DIVERSITY, TAXONOMY, AND SYSTEMATICS OF CHANTERELLES AND ALLIES (CANTHARELLALES)

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Rachel A. Swenie
August 2023

ACKNOWLEDGEMENTS

Thank you to my Ph.D. advisor, Dr. Brandon Matheny, for supporting and challenging me in equal measure. I have benefitted greatly from your mentorship, mycological expertise, and sense of humor.

Many thanks to my committee members, Drs. Liz Derryberry, Joe Williams, and Todd Reynolds, for providing suggestions and guidance on a variety of matters throughout my Ph.D.

I am grateful to my past and present lab mates, Marisol Sánchez-García, Hailee Korotkin, Brian Looney, Emma Harrower, Chance Noffsinger, and Django Grootmyers. Thank you for sharing your knowledge with me, and for your camaraderie in the field and lab.

I thank undergraduate researchers and lab technicians Alicia Hobbs and Noah Walker for contributing time and technical skills towards my research projects.

Warm acknowledgements to the Cumberland Mycological Society, the Asheville Mushroom Club, and the Wildacres Foray for organizing many fun and fruitful gatherings. I'm grateful for the connections I've made with individuals in these groups and the opportunities I've had to present my research findings to the broader southeastern mycological community.

Lastly, thank you Dan, Alex, Jenn, Anna, and my family for your unwavering support and encouragement.

ABSTRACT

The order Cantharellales is a lineage of approximately 1,000 fungal species that is sister to the rest of the mushroom-forming fungi (Agaricomycetes). Cantharellales species display a diverse array of morphologies and nutritional modes, from corticioid (crust-like) saprobes that decay dead wood, to biotrophic species that exist as parasites of plants, as well as mycorrhizal mushrooms that form mutualisms with forest trees. However, the evolutionary relationships among these lineages are poorly known.

Within the Cantharellales, I revised the taxonomy of the genus *Hydnum* (hedgehog mushrooms) in eastern North America by integrating morphological, ecological, and molecular phylogenetic data from modern and historical type collections. I found that sixteen *Hydnum* species occur in eastern North America alone, in contrast to the five species previously recognized, and all but one species is endemic to the region. I also conducted a systematic and floristic treatment of *Cantharellus* species (chanterelle mushrooms) from the southern Appalachian Mountains and found that while fruitbody macromorphology is highly variable within and across species, many *Cantharellus* species are identifiable by a combination of microscopic and ecological characteristics. I described one new species, *C. vicinus*, from east Tennessee and elevated another taxon to the species level. Furthermore, my results include the first report of the iconic European chanterelle *C. cibarius* from North America and suggest it may be conspecific with two previously described North American taxa.

To construct the first densely sampled multi-locus phylogeny of the Cantharellales, I developed a long-read, high-throughput sequencing method using the PacBio sequencing platform. This method produced clean sequences and allelic variants for multiple loci from hundreds of fresh and dried fungal specimens, cultures, and archival DNA extracts. Using this data, I measured intraspecific haplotype diversity to aid in species delimitation across the Cantharellales and found high levels of within-individual sequence divergence (up to 8.8%), particularly in corticioid Cantharellales with binucleate and multinucleate hyphae. I then combined PacBio and Sanger sequence data to generate the Cantharellales phylogeny, which supports four families in the order. Overall, *rpb2* is the most suitable locus for species recognition within Cantharellales.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Fungi are morphologically, ecologically, and functionally diverse organisms that comprise an estimated 2.2–3.8 million species worldwide. However, much of this diversity remains unrecognized; so far only 3–8% of fungal species have been named (Hawksworth & Lücking 2017). Species diversity is commonly underestimated even in mushroom-forming fungi, a group of particular importance to humans. Molecular sequencing techniques have led biologists to rethink traditional notions of what defines fungal species, since molecular species relationships are often at odds with traditional morphological species concepts.

The order Cantharellales exemplifies this dilemma. The order contains approximately 1,000 species and is sister to the rest of the mushroom-forming fungi (Agaricomycetes). Lineages within the Cantharellales are morphologically and ecologically diverse, including lichens that form symbioses with green algae, corticioid (crust-like) fungi that degrade dead organic carbon, pathogens that cause disease in agricultural crops, mutualists that inhabit orchid roots, species that grow on lichens, and tree-associated edible mushroom-forming fungi that are sold in markets worldwide (Moncalvo et al. 2006, Gónzalez et al. 2016, Lawrey et al. 2016, Oberwinkler et al. 2017, Olariaga et al. 2017). Despite recent advances in characterizing relationships across the fungal tree of life, evolutionary relationships in the Cantharellales remain poorly understood. From the handful of genomes that have been sequenced in the order, basic relationships have been inferred among the six named families in the order, but the genome-based phylogeny (https://mycocosm.jgi.doe.gov) has changed through time as genome sampling increases. A recent multi-gene systematic study supported combining three of these families into one broader family (Cao et al. 2021). One polyphyletic genus, *Sistotrema*, comprises five or more separate genera, four of which cannot be placed within existing families (Moncalvo et al. 2006). The polyphyletic genera *Ceratobasidium* and *Rhizoctonia* include important agricultural pathogens but have presented many challenges to delimiting species (Veldre et al. 2013, Gónzalez et al. 2016). Lichenicolous Cantharellales taxa are known solely from nuclear ribosomal DNA sequences, which do not resolve those species' phylogenetic positions (Lawrey et al. 2016). To date, there is no densely sampled phylogeny of the Cantharellales that resolves these family and generic-level relationships. A comprehensive Cantharellales phylogeny will also enable better sequence-based identification of species and allow future macroevolutionary studies examining biogeography and drivers of diversification.

This dissertation research utilizes molecular systematics, taxonomy, type studies, and a novel high-throughput sequencing method to examine species diversity and evolution across the Cantharellales. First, I examine the taxonomy and systematics of two economically important groups within the Cantharellales, the edible mushroom genera *Hydnum* and *Cantharellus*, in eastern North America. Next, I develop a new sequencing method utilizing the long-read PacBio platform for generating multi-locus sequence data from an array of source materials, from fresh and dried specimens to cultures and archival DNA extracts. This method yields high-quality sequence data suitable for systematic analysis and allelic sequence variants useful for understanding intraspecific

and intra-individual genetic variation. Lastly, I infer an evolutionary framework for the Cantharellales using single- and multi-locus phylogenies to evaluate family-level relationships and assess the utility of various loci for species recognition across the order.

REFERENCES

Cao, T., Hu, Y. P., Yu, J. R., Wei, T. Z., & Yuan, H. S. (2021). A phylogenetic overview of the Hydnaceae (Cantharellales, Basidiomycota) with new taxa from China. *Studies in Mycology*, *99*, 100–121. https://doi.org/10.1016/j.simyco.2021.100121

Feng, B., Wang, X.-H., Ratkowsky, D., Gates, G., Lee, S. S., Grebenc, T., & Yang, Z. L. (2016). Multilocus phylogenetic analyses reveal unexpected abundant diversity and significant disjunct distribution pattern of the Hedgehog Mushrooms (*Hydnum* L.). *Scientific Reports*, *6*(April), 25586. https://doi.org/10.1038/srep25586

Gónzalez, D., Rodriguez-Carres, M., Boekhout, T., Stalpers, J., Kuramae, E. E., Nakatani, A. K., Vilgalys, R., & Cubeta, M. A. (2016). Phylogenetic relationships of Rhizoctonia fungi within the Cantharellales. *Fungal Biology*, *120*(4), 603–619. https://doi.org/10.1016/j.funbio.2016.01.012

Hawksworth, D. L., & Lücking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol Spectrum*, *5*(4). https://doi.org/10.1128/microbiolspec.FUNK-0052-2016.

Lawrey, J. D., Zimmermann, E., Sikaroodi, M., & Diederich, P. (2016). Phylogenetic diversity of bulbil-forming lichenicolous fungi in Cantharellales including a new genus and species. *The Bryologist*, *119*(4), 341–349. https://doi.org/10.1639/0007-2745-119.4.341

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, *14*(12), 760–773. https://doi.org/10.1038/nrmicro.2016.149

Moncalvo, J.-M., Nilsson, R. H., Koster, B., Dunham, S. M., Bernauer, T., Matheny, P. B., Porter, T. M., Margaritescu, S., Weiss, M., Garnica, S., Danell, E., Langer, G., Langer, E., Larsson, E., Larsson, K.-H., & Vilgalys, R. (2006). The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia*, *98*(6), 937–948. https://doi.org/10.3852/mycologia.98.6.937

Oberwinkler, F., Cruz, D., & Suárez, J. P. (2017). Biogeography and Ecology of Tulasnellaceae. *Ecol. Stud.* 230: 237–271. https://doi.org/10.1007/978-3-319-56363-3

Olariaga, I., Moreno, G., Manjón, J. L., Salcedo, I., Hofstetter, V., Rodríguez, D., & Buyck, B. (2017). *Cantharellus* (Cantharellales, Basidiomycota) revisited in Europe through a multigene phylogeny. *Fungal Diversity*, *83*(1), 263–292. https://doi.org/10.1007/s13225-016-0376-7

Pilz, D., Norvell, L., Danell, E., & Molina, R. (2003). Ecology and management of commercially harvested chanterelle mushrooms. Portland (OR): US Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Veldre, V., Abarenkov, K., Bahram, M., Martos, F., Selosse, M. A., Tamm, H., Kõljalg, U., & Tedersoo, L. (2013). Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecology*, *6*(4), 256–268. https://doi.org/10.1016/j.funeco.2013.03.004

CHAPTER I
SIX NEW SPECIES AND REPORTS OF *HYDNUM* (CANTHARELLALES) FROM EASTERN NORTH AMERICA

A version of this chapter was originally published by Rachel A. Swenie, Timothy J. Baroni, and P. Brandon Matheny:

Rachel A. Swenie, Timothy J. Baroni, P. Brandon Matheny. “Six new species and reports of *Hydnum* (Cantharellales) from eastern North America.” *MycoKeys* 42 (2018): 35–72.

R.A.S. and P.B.M. designed the study. All authors contributed specimens. R.A.S. conducted laboratory work and phylogenetic analyses. R.A.S. wrote the manuscript and all authors provided manuscript edits.

