. 4). Although species accumulation curves were not shown for individual sampling regions, they too exhibited similar steep patterns because all but two regions were comprised of over 50% singletons (see Table 1).

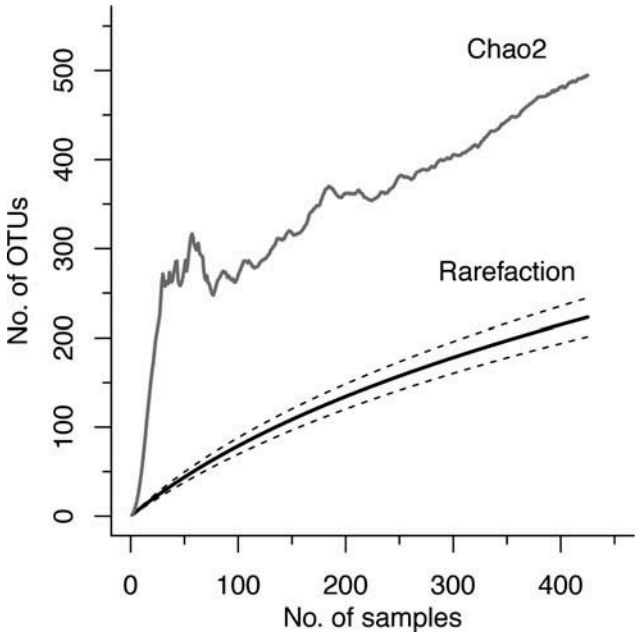

Figure 4. Rarefaction curve with 95% CI (black) and Chao 2 curve (gray) for entire voucher collection.

Ectomycorrhizal taxa.—Based on our conservative and relaxed criteria, we estimated that 14.8–24.7% of vouchered OTUs and 17.9–26.8% of collections were EM. Of the 108 OTUs that were also recovered in the soil, 25.9–34.3% belonged to EM genera. A larger proportion of the soil community was EM, constituting 40.5–58.6% of OTUs and 61.3–79.3% of samples. Several vouchered OTUs matched EM roots in GenBank, UNITE, our plots, or our seedling bioassays. Most of the identified taxa that matched root sequences were from known ectomycorrhizal genera: *Amphinema, Piloderma, Tylospora, Pseudotomentella, Thelephora, Tomentella, Tomentellopsis, Sistotrema*, and *Sebacina*. *Trechispora* was also found to match sequences from the soil data set and EM roots (Supplementary Table 1), and as discussed below, there is reason to reaffirm previous suspicions that some species are EM (see Dunham et al. 2007). *Hypochnicium, Scytinostroma*, and *Tubulicrinis* matched EM roots, but based on current knowledge of their ecology, we did not consider them potentially EM.

Distance-decay pattern.—There was a significant effect of distance decay on our soil collection of corticioid fungi (Figure 5A). Among the set of three beta-diversity indices analyzed at two rarefaction levels, Mantel r ranged between 0.348 to 0.589 and $P < 0.001$ (Supplementary Table 2), indicating that a distance-decay pattern was present regardless of the parameters. The mantel correlogram, analyzed using the Jaccard dissimilarity index at the lower rarefaction level, showed corticioid fungal assemblages to be significantly different after 1000 km (Fig. 5B, Supplementary Table 3).

DISCUSSION

Our continental survey of corticioid fungi added 425 new sequences representing 223 OTUs to the ITS database, and when these voucher sequences were combined with EM root sequences, our study enhances our understanding of the autoecology and distribution of corticioid fungi. For such an overlooked group of fungi whose morphology offers few diagnostic characters, the addition of 215 new OTUs identified to at least genus level is significant