Abstract

Five species of *Hydnum* have been generally recognized from eastern North America based on morphological recognition: *H. albidum*, *H. albomagnum*, *H. repandum* and varieties, *H. rufescens*, and *H. umbilicatum*. Other unique North American species, such as *H. caespitosum* and *H. washingtonianum*, are either illegitimately named or considered synonymous with European taxa. Here, seventeen phylogenetic species of *Hydnum* are detected from eastern North America based on a molecular phylogenetic survey of ITS sequences from herbarium collections and GenBank data, including environmental sequences. Based on current distribution results, sixteen of these species appear endemic to North America. Of these, six species are described as new: *H. alboaurantiacum*, *H. cuspidatum*, *H. ferruginescens*, *H. subconnatum*, *H. subtilior*, and *H. vagabundum*. Geographic range extensions and taxonomic notes are provided for five additional species recently described as new from eastern North America. A new name, *H. geminum*, is proposed for *H. caespitosum* Banning ex Peck, non Valenti. Overall, species of *Hydnum* are best recognized by a combination of morphological and molecular phylogenetic analyses. Taxonomic descriptions are provided for seventeen species, including epitype designations for *H. albidum*, *H. albomagnum*, and *H. umbilicatum*, taxa described more than 100 years ago, and molecular annotation of the isotype of *H. washingtonianum*. Photographs and a key to eastern North American *Hydnum* species are presented.

Introduction

Hydnum L: Fr (=*Dentinum* Gray) is a genus of ectomycorrhizal (ECM) mushroom-forming fungi found primarily in temperate forests of Asia, Australia, Europe, and North America. Until recently, only twelve species of *Hydnum* were commonly accepted world-wide. Initial phylogenetic studies of European *Hydnum* revealed higher than expected taxonomic diversity in the genus, with thirteen molecularly recognized clades masquerading under four morphologically-defined species names (Grebenc et al. 2009), which have since been described as new species (Olariaga et al. 2012, Vizzini et al. 2013, Niskanen et al. 2018). Following a global survey of diversity in the genus which estimated 31 species worldwide based on molecular phylogenetic analysis (Feng et al.

2016), additional taxonomic work in Europe and North America has raised the global species count to 34 (Buyck et al. 2017, Niskanen et al. 2018), which is estimated to be less than half of the total number of Hydnum species (Niskanen et al. 2018). This previously overlooked diversity may be due to morphological stasis in evolution of basidiome morphology and lack of attention to regional characterization of the Hydnum flora in locations outside Europe.

Hydnum, in its original Linnean concept, contained all species of mushroom-forming fungi with a spinose hymenophore (e.g., Fries 1821). Indeed, over 900 names have been attributed to the genus per Index Fungorum (www.indexfungorum.org). However, molecular phylogenetic analysis showed that the spinose hymenophore has evolved independently many times in distantly related taxa (Hibbett et al. 1997). As a consequence, most species formerly contained in *Hydnum* have been moved to other genera. Species of *Hydnum* in the contemporary sense (Donk 1956) and typified by *H. repandum* L.:Fr. are united by their smooth hyaline basidiospores, white to orange basidiomes, and stichic basidia, in which the meiotic spindle is vertically oriented (Donk 1933, Maas Geesteranus 1971, Restivo and Petersen 1976, Pine et al. 1999). Ecologically, *Hydnum* form ECM associations with a variety of vascular plant species including members of Pinaceae (Agerer et. al 1996), Myrtaceae (McNabb 1971), Fagales (McNabb 1971, Feng et. al 2016, Niskanen et al. 2018), Salicaceae (Niskanen et al. 2018), Malvaceae (Niskanen et al. 2018), and Dipterocarpaceae (Lee et al. 2002). The genus is distributed mostly in temperate areas, with a few reports from tropical and subtropical forests in southeast Asia (Lee et al. 2002, Feng et al. 2016) and the neotropics (Garibay-Orijel et al. 2006, Sarmiento and Fontecha 2013, Feng et al. 2016, Niskanen et al. 2018).

Previous analysis of global *Hydnum* diversity revealed the presence of six distinct clades of *Hydnum* in eastern North America, only one of which also occurs on another continent (Feng et al. 2016). Currently, five species have been described from eastern North America: *H. albidum* Peck, *H. albomagnum* Banker, *H. aerostatisporum* Buyck, D.P. Lewis & V. Hofst., *H. caespitosum* Banning ex Peck (non Valenti), and *H. umbilicatum* Peck. Banker (1906) and Harrison and Grund (1987) recognized six species of Hydnum from across North America in their taxonomic treatments. Of those species that occur in eastern North America, four were described over one hundred years ago, and the application of those names has not been clarified in light of molecular phylogenetic analyses. Here, we resolve those species names by taxonomic revision of type specimens and DNA sequencing of contemporary collections, as well as document *Hydnum* species diversity and distribution in eastern North America. Seventeen species are treated here, of which six are described as new. A taxonomic key to species from eastern North America is included.

Methods

Dried specimens of *Hydnum* were obtained from TENN, CORT, NYS, NY, WTU, and CSU. Herbarium abbreviations follow Thiers [continuously updated]. Additional

collections were borrowed from the personal herbarium of Michael Kuo (Charleston, Illinois). Fresh *Hydnum* specimens were collected from localities in the eastern United States (North Carolina, Tennessee, Georgia, Florida, Virginia). Color documentation of fresh material follows Kornerup and Wanscher (1967; e.g., 5A2), Munsell Soil Color Charts (1954; e.g., 10YR 4/7), or Ridgway (1912; e.g., "Ochraceous-Tawny"). Macroscopic descriptions were taken from fresh material. In some instances, 5% KOH and 10% $FeSO_4$ were applied to pilei to test for macrochemical reactions.

Microscopic features were examined on a Nikon Eclipse 80i microscope from dried material rehydrated in 5% KOH and stained with Congo red or phloxine. Measurements and photographs were taken using a Nikon DS-Fi1 camera and Nikon NIS Elements 3.1 software. Basidiospores were measured from spore prints where available or spine tissue, and Q (quotient of basidiospore length to width) was calculated foreach spore. The number of spores measured for each species is represented as n=total number/number of specimens (e.g., n=20/3). Measurements in excess of two standard deviations are denoted in parentheses and averages in italics.

DNA extractions were performed using two methods. Fresh or dried material less than five years old was extracted using an Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, MO, USA). Older dried specimens were extracted using an HP Fungal DNA Extraction Kit (Omega Bio-Tek, Norcross, Georgia, USA). For specimens >50 years old, 10–20 mg ground tissue was incubated in extraction buffer at 65 °C for 72 hours prior to the first extraction step. The extraction was performed in a laminar flow hood to minimize contamination.

Primers ITS1F and ITS4 (Gardes and Bruns 1993, White et al. 1990) were used to amplify and sequence the nuclear rDNA internal transcribed spacer 1, 5.8S rRNA, and internal transcribed spacer 2 (hereafter, ITS). For older materials, we amplified and sequenced the two spacer regions separately following Ammirati et al. (2007) using primers ITS1F/ITS2 and 5.8SR/ITS4. Sequencing was performed on an Applied Biosystems 3730 Genetic Analyzer at the University of Tennessee Genomics Core. Sequence reads were assembled using Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan, USA).

GenBank sequences labeled as *Hydnum*, as well as closely matching environmental sequences, were downloaded. Sequences were visualized in AliView 1.20 (Larsson 2014) and aligned using MUSCLE 3.8.31 (Edgar 2004). Minor adjustments were made manually to the alignment. The GTR+I+GAMMA substitution model was selected as the best-fit model for Bayesian inference (BI) analysis in PartitionFinder 2.1.1 (Lanfear et al. 2016). BI analyses were performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003); the global analysis ran for 10 million generations with sampling every 1000^{th} generation. Following global BI tree visualization, clades that did not contain sequences from eastern North America were pruned to form a second tree figure for easier graphical representation. Maximum Likelihood (ML) analysis was performed on the pruned dataset in RAxML 8.2.8 (Stamatakis 2014) using 1000 bootstraps under a GTRGAMMA model of nucleotide substitution following the RAxML user manual recommendation. BI analysis of the pruned data set ran for 5 million generations with sampling every 500^{th} generation.

The resulting phylogenies were visualized in FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Mislabeled sequences were omitted, as well as *Hydnum* from the southern hemisphere due to high levels of sequence divergence (Feng et al. 2016). *Sistotrema alboluteum* was chosen as an outgroup based on Feng et al. (2016). DNA alignments and tree files are available at TreeBase (submission 22888). Species are recognized here as monophyletic groups that differ in morphology, ecology, and/or geographic distribution.

Results

119 ITS sequences were produced for this study (Table 1). For the BI analyses, the average standard deviation of split frequencies reached below 0.01 after 4 million generations (global phylogeny) and 2 million generations (pruned phylogeny), so the first 40% of sampled trees were discarded as the burn-in for each analysis. Posterior probabilities (PP) for each analysis were calculated from 12002 samples from two independent runs for both the global and pruned analyses. In each case the potential scale reduction factor (PSRF) convergence diagnostic reached a value of 1.0 for all parameters, indicative of sufficient sample size.

The global phylogeny contained 397 sequences and 61 species-level clades. Species-level clades containing sequences from eastern United States and Canada are colored red in the global circle tree (Figure 1; all Figures are in Appendix). PP ≥ 0.95 for nodes of these clades are denoted with an asterisk. The pruned version of the global tree, including taxon tip labeling information, is shown in Figure 2. Four major clades of North American *Hydnum* were recovered, each with ML bootstrap support ≥ 70%. These are labeled by subgenus: *Alba, Pallida, Hydnum, Rufescentia* (Niskanen et al. 2018). A sister group relationship was recovered between subg. *Hydnum* and *Rufescentia*. In subg. *Alba* we recovered three monophyletic lineages that originate from eastern North America and correspond to phylogenetic species: *H. alboaurantiacum* sp. nov., *H. albidum*, and *H. albomagnum*. Subg. *Pallida* is represented by a single phylogenetic species from eastern North America: *H. subtilior* sp. nov. In subg. *Hydnum* three phylogenetic species from eastern North America were detected: *H. vagabundum* sp. nov., *H. washingtonianum*, and *H. subolympicum*. Subg. *Rufescentia*, centered around *H. rufescens*, contains the largest number of eastern North American phylogenetic species, including *H. ferruginescens* sp. nov., *H. aerostatisporum*, *H. canadense*, *H. mulsicolor*, *Hydnum* sp. AS30, *H. subconnatum* sp. nov., *H. quebecense*, *H. cuspidatum* sp. nov., and *H. umbilicatum*.

We were unable to produce ITS sequences from the holotypes of the following species: *H. albidum*, *H. albomagnum*, *H. umbilicatum* and *H. caespitosum*. As a result, we designated epitypes for three of those species – *H. umbilicatum* 10651TJB (CORT 012241), *H. albidum* 10640TJB (CORT 012029), and *H. albomagnum* RAS231 (TENN 073062), which are represented by ITS data. Each designated epitype was chosen based on morphological consistency and geographic proximity to sites containing holotypes. We were able to sequence partial ITS regions from two historical Peck collections

labeled *H. albidum*, but based on morphology those specimens represent a separate, larger, white species that we describe here as *H. vagabundum*.

A total of sixteen species-level monophyletic groups from eastern North America were recovered, representing ten described and six undescribed species. One additional species, *H. geminum*, was not represented by any modern collections, and thus its phylogenetic position is unconfirmed. Below, taxonomic descriptions are presented for eastern North American species by subgenus. Of the recovered species, only one eastern North American *Hydnum*, *H. mulsicolor*, also occurs outside North America. This result is consistent with recent work that showed eastern North American clades of *Hydnum* are largely endemic (Feng et al. 2016, Niskanen et al. 2018).

None of the species studied display a clear preference for tree host genus. Many occupy a wide geographic range within eastern North America, with three species (*H. albidum*, *H. subtilior*, *H. vagabundum*) also found in Central America. One species, *H. washingtonianum*, occurs in both eastern and western North America.

Basidia in *Hydnum* were consistently suburniform, often undulating, and tapered to a narrow pedicel. Basidium shape was not considered a diagnostic trait and thus omitted from taxonomic descriptions below. Basidiospores of *Hydnum* were inamyloid and acyanophilous (Hall and Stuntz 1971). Morphological variation across *Hydnum* was low compared to other genera, with few variable microscopic features. As a consequence, species varied by differences in basidiospore size and shape, number of sterigmata, and pileipellis elements. Several taxa in subg. *Rufescentia* were nearly morphologically indistinct from one another and could be identified in the field only by a combination of morphology and distribution/habitat data. Even so, ITS sequencing is necessary to confidently identify those species.

Taxonomy

Hydnum subg. *Alba* Niskanen & Liimat., Mycologia 110(5): 896 (2018)

Hydnum albidum Peck, Bulletin of the New York State Museum 1(2): 10 (1887)
MycoBank Epitypification: MBT381859
GenBank: MH379883
Figures 3A, 3B, 6A

= *Hydnum repandum* var. *albidum* (Peck) Bres., Iconographia Mycologica 21: 1045 (1932)
= *Dentinum albidum* (Peck) Snell, Mycologia 37: 51 (1945)
= *Hydnum repandum* f. *albidum* (Peck) Nikol., Flora Plantarum Cryptogamarum URSS. Fungi. Familia Hydnaceae 6(2): 306 (1961)

Type: UNITED STATES. New York: Rensselaer County, Sandlake, ground in thin woods, Jul ca. 1886, C.H. Peck (holotype: NYS-F-134). Epitype: UNITED STATES. New York: Cortland County, Kennedy State Forest, Scutt Road (42.4685; -76.1656), on

humus in forest with Quercus rubra, Fagus, Acer, 550 m, 30 Jul 2014, T.J. Baroni 10640TJB (CORT 012029).

Description: Pileus 15–50 mm wide, round to reniform, convex to plano-convex or uplifted, disc sometimes shallowly depressed; surface glabrous, sometimes irregularly bumpy or mottled in appearance, bright white becoming cream or cream-peach (10YR 8/4), no reaction to KOH; margin entire and incurved when young, undulating in age. Spines 1–6 mm long, easily rubbing off, subdecurrent to decurrent, white to cream white (10YR 8/3). Stipe 15–45 × 5–15 mm, central or eccentric, equal to slightly enlarged or bulbous at the base, then tapering into ground, concolorous with the pileus, staining orange-ochre (5A4–5B7 or "Yellow Ochre"). Basal mycelium white when present. Context white to pale cream, staining slowly orange (5A6) after five mins. Odor mild at first, then pleasantly fruity like apricots when stored in foil. Taste mild, pleasant, or occasionally peppery.

Basidiospores 4.5–5.2–6 μm × 3–4–4.5(5) μm, Q=(1.05)1.07–1.33–1.58(1.74) (n=72/5), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 28–36(40) × 6–7(8) μm with 5–6(7) sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–6 μm wide. Clamp connections present.

Distribution: Eastern Canada and U.S. and central Mexico – Nova Scotia, Vermont, New York (type), Tennessee, North Carolina. Also Veracruz, Mexico (GenBank KC152123).

Ecology: In hardwood and mixed woods with *Betula*, *Quercus*, *Fagus*, *Tsuga*, *Pinus*, *Abies*. June to early September.

Other specimens examined: CANADA. Nova Scotia: Victoria County, Cape North, Grey Glen Brook, Farm lot, with *Abies*, *Betula*, 80 m, 8 Sep 1973, R.H. Petersen TFB38198 (TENN 038198). UNITED STATES. New York: Cortland Co., Kennedy Forest, Scutt Hill Road, on humus under *Quercus*, *Fagus*, *Acer*, 550 m, 10 Aug 2003, T.J. Baroni 9623TJB (CORT 014489). North Carolina: Great Smoky Mountains National Park, Heintooga Round Bottom Road, top of road on embankment with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS204 (TENN 071752). Great Smoky Mountains National Park, Heintooga Round Bottom Rd., on embankment with *Betula*, *Quercus*, *Tsuga*, 1525 m, 17 Aug 2017, R.A. Swenie RAS210 (TENN 073173). Tennessee: Great Smoky Mountains National Park, Ogle Place Nature Trail, near stream in soil among leaf litter with *Tsuga*, *Betula*, 670 m, 5 Jun 2016, R.A. Swenie RAS058 (TENN 072000). Great Smoky Mountains National Park, Schoolhouse Gap Trail, on embankment with *Quercus*, *Pinus*, *Betula*, 550 m, 8 Jul 2017, R.A. Swenie RAS158 (TENN 073041). Vermont: Windham County, Stratton Mountain Resort area, 650 m, 28 Jul 2017, T.J. Baroni 10788TJB (CORT 014475).

Discussion: *Hydnum albidum* was originally described from New York by Peck (1887) and produces small white to cream-colored basidiomes with small subglobose to broadly elliptic basidiospores. In the protologue Peck distinguishes *H. albidum* from *H. repandum* by the smaller basidiomes and spores, as well as white coloration. In a later description Peck (1897) added that *H. albidum* is an edible but uncommon species

"uniformly colored in all its parts". Of the two small white species of *Hydnum* that occur in eastern North America (Figures 3A–D), both *H. albidum* and *H. alboaurantiacum* have similarly small subglobose basdiospores (Figures 6A–B). However, *H. alboaurantiacum* quickly stains bright orange within minutes wherever handled, while *H. albidum* stains much less vividly brown-orange, sometimes only hours after handling. In addition, *H. alboaurantiacum* is only known from the southeastern US. While we were unable to successfully sequence DNA from the holotype of *H. albidum*, several collections from the region of the type locality are consistent with the morphology of the holotype of *H. albidum*, and one of these is designated as an epitype.

In addition to the holotype, there are several other historical collections made by Peck to which he applied the name *H. albidum*. Based on basidiospore measurements alone, it is clear three of the eight collections have much larger spores than *H. albidum*. We successfully sequenced partial ITS from two of those three collections, which matched modern specimens from Texas, New York, and Honduras belonging to a species more closely related to *H. repandum* (see discussion of *H. vagabundum*).

Hydnum repandum var. *album* (Quél.) Rea is a European variety, the name of which has been widely applied in North America (Coker and Beers 1951, Smith et al. 1981, Harrison and Grund 1987, Roody 2003). The description of *H. repandum* var. *album* by Roody (2003) appears to refer to *H. subtilior*, while displaying a photo of what is perhaps *H. albidum*. However, the spores of *H. albidum* are smaller than the 7–8.5 × 5.5–7 μm listed by Roody (2003), Harrison and Grund (1987), Smith et al. (1981), and Coker and Beers (1951). Thus, the American concept of *H. repandum* var. *album* is best interpreted as *H. subtilior*, described below.

***Hydnum alboaurantiacum* Swenie & Matheny, MycoKeys 42: 45 (2018)**
MycoBank No: MB825492
GenBank: MH379955
Figures 3C, 3D, 6B

Diagnosis: Most similar to *Hydnum albidum* but differs from it by the slightly stouter basidiomes that stain bright orange within minutes of handling. Differs from *H. subtilior* and *H. vesterholtii* by smaller basidiospores.

Type: UNITED STATES. North Carolina: Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail (35.5634; -83.3092), scattered under *Betula*, *Fagus*, with *Tsuga* nearby, 28 Jul 2017, R.A. Swenie RAS186 (holotype: TENN 073053).

Etymology: *alboaurantiacum* (L.) white-orange, referring to the coloration of the basidiomes, which stain bright orange.

Description: Pileus 20–70 mm wide, irregularly round, convex, becoming shallowly convex to depressed, occasionally umbilicate; margin thin, wavy to lobed, incurved becoming decurved; surface matt, glabrous, pale to cream white ("Pale Ochraceous Buff"), quickly bruising orange ("Zinc Orange" or "Xanthine Orange", 6A6-8). Spines 1–7 mm long, brittle in mass and breaking easily, adnate to subdecurrent, white to cream-orange ("Pale Ochraceous Buff" to "Light Ochraceous Buff", 4A2–5A3). Stipe 17–50 × 6–21 mm, central or eccentric, terete or clavate, concolorous with the

pileus, easily bruising orange (5A2). Context thin, firm, cream white, staining orange ("Xanthine Orange" to "Mars Yellow", 6A8 to 5B6–B7), especially in young specimens at base of stipe within five minutes when cut in half. Odor mild or sweet and fruity. Taste mild.

Basidiospores 4–4.8–6(7) µm × 3–3.9–5(6) µm, Q=1.00–1.25–1.52(1.54) (n=44/6), globose to ellipsoid, smooth, hyaline in KOH. Basidia 36–42 × 4.5–7 µm with 5–7 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–5 µm wide. Clamp connections present.

Distribution: Southeastern U.S. – North Carolina (type), Tennessee, and West Virginia (GenBank KU612600).

Ecology: In mixed woods with *Quercus*, *Tsuga*, *Pinus*, *Betula*, *Liriodendron*, *Fagus*. May to August.