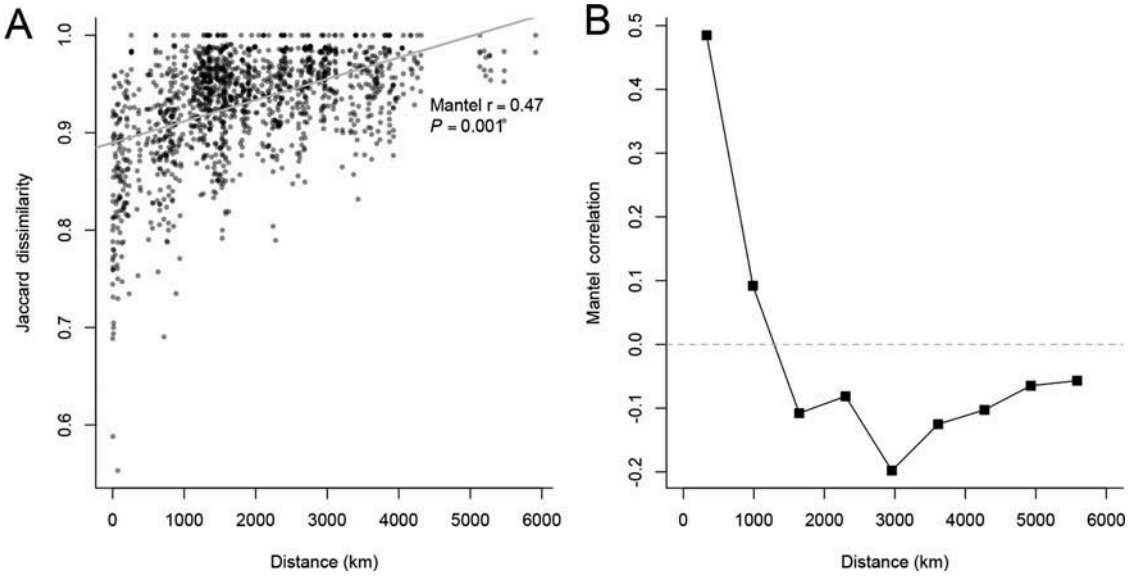

Figure 5. Analysis of spatial autocorrelation. A. Mantel test of Jaccard community dissimilarity versus geographic distance (m). Points represent pairwise comparisons between sites. Points are semi-transparent in order to better display those that are overlapping. B. Mantel correlogram shows the scale of species turnover. All points were significant ($P < 0.05$). Those above the zero line are more similar and those below more dissimilar than expected from a random distribution.

because molecular identification is the common method through which these taxa are now encountered in ecological studies. Furthermore, our results reinforce the view that a large proportion of corticioid fungi are present in EM communities (Kõljalg et al. 2000).

Among the EM corticioid fungi in our study, *Trechispora stellulata* emerged as potentially EM because sequences from basidiomata matched those of EM root tips. The nutritional modes of *Trechispora* are still inconclusive, but recent findings suggest that some species, including *T. stellulata*, may be mycorrhizal. An unidentified ericoid mycorrhizal Agaricomycete was found to be sister to Trechisporales (Vohník et al. 2012). Assuming ericoid mycorrhizae are associated with this clade, a switchover to EM habit could have evolved independently, as this is a common pattern (Tedersoo et al. 2010). Additionally, Dunham et al. (2007) found *T.* cf. *stellulata* forming EM mats in pinaceous forests. Tedersoo et al. (2010) disputed its EM status, claiming that tissue was collected from mycelial mats rather than root tips. In the study by Dunham et al. (2007), root tips were indeed collected from every EM mat and morphologically confirmed identical to rhizomorphs before DNA extraction. Here we were not sampling in mats, but only root tips and basidiomata. *T. stellulata* from our collection had a 99% sequence similarity to Pinaceae EM root tips collected by Dunham et al. (2007) and Peay et al. (2010).

OTUs of *Hypochnicium geogenium, Scytinostroma* sp., and *Tubulicrinis* cf. *sororius* also matched some root samples, but in these cases, we find no convincing data supporting that they may be mycorrhizal. *Hypochnicium* species have been found as root endophytes (Chlebicki 2009), perhaps explaining why its sequences were retrieved from EM roots. Previous studies have posited *Scytinostroma* and *Tubulicrinis* as EM based on root tip morphology (Rinaldi et al. 2008). De Román and De Miguel (2005) morphotyped *Quercus* EM root tips, assigning one type to *Scytinostroma*, but there was no apparent effort made to link the morphotype to a molecular fungal identity. EM status of *Scytinostroma* is not supported based on its phylogenetic position (Miller et al. 2006), but given the polyphyletic nature of EM fungi, this conclusion is not entirely convincing. Kernaghan (2001) tentatively identified *Tubulicrinis* as EM due to its distinctive amyloid lyocystidia, but in our samples only 1 out of 18 *Tubulicrinis* OTUs matched to any root sequence. Our observations in conjunction with these previous reports leave the EM status of these three taxa ambiguous.

Although a substantial subset of corticioid basidiomata was found to be EM, roughly 3 times as many soil samples were found to be EM. In fact, 50% of soil corticioid samples were composed of four known EM genera, *Piloderma, Amphinema, Tomentella*, and *Pseudotomentella*. On the other hand, out of the nine genera that make up half of the vouchered samples, only *Piloderma* and *Ampinema* are known EM fungi.

The disconnect between the aboveground and belowground fungal communities has been described on multiple accounts (Gardes and Bruns 1996; Smith et al. 2011) and was not unexpected. Still the pattern is striking, with

less than 50% of voucher-based OTUs shared in common with the soil and large differences in frequency of the taxa that do overlap (Fig. 2). In our study, these differences are likely due to both sampling methods and autecology of the taxa involved. Taxa found in the soil but not in the fruiting record are likely attributable in part to a sampling artifact. By visiting each plot only once to sample basidiomata, which are known to be temporally sensitive (Halme and Kotiaho 2012), we obtained an incomplete snapshot of the entire aboveground diversity. In contrast, the belowground diversity varies much less throughout the seasons (Smith et al. 2011), possibly due in part to soil samples detecting metabolically inactive fungi (Baldrian et al. 2012). A more important factor is that our selective sampling scheme targeted fungi that looked similar to the largely EM Atheliales. Because corticioid fungal groups can seldom be identified by macromorphology, especially in the field, we recovered a high proportion of specimens with gross similarity to the Atheliales that belonged to distant orders. Ultimately, our collection represented a phylogenetically diverse set of corticoid fungi that were biased towards light-colored and thin morphologies (57% of samples). Dark-colored species are underrepresented, most notably excluding many EM Thelephorales and perhaps *Byssocorticium*. Therefore, a cautious interpretation of comparisons of above- and belowground communities is advised.