Other specimens examined: UNITED STATES. North Carolina: Great Smoky Mountains National Park, Deep Creek, Indian Creek, on soil in mixed woods, 610 m, 9 Jul 1974, J.H. Restivo JHR1459 (TENN 040599). Great Smoky Mountains Na- tional Park, Smokemont, Bradley Fork Trail, scattered under *Betula*, *Fagus*, *Tsuga*, 700 m, 28 Jul 2017, R.A. Swenie RAS186 (TENN 073053). Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on embankment with *Quercus*, *Betula*, *Tsuga*, 1525 m, 17 Aug 2017, R.A. Swenie RAS208 (TENN 071751). Macon County, vicinity of Highlands, Glen Falls Trail, 1100 m, 14 Jul 2000, E.B. Lickey TFB9833 (TENN 058812). Transylvania County, Pisgah National Forest, Yellow Gap Road, 310 m, 18 Jul 2000, R.H. Petersen TFB9764 (TENN 058665). Duke Forest, scattered in mixed duff with *Quercus*, *Pinus*, 150 m, 25 May 2016, B.P. Looney BPL876 (TENN 073003). Blue Ridge Parkway, near mile marker 342, side of road on embankment, deciduous woodlot, 1225 m, 19 Aug 2016, J. Schieb RAS104 (TENN 073014). Buncombe County, Bent Creek Experimental Forest, near Boyd Branch Road, mossy acidic forest with *Quercus alba*, *Q. rubra*, *Liriodendron tulipifera*, *Betula lenta*, *Tsuga canadensis*, 670 m, 22 Aug 2016, M. Hopping MH16004 (TENN 073548). Tennessee: Great Smoky Mountains National Park, Cades Cove, Gregory Ridge Trail, 550 m, 18 Aug 2005, E.B. Lickey TFB12761 (TENN 061328). Great Smoky Mountains National Park, Maddron Bald Trail, on mossy soil with *Tsuga*, *Quercus*, *Fagus*, *Pinus*, 550 m, 4 Aug 2012, S.A. Trudell SAT1221712 (TENN 067355). Sevier County, University of Tennessee Biology Field Station, on mossy soil with *Quercus*, *Tsuga*, *Pinus*, 450 m, 27 Jul 2009, A.D. Wolfenbarger AW0119 (TENN 064272).

Discussion: *Hydnum alboaurantiacum* has probably been mistaken for the closely related *H. albidum* due to the initial pale white coloration and similar basidiospore size and shape. However, *H. alboaurantiacum* quickly stains bright rusty orange on all parts of the basidiomes where handled, whereas *H. albidum* slowly stains a lighter brown-orange hue. In addition, *H. alboaurantiacum* often displays a larger and more stout stature than *H. albidum*. The two species are readily distinguished as separate clades in Figure 2. *Hydnum alboaurantiacum* is known only from the southeastern US and appears derived from a grade of Central American taxa including the recently described *H. zongolicense* (Niskanen et al. 2018).

Hydnum albomagnum Banker, Bulletin of the Torrey Botanical Club 28(4): 207 (1901)
MycoBank Epitypification: MBT381860
GenBank: MH379943
Figures 3E, 3F, 6C

= *Dentinum albomagnum* (Banker) Pouzar, Ceská Mykologie 10 (2): 76 (1956)

Type: UNITED STATES. Alabama: Lee County, Auburn, Dec 1896, F.S. Earle (holotype: NY 776138). Epitype: UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, Bandy Creek area (36.4920; -84.6950), solitary on soil with *Quercus*, *Pinus*, 450 m, 23 Sep 2017, RAS231 (TENN 073062).

Description: Pileus 60–110 mm wide, irregularly round, irregularly convex to plano-convex or uplifted; surface dull, glabrous with adhering debris, uneven and sometimes pitted in places, cream white (4A4) with patches of cottony white, becoming light tan in age (10 YR 6/4); margin thin, wavy to slightly lobed, incurved when young then raised in age. Spines 1–6 mm long, brittle in mass, adnate to subdecurrent, white to cream white (4A3–5A3). Stipe 20–40 × 13–20 mm thick, central or eccentric, clavate, occasionally split in two towards the apex; surface smooth, concolorous with spines, if bruising then only very slightly an hour or more after handling ("Yellow-Ocher" to "Ochraceous-Tawny"). Context fleshy, white, unchanging when cut. Odor mild or slightly acidic at first, then pleasantly fruity like apricots when stored in foil. Taste mild.

Basidiospores 5.5–6.2–7 μm × 3–3.8–5 μm, Q=1.24–1.66–2.07(2.17) (n=45/3), ellipsoid to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 38–46 × 5–6 μm with 4–5(6) sterigmata. Pileipellis a tightly interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 2.5–5 μm wide. Clamp connections present.

Distribution: Eastern U.S. – Massachusetts, North Carolina, Tennessee (epitype), and Alabama (holotype).

Ecology: In hardwood and mixed woods with *Quercus*, *Pinus*. September to December.

Other specimens examined: UNITED STATES. Massachusetts: Worcester County, Rutland State Park, gregarious under litter layer along edge of road with *Quercus*, *Pinus strobus*, 260 m, 1 Nov 2003, P.B. Matheny PBM2512 (TENN 066858). North Carolina: Buncombe County, Black Mountain YMCA Blue Ridge Assembly, Wolfpit Loop, in leaf litter in broadleaf woods with *Quercus*, *Acer*, 900 m, 24 Sep 2015, B. Moerk NAMA 2015-050 (F C0305359F). Tennessee: Knox County, New Hopewell, on soil in mixed woods, 300 m, 2 Dec 1951, L.R. Hesler (TENN 020243).

Discussion: The original description of *Hydnum albomagnum* by Banker (1901) was based solely on dried material. As mentioned in the protologue, this species has a shorter stipe and overall stouter appearance than other *Hydnum*. In addition, mature basidiomes are much larger than other whitish small-spored species. *Hydnum* albomagnum also has more strongly ellipsoid spores (Q values averaging 1.66) than other species. Historically, *H. albomagnum* has been less frequently collected than other pale species of Hydnum, perhaps because the basidiomes are often buried underneath layers of needle and leaf litter and thus overlooked. As basidiomes emerge from the ground, leaf

litter remains stuck to the matt glabrous surface of the pileus, masking its appearance. This characteristic, along with the creamy white coloration, large basidiome size, and small ellipsoid spores, makes this one of the easier *Hydnum* species to identify. DNA sequencing of the type specimen of *H. albomagnum* was not successful; however, all modern collections reported under this species name have identical ITS sequences and match the morphology of the original species description. An epitype is chosen from Tennessee material, closest to the location of the holotype from Alabama.

Collections that are likely *H. alboaurantiacum* (TENN 041525) and *H. vagabundum* (TENN 003140) have been misidentified as *H. albomagnum*, perhaps due to the larger white appearance of those specimens. However, those collections differ from *H. albomagnum* in spore dimensions and lack litter debris on the pileus, which persists even after drying in *H. albomagnum*.

Hydnum subg. *Pallida* Niskanen & Liimat., Mycologia 110(5): 899 (2018)

***Hydnum subtilior* Swenie & Matheny, MycoKeys 42: 48 (2018)**
MycoBank No: MB825493
GenBank: MH379913
Figures 3G, 3H, 6D

= *Hydnum repandum* var. *album* (Quél.) Rea sensu Am. auct.

Diagnosis: *Hydnum subtilior* is most closely related to European *H. vesterholtii* but differs from it based on ITS molecular data and geographic distribution in eastern North America.

Type: UNITED STATES. Tennessee: Anderson County, Norris Dam State Park, Clear Creek Trail (36.2124; -84.0681), scattered on soil along trail under *Fagus*, *Carya*, *Quercus*, 24 Jun 2017, P.B. Matheny PBM4093 (holotype: TENN 073034).

Etymology: *subtilior* (L.) finer, more slender, in reference to the slim basidiomes.

Description: Pileus 20–90 mm wide, round or occasionally reniform, convex becoming plano-convex to depressed, sometimes umbilicate; surface matt, glabrous, sometimes cracking into scales at the center, light cream yellow to cream orange buff ("Marguerite Yellow" to "Light Ochraceous Buff", 4A3–A5 to 5A2–A4), yellow with KOH, negative with FeSO4; margin thin, entire, incurved when young then decurved and sometimes wavy in age, staining rusty orange-brown ("Ochraceous-Orange" to "Mars Yellow", 6A5 to 5B6–B7). Spines 1–8 mm long, adnexed to decurrent, cream white to pale orange-cream (5A1–A2). Stipe 20–60 × 5–21 mm, central or eccentric, sometimes curving, equal or enlarging towards base, cream white or slightly lighter than pileus, staining rusty orange-brown (5B6-B8). Context spongy, cream white to pale orange-cream, slowly staining orange (5A4–6) throughout after five minutes where cut. Odor mild or sweet. Taste mild or pleasant.

Basidiospores 7–8–9 μm × 5–6.3–7.5 μm, Q=1.07–1.27–1.52 (n=51/5), subglo-

bose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 32–44 × 7–9 μm with 3–5(6) sterigmata. Pileipellis an interwoven cutis. Hyphae smooth, cylindri- cal, thin-walled, mostly 3–7 μm wide. Clamp connections present.

Distribution: Eastern U.S. – Illinois, North Carolina, Tennessee (type), Georgia, and Florida. Also Michoacán, Mexico (GenBank KY574324).

Ecology: In hardwoods under *Quercus*, *Carya*, *Fagus*, *Carpinus* or in mixed woods with these trees or *Betula* and conifers such as *Tsuga* or *Pinus* or less frequently *Picea*. June to August.

Other specimens examined: UNITED STATES. Florida: Alachua County, San Felasco Hammock Preserve State Park, Moonshine Sink Trail, in soil with deep layer of leaf litter, forest almost entirely *Carya*, 75 m, 23 Jul 2017, R.A. Swenie RAS180 (TENN 073050). Alachua County, Sweetwater Preserve off 16th Street entrance, mixed hardwood forest of *Quercus*, *Carya*, *Carpinus* and occasionally *Pinus*, 35 m, 6 Aug 2017, L. Kaminsky & G. LaPierre (FLAS 61253). Georgia: Putnam County, Rock Eagle 4-H Camp, with *Quercus*, *Pinus*, *Carpinus*, 200 m, 20 Jul 2017, R.A. Swenie RAS170 (TENN 073049). Illinois: Coles County, Lakeview Park, scattered under *Quercus alba*, *Carya*, 215 m, 8 Aug 2009, M. Kuo MK08080904. North Carolina: Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail to Mt. Sterling, on soil under *Tsuga*, 500 m, 9 Aug 2012, P.B. Matheny PBM3868 (TENN 067482). Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail, scattered under *Betula*, *Fagus*, *Quercus*, *Tsuga*, 700 m, 28 Jul 2017, R.A. Swenie RAS184 (TENN 073051). Great Smoky Mountains National Park, Heintooga Round Bottom Road, solitary with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS207 (TENN 073057). Tennessee: Great Smoky Mountains National Park, Tremont, Middle Prong Trail, scattered singly in mixed woods next to river with *Tsuga*, *Betula*, 450 m, 14 Jul 2013, P.B. Matheny PBM3923 (TENN 071999). Great Smoky Moun- tains National Park, Cades Cove Road, 610 m, 31 Jul 2004, R.H. Petersen TFB12107 (TENN 060045). Great Smoky Mountains National Park, Elkmont, solitary under *Quercus*, *Tsuga*, 670 m, 4 Aug 2009, J.M. Birkebak JMB080409-09 (TENN 064273). Great Smoky Mountains National Park, Tremont Institute, Lagoon Trail, solitary under *Tsuga*, *Carpinus*, *Betula*, 450 m, 23 Jun 2017, R.A. Swenie RAS148 (TENN 073035). Great Smoky Mountains National Park, Greenbrier picnic area, solitary in riparian forest under *Tsuga*, *Betula*, 500 m, 28 Jul 2017, B.P. Looney BPL987 (TENN 073032). Anderson County, Norris Dam State Park, Clear Creek Trail, solitary in litter on slope under *Quercus*, *Carya*, *Fagus*, 275 m, 31 Aug 2009, A.J. Floden AJF2 (TENN 069607).

Discussion: *Hydnum subtilior* is a common species in the southeastern U.S. found in deciduous and mixed forests with a variety of tree associates, often in deep layers of leaf litter. Environmental sequencing has recovered this species from *Quercus* root tips in central Mexico (García-Guzmán et al. 2017). The stipe is usually longer than the diameter of the pileus, and the overall coloration can range from light cream-yellow to peach or tan. The best diagnostic features for this species are the coloration and often elongated stature in combination with broadly ellipsoid spores averaging 8 × 6.3 μm. In addition, the context of fresh basidiomes stains orange throughout within five minutes when cut in half (Figure 3H).

Earlier authors (Coker and Beers 1951, Smith et al. 1981, Harrison and Grund 1987, Roody 2003) referred to this species as *H. repandum* var. *album*, a taxon originally described from Europe.

Hydnum subg. *Hydnum* L.

Hydnum subolympicum Liimat., Niskanen, R.E. Baird & Voitk, Mycologia 110(5): 903 (2018)

= *Hydnum repandum* sensu Coker & Beers, 1951

Type: CANADA. Newfoundland and Labrador: Near Humber Village, trail to Barry's Lookout (48.9860; -57.7600), mature secondary growth of *Betula papyrifera* and *B. alleghaniensis*, also with *Cantharellus amethysteus*, 2 Sept 2012, A. Voitk 12.09.02. av12 (holotype DAOM744368, isotype K(M)249002).

Description: Pileus 80–130 mm wide, round, convex, becoming plano-convex; surface dry, glabrous, dull reddish-orange when young (5 YR 5/6) then cream to peach or dull orange in age (5A2-3), sometimes cracking in age to reveal white color of flesh; margin incurved and entire, becoming wavy and decurved, staining ochre to rusty brown very slowly after handling ("Yellow Ochre" to 5B8). Spines 1–7 mm long, close, subdecurrent, cream-yellow to pinkish cream. Stipe 30–100 × 20–40 mm, central or eccentric, tapering downwards to a slightly bulbous base, texture firm, smooth, white or off-white, staining orange cream to rusty orange, then yellow-brown when handled ("Mars Yellow", 10YR 5/8). Context white to cream, dry, firm, brittle, discoloration not observed. Odor mild or fruity and reminiscent of apricots. Taste mild or slowly bitter or peppery.

Basidiospores 6–7.5–9 μm × 5–6.1–7 μm, Q=1.07–1.23–1.46 (n=38/3), subglobose to broadly ellipsoid, smooth, hyaline in KOH. Basidia 36–42 × 6.5–8 μm with (3)4–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin- walled, mostly 4–6 μm wide. Clamp connections present.

Distribution: Eastern North America – Newfoundland and Labrador (type), Quebec (GenBank No. KM406979), Maryland, Virginia, North Carolina, Tennessee, and Georgia.

Ecology: In hardwood or mixed woods with *Quercus*, *Betula*. August to October.

Other specimens examined: UNITED STATES. Georgia: White County, Unicoi State Park, Unicoi to Helen Trail, hidden in soil embankment under roots, 460 m, 16 Jul 2017, R. Healy RAS168 (TENN 073047). Maryland: Harford County, Susquehanna State Park, 60 m, 9 Sep 2016, RAS118 (TENN 073021). North Carolina: Buncombe County, Bent Creek Experimental Forest, Boyd Branch, solitary in mature bottomland forest including *Liriodendron tulipifera*, 670 m, 19 Sep 2016, M. Hopping MH16009 (TENN 073551). Tennessee: Great Smoky Mountains National Park, Schoolhouse Gap Trail, solitary in mixed woods under *Quercus*, 490 m, 26 Oct 2013, A.J. Ramsey AJR14 (TENN 073004). Virginia: Grayson County, Mount Rogers National Recreation Area,

Mt. Rogers Trail, scattered along trail in mixed hardwood forest with *Betula*, *Quercus*, 1225 m, 17 Aug 2015, R.A. Swenie RAS029 (TENN 070845).

Discussion: This eastern North American species is phylogenetically allied with *H. repandum* and relatives in subgenus *Hydnum* along with *H. vagabundum* sp. nov. (described below) and *H. washingtonianum*. It can be distinguished from European *H. repandum* mainly by the different geographic distribution (eastern North America). This species differs from *H. vagabundum* by the smooth yellow-peach pileus that tends to crack in age and mostly non-lobate pileus margin. In comparison to *H. washingtonianum*, *H. subolympicum* produces smaller spores. Because of the shape and coloration, basidiomes of *H. subolympicum* in the field can resemble large chanterelles from above. Like many other species of *Hydnum*, basidiomes of this species often possess the sweet apricot-like odor that is characteristic of chanterelles. In our experience, this species is a choice edible. Coker and Beers (1951) likely referred to this taxon under the name *H. repandum*.

***Hydnum vagabundum* Swenie, Ovrebo & Matheny, sp. nov.**
MycoBank: MB825494
GenBank: MH379909
Figures 4B, 4C, 6F

Diagnosis: Closely related to *Hydnum subolympicum* but differs from it by the paler, more lobate pileus and ITS sequence divergence.

Type: UNITED STATES. Texas: Newton County, State Highway 87 and County Road 3062 (30.7080; -93.8270), scattered in soil under *Fagus*, *Pinus*, *Quercus*, 29 Dec 2011, C.L. Ovrebo CLO4985 (holotype: TENN 074443).

Etymology: *vagabundum* (L.), wandering, roving about, in reference to the broad distribution of this species in North America.

Description: Pileus 30–140 mm wide, round, convex, becoming plano-convex to broadly depressed; margin incurved and often lobed when young, then decurved or straight and wavy in age; surface matted tomentose or glabrous and pitted-grooved to bumpy in areas, off-white with pale pinkish buff tones (5A2), sometimes with slight ochre hues (5C4–C5), staining ochre where bruised. Spines 1–12 mm long, shortest near the pileus margin, adnate to subdecurrent, concolorous with the pileus or slightly darker pinkish-orange (5A3). Stipe 20–60 × 10–30 mm, central or eccentric, equal or with a swollen base, surface smooth or soft matted-tomentose, white or concolorous with the pileus, pinkish tan in areas, slowly staining ochre where bruised. Context solid, white, discoloring slight ochre (5C4–C5) where cut in half. Odor not distinctive. Taste mild or sweet-nutty, then slowly slightly acidulous.

Basidiospores 6.5–7.4–8.5 × 5–6.1–7.5 μm, Q=1.03–1.22–1.43(1.60) (n=97/4), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 39–57 × 8–10.5 μm with (3)4–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–7 μm wide. Clamp connections present.

Distribution: Eastern U.S. and Central America – New York, Texas (type), Honduras (GenBank HM639267–HM639268).

Ecology: In mixed woods with *Fagus*, *Pinus*, *Quercus*, *Tsuga*, *Picea*, *Betula*. May to December.

Other specimens examined: UNITED STATES. New York: Bolton, Sand Lake, August, C.H. Peck (NYS-D-7819). Rensselaer County, Burden Lake, 1 Sep, C.H. Peck (NYS-D-7820). Tompkins County, Hammond Hill State Forest, Red Man Run Rd., on humus in mixed woods with *Tsuga*, *Fagus*, *Picea*, *Betula alleghaniensis*, 520 m, 1 Sep 2017, T.J. Baroni 10782TJB (CORT 014461).

Discussion: *Hydnum vagabundum* is a large whitish species in subgenus *Hydnum*. Two collections of this species at NYS from the late 1800s and early 1900s were misidentified as *H. albidum* by Peck but feature distinctly larger basidiospores than the holotype of *H. albidum*. In addition, basidiomes of this species are much larger and fleshier than those of *H. albidum*. Partial ITS sequences obtained from Peck's specimens match two extant collections from New York and Texas, as well as GenBank sequences from western Honduras (HM639268, HM639267; Sarmiento and Fontecha 2013). In Honduras this species can be found in May and June, where it is a common edible (Sarmiento and Fontecha 2013). To date, *H. vagabundum* has the widest known range among the endemic eastern North American Hydnum species.

Peck's notes of what is described here as *H. vagabundum* indicate it as a "white, edible *Hydnum*". Another large whitish species, *H. albomagnum*, can be distinguished from *H. vagabundum* by the copious amount of leaf and needle debris that adheres to the pileus surface and smaller basidiospores.

Hydnum washingtonianum Ellis & Everhart, Proc. Phila. Acad. 1894: 323 (1894)

= *Hydnum neorepandum* Niskanen & Liimat., Mycologia 110(5): 901 (2018)

Type: UNITED STATES. Washington: Kitsap County, Tracyton (47.6090; -122.6540), on ground in deep coniferous woods, 27 Dec 1893, A.M. Parker (holotype: NY 776185, isotype: WTU-F-14341).

Description: Pileus up to 40 mm wide, subplane, slightly depressed, thin, irregular; surface glabrous, "subviscose", wrinkled when dry, pale orange. Spines 3–5 mm long, terete, slender, acute, decurrent halfway down the stipe, pale yellow but nearly white when fresh. Stipe up to 40 × 5–10 mm, subcylindrical, tapering slightly towards the base, central or slightly eccentric, pale orange. Context fleshy.

Basidiospores 7–7.7–8.5 μm × 6–6.8–7.5(8) μm, Q=1.04–1.13–1.22 (n=40/2), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 31–41 × 7.5– 8.5 μm with 4 sterigmata. Pileipellis hyphae not reviving. Clamp connections present.

Distribution: Western North America and eastern Canada – British Columbia, Washington (type), California (GenBank GU180269, MG972632), and Newfoundland and Labrador.

Ecology: On ground in coniferous woods. December.

Discussion: *Hydnum washingtonianum*, originally described from the Puget Sound region of Washington, is characterized by the pale orange pileus, yellowish

decurrent spines, small globose basidiospores, and tough flesh. The species was considered synonymous with *H. repandum* by Maas Geesteranus (1964) and Hall and Stuntz (1971). However, we were able to produce a partial ITS sequence from the isotype (GenBank MH379846), which does not match European *H. repandum* sequences. Thus, we consider this species as an autonomous taxon with a mostly northern geographic distribution in North America. Phylogenetic analysis of the ITS sequence confirms this species from Washington, British Columbia, California, and Newfoundland and Labrador. *Hydnum washingtonianum* is associated with coniferous forests on both coasts, and one environmental sequence (GenBank GU180269) recovered this species on root tips of *Pinus muricata* in California.