The reverse pattern—taxa found fruiting but not in the soil—cannot be explained by the same sampling biases and instead points toward a biological difference in the two sample types. This conjecture is supported by the fact that the soil samples comprise primarily EM fungi, whereas the fruiting samples have more saprotrophs. We acknowledge that the Thelephorales are underrepresented in the fruiting samples, therefore artificially reducing the proportion of EM samples. If we take this sampling bias into account by removing samples in the Thelephorales from both the above- and belowground collections, the proportion of EM samples in the soil is still appreciably greater (14.9–24.1% vs. 52.7–74.7%). This observation is consistent with the idea that many decomposers may be largely confined to their carbon resources and do not send mycelium into the soil. In fact, all of the 17 genera that were found exclusively in the voucher collection are thought to be wood decayers or other saprotrophs (Fig. 2, bolded text). Conversely, mycorrhizal fungi explore the soil to scavenge for mineral nutrients and to locate uncolonized root tips and for that reason are more likely to be detected in the soil sequences. In corroboration of this explanation, the OTUs that were shared between the soil and voucher datasets were composed of a higher proportion of EM taxa than the entire set of voucher-based OTUs (25.9–34.3% vs. 15.7–23.3%), and every EM or potentially EM genus found as a basidioma was also recovered in the soil. This pattern indicates that the frequency in soil may be one additional way to crudely identify other species as potentially ectomycorrhizal.

Studies have increasingly demonstrated that fungal community composition is spatially autocorrelated (Lilleskov et al. 2004; Bahram et al. 2013), and the corticioid guild again demonstrates this pattern. Soil fungal communities (Talbot et al. 2014) and EM spore bank communities (Glassman et al. 2015) sampled at the same 2012 sites were found to have high levels of geographic endemism across North America, and strong distance-decay relationships were consistent throughout the local meter scale to the continental scale. Likewise, our study showed that corticioid fungi exhibited a significant distance-decay relationship at the continental scale. Given that our sequences were partially derived from the same soil sequences from Talbot et al. (2014), we are not providing independent evidence of the pattern, but we are showing that corticioid fungi are another group concordant with it, and the spatial turnover in species that we observe likely explains at least part of the high species richness seen in our survey (Qian et al. 2005). It is generally understood that both environmental heterogeneity and dispersal limitations dictate the extent of distance-decay patterns (Verleyen et al. 2009; Hanson et al. 2012), but several microbial studies conducted on the regional to the global scale identify dispersal limitations as the primary cause (Whitaker et al. 2003; Green et al. 2004; Reche et al. 2005; Talbot et al. 2014). With our corticioid sample we cannot separate environmental drivers from dispersal limitation, but given the mode of spore dispersal in these fungi, it would not be surprising if dispersal limitation was the dominant factor causing the distance-decay relationship.

Both rarefaction and Chao2 species estimator curves did not plateau, demonstrating an undersampled, hyperdiverse community. These curves closely corresponded to those based on other hyperdiverse fungal systems, such as morphotypes of tropical endophytes and pyrosequences of *Quercus* leaf phyllospheres (Arnold et al. 2000; Jumpponen and Jones 2009). Although it is not possible to directly compare our data with these previous studies due to sampling and methodological differences, it is worth noting the similarities. Jumpponen and Jones (2009) drew parallels between their *Quercus* leaf results and the tropical endophytes from the Arnold et al. (2000) study, arguing that the fungal phyllosphere community of temperate *Quercus* leaves was also hyperdiverse based on an equally high proportion of singletons.

In both of these studies, over half of the fungi were encountered as singletons, which is similar to the 62% singleton rate in our corticioid collections.

Initial comparisons against public sequence databases led to a majority of our collections insufficiently identified, and only after the UNITE database was updated during the last few months with annotated sequences from Europe were we able to attain species names for a significant portion. Although this was not unforeseen, especially for a neglected group such as corticioid fungi, it is indicative of a major problem because sequences are becoming the standard means for fungal species identification. Although much can be learned about the patterns of fungal diversity without attaching species names, what is missed with anonymous sequences is the ability to retrieve and organize information about the autecology of the individual species (Peay 2014).

We picked up a significant diversity in our basidioma surveys that was not recovered in the soil samples, despite the enormous sequencing capabilities of next-generation sequencing techniques. We were able to apply names to many of these samples and document their occurrence across a large area of the continent. Perhaps more importantly, the deposited sequences will allow others to objectively compare what we found, independent of the names we apply, and then retrieve and study the collections of interest. We hope this survey paves the way for future studies on these diverse, important, and widespread fungi.

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