Hydnum neorepandum, a recently described species from Newfoundland and Labrador (Niskanen et al. 2018), has an ITS sequence that differs by a single base pair from that of the isotype of *Hydnum washingtonianum*. The morphology of both protologues is also in agreement. Thus, we consider *H. neorepandum* a taxonomic synonym of *H. washingtonianum*.

Hydnum subg. *Rufescentia* Niskanen & Liimat., Mycologia 110(5): 905 (2018)

***Hydnum ferruginescens* Swenie & Matheny, sp. nov.**
MycoBank: MB825495
GenBank: MH379905
Figures 3D, 5G

Diagnosis: Most closely related to the Eurasian *Hydnum magnorufescens* but differs from it by somewhat smaller basidiospores, ITS sequence divergence, and geographic distribution in the southeastern U.S.

Type: UNITED STATES. North Carolina: Buncombe County, Tanbark Ridge (35.6535; -82.4853), growing singly or conjoined in moss along trail with *Pinus strobus*, *Quercus prinus*, *Kalmia latifolia*, 915 m, 4 Sep 2016, M. Hopping MH16005 (holotype: TENN 073549).

Etymology: *ferruginescens* (L.), becoming ferruginous or rust-colored, in reference to the overall coloration of this species.

Description: Pileus 22–60 mm wide, round, convex, becoming depressed; margin incurved and entire when young, then irregularly lobed or degraded in age; surface dry, glabrous, tawny to fulvous (5YR 5/8 to 6/8), discoloring slightly darker when handled. Spines 1–4 mm long, shorter near the margin, adnate to subdecurrent, white to cream (5A2–A4), bruising orange. Stipe 15–40 × 5–12 mm, central or eccentric, equal or slightly wider at apex, texture smooth, white or cream, lightly bruising orange (7.5 YR 7/8, 5A6-7); thick white mycelial mat sometimes present at base of stipe. Context white, unchanging after 5 minutes where cut in half. Odor not distinctive. Taste not distinctive or mildly fruity.

Basidiospores (5.5)6–6.9–8 μm × 5–6.3–7.5 μm, Q=1.01–1.09–1.22, (n=45/2), globose to subglobose, smooth, hyaline in KOH. Basidia 39–56 × 7.5–9 μm with (3)4–5

sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin- walled, mostly 5–7 μm wide. Clamp connections present.

Distribution: Southeastern U.S. – North Carolina (type), Tennessee, Arkansas (GenBank KX358033).

Ecology: In mixed woods with *Quercus*, *Pinus*, *Carya*, *Tsuga*. September.

Other specimens examined: UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, West Bandy Trail, scattered along trail with *Quercus*, *Carya*, *Pinus*, *Tsuga*, 450 m, 23 Sep 2017, R.A. Swenie RAS229 (TENN 073061).

Discussion: *Hydnum ferruginescens* is known only from three occurrences in the southeastern U.S. This species is similar to *H. magnorufescens*, which has similarly sized basidiomes but slightly larger spores and is known from Europe and Asia.

Hydnum aerostatisporum Buyck, Lewis & V. Hofstetter, Crypt. Mycologie, 38: 101–146 (2017)
Figures 3E, 3F, 5H

= *Hydnum subrufescens* Niskanen & Liimat., Mycologia 110(5): 911 (2018)

Type: UNITED STATES. Texas: Polk County, Big Thicket Natural Preserve, Big Sandy Creek Unit, Beaver Slide Trail (30.6150; -94.6700), 4 Jul 2014, Buyck 14.156 (PC0142475).

Description: Pileus (20)30–100 mm wide, irregularly round or sometimes reniform, convex to plano-convex, becoming funnel-shaped in age, sometimes with slit or umbilicus forming over stipe, surface dry, glabrous, subzonate when young, then cracking to coarsely scurfy in age, bright to medium brownish orange ("Xanthine Orange" to "Orange Rufous"), paler when young ("Salmon-Orange"), often cracking in age to reveal lighter color of context ("Pale Pinkish Buff"); margin incurved and entire when young, then wavy, irregular or degraded in age, discoloring slightly darker after handling. Spines 1–9 mm long, close, mostly awl-shaped but occasionally spathulate, adnate to subdecurrent, buff to peach ("Light Buff" to "Pinkish Buff"). Stipe 25–80 × (3)7–25 mm, central or eccentric, equal or slight bulbous at base in younger specimens, smooth, often with white hazy or cottony patches overlaid on surface, cream white to pale orange in younger basidiomes, then darker tan orange with age, discoloring very slightly brownish orange when handled. Context cream to peach colored, firm, sometimes hollow with age, unchanging after 5 minutes when cut in half. Odor mild or sweet at first, then pleasantly fruity like apricots when stored in foil. Taste mild or weakly acrid.

Basidiospores 7–8.1–8.5 × 6–7–8 μm, Q=1.01–1.15–1.33 (n=38/3), mostly broadly ellipsoid, smooth, hyaline in KOH. Basidia 40–47 × 8–10 μm with (2)3–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 μm wide. Clamp connections present.

Distribution: Eastern U.S. – Illinois, Texas (type), North Carolina, Tennessee, Virginia, and Florida.

Ecology: In hardwoods of *Quercus*, *Carya*, *Ulmus* or mixed woods including *Betula*, *Picea*, *Tsuga*. June to October.

Specimens examined: UNITED STATES. Florida: Alachua County, Gainesville, University of Florida Natural Area Teaching Laboratory, 30 m, 9 Sept 2016, A. I. Zuniga AIZ-021 (FLAS 60406). Alachua County, Gainesville, Possum Creek Park, in deep woods with *Carya* and some *Quercus*, soil rich and not sandy, 55 m, 9 Oct 2015, M.E. Smith MES1432 (FLAS 59996). Illinois: Coles County, Fox Ridge State Park, 230 m, 28 Sep 1996, M. Kuo MK09289621. Dewitt County, Weldon Springs State Recreation Area, gregarious under *Carya* with *Quercus alba, Quercus rubra, Ulmus* nearby, 215 m, 18 Aug 2014, M. Kuo MK09181403. North Carolina: Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on mossy bank with *Picea, Betula*, other hardwoods, 1580 m, 24 Jul 2016, R.A. Swenie RAS071 (TENN 073001). Great Smoky Mountains National Park, Heintooga Round Bottom Road, solitary on embankment with *Picea, Betula*, 1525 m, 17 Aug 2017, R.A. Swenie RAS211 (TENN 073174). Great Smoky Mountains National Park, Cataloochee, Big Fork Ridge Trail, 1100 m, 18 Jun 2005, E.B. Lickey TFB12514 (TENN 060681). McDowell County, near Little Switzerland, with *Tsuga*, ca. 1000 m, 19 Aug 2016, A. Funston RAS107 (TENN 073017). Tennessee: Great Smoky Mountains National Park, Cades Cove Road, 1 mile before Schoolhouse Gap Rd, 610 m, 31 Jul 2004, R.H. Petersen TFB12108 (TENN 060046). Great Smoky Mountains National Park, Tremont River Trail, solitary on slope in mossy area in mixed woods with *Tsuga* and hardwoods, 450 m, 4 Jul 2017, B. Teresi & K. Hucks RAS157 (TENN 073040). Texas: Polk County, Big Thicket National Preserve, Beaverslide Trail, on ground in mixed woods, 30 m, 12 Jun 2017, R.L. Pastorino RLP61217D (TENN 073547). Virginia: Shenandoah National Park, Hogback Mountain, 600 m, 9 Sept 2016, RAS121 (TENN 073024).

Discussion: *Hydnum aerostatisporum* is a commonly encountered species in the eastern U.S. It has been found primarily in hardwoods and mixed woods including conifers at high and low elevations on both sandy and non-sandy soils. The vibrant medium to dark orange pileus transitions from smooth in young specimens to conspicuously cracked and scurfy in age, often becoming funnel-shaped, occasionally with a hole or umbilicus. The stipe frequently becomes darker tan-orange in age, which is unusual in other medium-sized orange-pileate species of *Hydnum*. Basidiomes, particularly older specimens, often have patches of hazy white on the stipe surface.

Hydnum aerostatisporum was recently re-described as a new species from Quebec – *H. subrufescens* (Niskanen et al. 2018). The ITS sequence of the holotype of *H. subrufescens* differs from that of the holotype of the earlier described *H. aerostatisporum* by seven base pairs, but *H. subrufescens* does not form a well-supported monophyletic group in our phylogenetic analyses and recognition of *H. subrufescens* as a separate species would render *H. aerostatisporum* paraphyletic (Figure 2). The morphology of both is consistent, including the similarly sized globose to subglobose spores. For these reasons, we consider *H. subrufescens* a taxonomic synonym of *H. aerostatisporum*.

Hydnum canadense Niskanen & Liimat., Mycologia 110(5): 908 (2018)
Figures 3G, 5I

Type: CANADA. Newfoundland and Labrador: Near Grand Falls, south of the Exploits River, west of Hwy 360, south of Hwy 1, along a gravel road beside Moccasin Lake (48.9030; -55.5580), in conifer-dominated forest, 9 Sep 2009, K. Liimatainen & T. Niskanen 09-006 (holotype H7043727, isotype K(M)248978, isotype NY).

Description: Pileus 12–25 mm wide, irregularly round to slightly reniform, convex to plano-convex, surface dry, glabrous, orange ("Zinc Orange" to "Xanthine Orange"), sometimes cracking in age near central depression; margin incurved and entire or slightly degraded. Spines 1–3 mm long, adnate, cream-colored, at times thick and somewhat flattened. Stipe 15–35 × 5–8 mm, central or eccentric, equal or widening at base, firm, smooth, white to cream, lightly staining ochre to medium brownish orange ("Mars Yellow" to "Orange Rufous") where handled. Context not observed. Odor and taste mild.

Basidiospores 7–8–9(9.5) μm × 7–7.6–9 μm, Q=1.00–1.05–1.11, (n=38/1), globose to subglobose, smooth, hyaline in KOH. Basidia 38–46 × 7.5–9.5 μm with 3–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 5–7 μm wide. Clamp connections present.

Distribution: Eastern North America – Newfoundland and Labrador (type, GenBank KX388681) and North Carolina.

Ecology: In conifer forest with *Abies fraseri*. August to September.

Specimen examined: UNITED STATES. North Carolina: Yancey County, Mount Mitchell State Park, in fir forest, 2000 m, 19 Aug 2016, R.A. Swenie RAS100 (TENN 073010).

Discussion: *Hydnum canadense* is only known from high-elevation and higher latitude conifer forests in North Carolina and eastern Canada. The North Carolina collection was found among several basidiomes of *H. umbilicatum*. *Hydnum canadense* can be distinguished from *H. umbilicatum* by the 3–5 sterigmata (versus 2–4 sterigmata in *H. umbilicatum*) and ITS sequence divergence. *Hydnum canadense* differs from the closely related *H. mulsicolor* by the association with conifers and larger spore size. Another closely related species, *H. submulsicolor*, is morphologically indistinguishable from *H. canadense* according to Niskanen et al. (2018), and ITS sequencing is likely necessary to reliably differentiate it from *H. canadense*. The spores of the North Carolina collection of *H. canadense* reported here are more globose with a lower average Q value than that of the Newfoundland and Labrador collections reported by Niskanen et al. (2018).

Hydnum mulsicolor Liimat. & Niskanen, Mycologia 110(5): 908 (2018)
Figures 3H, 5J

Type: SLOVENIA. Velike Lašče (45.8500; 14.6000): In forest of *Picea abies*, *Fagus sylvatica*, and *Corylus avellana*, GIS 1336 (holotype LJF1057).

Description: Pileus 30–45 mm wide, round, convex when young, becoming plano-convex to funnel-shaped; surface dry, glabrous or matted-tomentose, bright orange to tan ("Zinc Orange" to "Ochraceous-Tawny") becoming subzonate towards margin, sometimes distinctly umbilicate at center; margin incurved at first, becoming decurved,

wavy, and lightening in color. Spines 1–7mm long, decurrent, pinkish cream with white tips. Stipe 25–45 × 5–8 mm, central or eccentric, equal or enlarging downwards, texture firm, smooth, with aborted spines at stipe apex and some texturing below, cream white, sometimes with small white cottony patches, staining orange when handled, a dense mat of basal mycelium present at base. Context not observed. Odor mild or pleasant. Taste not distinctive.

Basidiospores 6.5–7.7–8.5 μm × (5.5)6–7.1–8.5 μm, Q=1.00–1.08–1.19(1.24) (n=46/3), subglobose, smooth, hyaline in KOH. Basidia 52–60 × 7.5–9.5 μm with 3–4(5) sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin- walled, mostly 4–6 μm wide. Clamp connections present.

Distribution: Eastern U.S. and Central Europe – Ohio, Virginia, North Carolina, Tennessee (GenBank AJ547885, AJ547868, AJ783969), and Slovenia (type), Switzerland (GenBank KU612545, KX086216).

Ecology: In deciduous or mixed forests with *Quercus*. July to September.

Other specimens examined: UNITED STATES. North Carolina: Blue Ridge Parkway near Little Switzerland, deciduous woodlot with *Quercus*, 1100 m, 19 Aug 2016, M. Hopping RAS105 (TENN 073015). Blue Ridge Parkway near Little Switzerland, in mixed woods, 1100 m, 19 Aug 2016, D. Boes RAS108 (TENN 073018). Buncombe County, Tanbark Ridge, growing singly in mature acidic cove forest with *Quercus* and *Acer*, 915 m, 4 Sep 2016, M. Hopping MH16006 (TENN 073550). Tennessee: Great Smoky Mountains National Park, Cherokee Orchard, Bullhead Trail, on soil in mixed forest, 1340 m, 16 Jul 2015, R.A. Swenie RAS023 (TENN 070321). Virginia: Shenandoah National Park, milepost 21, 1000 m, 9 Sep 2016, RAS120 (TENN 073023).

Discussion: *Hydnum mulsicolor* is the only species of *Hydnum* in eastern North America that is also known to occur in Europe based on ITS phylogenetic analysis. The basidiomes are small to medium-sized with strongly decurrent spines, and the pileus color ranges from strikingly orange to tan. Prominent basal mycelium is also present as a dense mat or as distinct rhizomorphs at the stipe base. In the eastern US, *H. mulsicolor* is often found with *Quercus* in mixed or hardwood forests. It is closely related to *H. submulsicolor* and *H. canadense*, both of which are known only from coniferous forests in eastern North America and have slightly larger spores than *H. mulsicolor* (Niskanen et al. 2018).

Hydnum quebecense Niskanen & Liimat., Mycologia 110(5): 912 (2018)
Figure 5K

Type: CANADA. Québec: Saint-Donat (46.3000; -74.2000), in conifer-dominated forest (*Tsuga*, *Abies*, *Picea*, *Betula*, and *Populus*), 5 Sep 2010, anonymous, T. Niskanen 10-064 (holotype H7043948, isotype K(M)248983, isotype NY).

Description: Pileus 2–20 mm wide, round or sometimes irregularly so, convex, apex sometimes depressed or umbilicate, surface dry, tomentose or velutinous, tan orange-brown to warm reddish-brown; margin incurved, becoming decurved and wavy. Spines 1–2 mm long, adnate when young, subdecurrent with age, cream-white to peach. Stipe 15–45 × 3–10 mm, central, equal to subclavate, glabrous to minutely velutinous,

cream white to very light buff orange, staining buff orange when handled. Context solid, cream to tan. Odor and taste mild.

Basidiospores 8–8.4–9.5 μm × 7–7.8–9 μm, Q=1.00–1.09–1.28 (n=19/2), globose to subglobose, smooth, hyaline in KOH. Basidia 40–51 × 7–8 μm with 2–3 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–8 μm wide. Clamp connections present.

Distribution: Eastern U.S. and eastern Canada – New York, Quebec (type, GenBank KX388662).

Ecology: In moss, especially *Sphagnum*, under *Fagus* and *Picea*. July.

Other specimens examined: UNITED STATES. New York: Hamilton County, Raquette Lake, Long Point, Blue Mountain Trail, in moss under *Fagus grandifolia*, 550 m, 25 Jul 2001, J. D'Apice 49JD (CORT 7367). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum*, 550 m, 22 Jul 1997, J. Guardino JG003 (CORT 7330). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum*, 550 m, 23 Jul 1988, C. Nelson CN9 (CORT 7365). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum* under *Picea*, 550 m, 26 July 1993, K. Hodge KH7 (CORT 7322).

Discussion: This species is closely related to H. umbilicatum but is characterized by the apparent habitat preference for *Sphagnum* bogs. The holotype from Québec (KX388662) was described among *Sphagnum* in association with conifers and northern hardwoods (mainly *Picea*, but also *Tsuga*, *Abies*, *Betula*, *Populus*).

Hydnum sp. AS 30

Description: Pileus 20 mm wide, round, umbilicate, deep buff. Stipe less than 10mm long, central.

Basidiospores 6.5–7.2–8 μm × 6–6.9–7.5 μm, Q=1.00–1.05–1.1 (n=12/1), globose to subglobose, smooth, hyaline in KOH. Basidia 44–48 × 8–10.5 μm with (2) 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–7 μm wide. Clamp connections present.

Distribution: Eastern U.S. – New York.

Ecology: In Sphagnum, under *Tsuga* and *Larix*. July.

Specimen examined: UNITED STATES. New York: Hamilton County, Raquette Lake, Silver Beach Bog, *Sphagnum* substrate under *Tsuga*, *Larix*, 550 m, 28 Jul 1982, A. Sabol AS30 (CORT 007356).

Discussion: This species is known only from a single basidiome collected in New York. The description is drawn from the original collection notes. It is closely related to *H. subconnatum* but differs from it by the smaller spore size, shorter stipe, and association with *Sphagnum*. It is recorded from the same locality and habitat as *H. quebecense* but differs from that species by ITS sequence and smaller spores. We refrain from describing the taxon as new until confirmed by additional collections and sequence data.

***Hydnum subconnatum* Swenie & Matheny, sp. nov.**
MycoBank: MB825496

GenBank: MH379930
Figures 4A, 5L

Diagnosis: Closely related to *Hydnum oregonense* but differs from it by ITS sequence divergence and geographic distribution. *Hydnum subconnatum* is known only from the southeastern U.S.

Type: UNITED STATES. North Carolina: Yancey County, Carolina Hemlocks Recreation Area, picnic area (35.8057; -82.2047), on soil growing in fused cluster and singly with *Tsuga carolinensis*, *Quercus*, *Liriodendron*, 840 m, 29 Sep 2017, R.A. Swenie RAS235 (holotype: TENN 073064).

Etymology. *subconnatum* (L.), born together, in reference to the fused stipe bases of multiple basidiomes.

Description: Pileus 10–75 mm wide, more or less round, broadly convex to plane, surface usually dry but sometimes slightly hygrophanous, glabrous, occasionally with shallow cracks or pits in age, sometimes umbilicate or with central depression, peach orange (6C8–6B7) to reddish-brown ("Cinnamon-Rufous"); margin incurved and entire, becoming eroded or split and sometimes wavy in age. Spines 1–8 mm long, shortest near margin, adnate to subdecurrent, white to pale orange (5A4, 6A3). Stipe 15–60 × 5–20(30) mm, central or eccentric, equal or widening to bulbous base, sometimes with up to four basidiomes fused together at base, texture smooth, white to dull tan, bruising orange-brown (6B8–7D7 or "Ochraceous- Buff"). Context fleshy, white to dull cream-brown, staining not observed. Odor mild or slightly sweet. Taste mild.

Basidiospores 8.5–8.9–10 µm × 7.5–8.5–9.5(10) µm, Q= 1.00–1.05–1.14(1.20) (n=34/3), globose to subglobose, smooth, hyaline in KOH. Basidia 48–61 × 8.5–10.5 µm with 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 µm wide. Clamp connections present.

Distribution: Southeastern U.S. – North Carolina (type), Tennessee, and Georgia.

Ecology: In mixed woods with *Quercus*, *Pinus*, *Tsuga*, *Fagus*, *Betula*, *Carya*, *Liriodendron*. July to December.

Other specimens examined: UNITED STATES. Georgia: Chatham County, Wormsloe Plantation, in duff with *Quercus*, *Pinus*, 8 m, 26 Dec 1976, J.H. Restivo JHR40605 (TENN 040605). White County, Unicoi State Park, Unicoi to Helen Trail, solitary beside trail with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, J.E. Uehling RAS165 (TENN 073026). White County, Unicoi State Park, Unicoi to Helen Trail, solitary beside trail with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, R.A. Swenie RAS169 (TENN 073048). Tennessee: Great Smoky Mountains National Park, under *Pinus*, *Tsuga*, *Quercus*, *Acer*, Meig's Creek Trail, 580 m, 30 Sep 2004, E.B. Lickey TFB12311 (TENN 060359). Great Smoky Mountains National Park, Cades Cove, scattered in mixed forest with *Fagus, Quercus, Carya, Pinus*, 520 m, 23 Nov 2013, R.A. Walter RAW18 (TENN 073005). Great Smoky Mountains National Park, Schoolhouse Gap Trail, scattered beneath *Pinus, Tsuga, Quercus, Betula*, 550 m, 26 Oct 2013, K.E. Rewcastle KER016 (TENN 073006). Great Smoky Mountains National Park, trail to Look Rock fire tower, some caespitose and forming a ring among *Quercus* litter on trail, 800 m, 17 Nov 2009, E.E. Austin EEA171109-1 (073744). Great Smoky Mountains

National Park, Trillium Gap Trail, 9 Jul 2017, B.P. Looney BPL931 (TENN 073028). Big Ridge State Park, Ghost House Loop Trail, scattered to caespitose under *Quercus*, *Fagus*, *Carya*, *Pinus virginiana*, 320 m, 9 Nov 2015, R.A. Swenie RAS053 (TENN 070846).

Discussion: *Hydnum subconnatum* is known from the southeastern U.S. in a range of low elevation mixed forests such as oak-pine or hemlock-pine mixed with oak and beech. All known specimens are reported under 1000 m elevation. Basidiomes can occur in caespitose clusters with the stipe bases and two or more pilei fused together. The pileus coloration is highly variable, however, ranging from deep orange to pale peach and fading to tan towards the margin, making this a difficult species to distinguish at a glance. *Hydnum caespitosum* Banning ex Peck (non *H. caespitosum* Valenti), described from Maryland, occurs "at roots of trees and near old stumps" and is much paler in coloration, depicted by Banning in her painting as a yellowish species. Furthermore, our examination of the holotype of *H. caespitosum* revealed that basidiospores are much smaller in that species than in *Hydnum subconnatum*. The new name *H. geminum* is proposed below for *H. caespitosum* Banning ex Peck.

Specimens of *H. subconnatum* form a monophyletic group with support values <70% (ML) and <0.95 (BI). ITS sequence variation is relatively low (<1%) among sampled specimens of *H. subconnatum*, but the clade is highly dissimilar (8% sequence divergence) from Mexican taxa (Genbank KR135344-KR135345) that form a well-supported sister lineage.

***Hydnum cuspidatum* Swenie & Matheny, sp. nov.**
MycoBank: MB825497
GenBank: MH379944
Figures 4B, 5M

Diagnosis: Closely related to *Hydnum umbilicatum* but differs from it by ITS sequence divergence as well as more elliptic basidiospores. Known so far in the southeastern and upper midwest United States. Differs from *H. aerostatisporum* by the smaller basidiomes and slightly larger basidiospores.

Type: UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, John Litton Farm Trail (36.4960; -84.6700), on soil with *Quercus*, *Tsuga*, *Pinus*, 425 m, 29 Oct 2017, R.A. Swenie RAS246 (holotype: TENN 073068).

Etymology: *cuspidatum* (L.), tapering to a fine, sharp point, in reference to the spines.

Description: Pileus (11)15–50 mm wide, round to oval or irregular and reniform, convex when young, becoming plane or depressed, margin incurved and entire, becoming irregularly wavy or degraded; surface glabrous, sometimes floccose-scaly or scabrous near the umbilicus, dull orange to deep orange-brown (5A6–6B7–6D8, "Tawny" to "Mikado Brown"), olive-brown with KOH, at times faded in color towards the margin. Spines 1–8 mm long, shorter near the margin, adnate, pale buff, cream-orange, or tan-orange (7.5YR 8/4–8/6 or 5A3–A5). Stipe 15–50 × 3–10(12) mm, central or eccentric, equal or enlarged towards base, sometimes curved, texture smooth, buff to peach-brown

(5A2–A3 to 5B6–C6), sometimes with hazy thin white patches especially towards apex, staining only very slightly light brown (10YR 7/4–7/6 or 5A–B7); cottony white basal mycelium often present. Context often hollow, flesh white to cream. Odor not distinctive or sweet and fruity. Taste not distinctive.

Basidiospores (7)7.5–8.5–9.5(10.0) μm × 6–7.2–8.5 μm, Q=1.01–1.18–1.38(1.52) (n=99/6), subglobose to irregularly rounded-elliptic, smooth, thin-walled, hyaline in KOH. Basidia 39–56 × 7–9 μm with 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–7 μm wide. Clamp connections present.

Distribution: Eastern U.S. – Michigan (KU612606, MG162266), North Carolina, Tennessee (type), and Georgia.

Ecology: In deciduous or mixed woods with *Quercus*, *Pinus*, *Tsuga*, *Fagus*, *Betula*, *Carya*, *Carpinus*, *Picea*. June to October.

Other specimens examined: UNITED STATES. Georgia: White County, Unicoi State Park, Unicoi to Helen Trail, solitary with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, R.A. Swenie RAS167 (TENN 073046). North Carolina: Blue Ridge Parkway near Little Switzerland, deciduous woodlot with *Quercus* and *Rhododendron maximum*, 1050 m, 19 Aug 2016, H. Hopping RAS106 (TENN 073016). McDowell County, Armstrong Creek, mixed woods, 450 m, 19 Aug 2016, J. Roberts RAS098 (TENN 073008). Great Smoky Mountains National Park, Big Creek Campground, under *Tsuga*, *Carpinus*, *Betula*, *Fagus*, *Quercus*, 525 m, 29 Jul 2017, B.P. Looney BPL989 (TENN 073033). Great Smoky Mountains National Park, Cataloochee Divide Trail, solitary with *Quercus*, *Betula*, *Tsuga*, 1525 m, 9 Sep 2017, RAS218 (TENN 073059). Great Smoky Mountains National Park, Balsam Mountain Road, 1525 m, 15 Aug 2005, E.B. Lickey TFB12725 (TENN 073033). Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on embankment with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS205 (TENN 073056). Tennessee: Great Smoky Mountains National Park, Tremont, Buckeye Trail, with *Tsuga*, *Betula*, 490 m, 23 Jun 2017, R.A. Swenie RAS151 (TENN 073038). Great Smoky Mountains National Park, Schoolhouse Gap Trail, scattered on embankment with *Quercus*, *Betula*, *Pinus*, 550 m, 18 Jul 2017, R.A. Swenie RAS160 (TENN 073043). Anderson County, Oak Ridge, UT Arboretum, on soil in forest with *Quercus*, *Carya*, 300 m, 26 Oct 2009, J. Heggan JRH102609-3 (TENN 071998).

Discussion: *Hydnum cuspidatum* is closely related to *H. umbilicatum* and is difficult to distinguish by morphology alone. As in *H. umbilicatum*, there is high variability in basidiome stature and color. The basidiospores of *H. cuspidatum* have a slightly higher Q value on average (1.18) than *H. umbilicatum* (1.06), otherwise phylogenetic analysis of ITS data is needed to distinguish the two species reliably. *Hydnum cuspidatum* occurs in deciduous or mixed forests in the midwest and southeastern U.S., where it can co-occur with *H. umbilicatum*. The ITS sequences of *H. cuspidatum* have relatively high intraspecific variation (up to 3%) compared to other North American *Hydnum* species. The lack of distinguishing morphological or ecological features deters further differentiation into separate taxa at this time.

Hydnum umbilicatum Peck, Ann. Rep. N.Y. St. Mus. 54: 953 (1902)
MycoBank Epitypification: MBT381861
GenBank: MH379890
Figures 5C, 5D, 6N, 6O

Type: UNITED STATES. New York: Rensselaer County, Sandlake, ground in thin woods, September, ca. 1901, C.H. Peck (holotype: NYS-F-3258). Epitype: UNITED STATES. New York: Cortland County, Lime Hollow Nature Center Tunison Aquatic Lab (42.5578; -76.2486), on humus in wet, boggy area with *Tsuga*, *Betula alleghaniensis*, 27 Aug 2014, T.J. Baroni 10651TJB (CORT 012241).

Description: Pileus 15–70 mm wide, round, conico-campanulate to irregularly convex, disc shallowly depressed to umbilicate; surface matt, glabrous or felty-fibrillose, orange-cream to orange-brown (5C6–8); margin entire and incurved when young to undulating in age, often paler in color than the rest of the pileus. Spines 1–8 mm long, aculeate, adnexed, fleshy pinkish to light orange (5A2–3). Stipe 20–80 × 4–15 mm, central or eccentric, equal to slightly enlarged downwards, glabrous or densely matted with fluffy fibrillose; white to peachy-pallid buff, staining ochre to medium brownish orange ("Mars Yellow" to "Orange Rufous"). Context white. Odor mild or pleasant. Taste mild, sometimes with nutty aftertaste.

Basidiospores 7.5–8.4–9.5 µm × 7–8–9 µm, Q=1.00–1.06–1.18 (n=97/5), globose to subglobose, smooth, thin-walled, hyaline in KOH. Basidia 43–52 × 7.5–10 µm with (1)2–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–8 µm wide. Clamp connections present.

Distribution: Eastern North America – Michigan, Massachusetts, New York (type), Tennessee, North Carolina, Newfoundland and Labrador (GenBank KX388676), and Quebec (GenBank KX388675).

Ecology: In coniferous or mixed woods with *Tsuga*, *Pinus*, *Abies*, *Quercus*, *Betula*, *Fagus*. July to November.

Other specimens examined: UNITED STATES. Massachusetts: Worcester County, Rutland State Park, in arcs scattered singly or gregarious with *Quercus*, *Pinus strobus*, 260 m, 1 Nov 2003, P.B. Matheny PBM2511 (TENN 066875). Michigan: Marquette County, Big Bay, Alder Creek, 240 m, 2 Sep 1971, R.H. Petersen TFB36346 (TENN 036346). New York: Tompkins County, Ridgewood Reserve, 305 m, 13 Sep 2007, O. Akinyemi OA14 (CORT 008175). Tompkins County, Eames Bog, 325 m, 22 Sep 2011, B. Demo BD14 (CORT 007319). North Carolina: Transylvania County, Pisgah National Forest, Yellow Gap Road, under *Quercus*, 850 m, 18 Jul 2000, Jason TFB9766 (TENN 058667). Blue Ridge Parkway near Little Switzerland, coniferous woodlot, 1000 m, 19 Aug 2016, N. Byers RAS103 (TENN 073013). Yancey County, Carolina Hemlocks Recreation Area, picnic area under *Tsuga carolinensis*, *Quercus*, possibly *Betula* or *Carpinus*, 840 m, 29 Sep 2017, R.A. Swenie RAS234 (TENN 058667). Yancey County, Mount Mitchell State Park, Balsam Mountain trail, under *Abies fraseri*, 1920 m, 19 Aug 2016, R.A. Swenie RAS099 (TENN 073009). Yancey County, Mount Mitchell State Park, Balsam Mountain trail, under *Abies fraseri*, 1920 m, 19 Aug 2016, R.A. Swenie RAS101 (TENN 073011). Yancey County, Mount Mitchell State Park, Balsam

Mountain trail, under *Abies fraseri*, 1920 m, 29 Sep 2017, R.A. Swenie RAS238 (TENN 073066). McDowell County, Armstrong Creek trail, under *Quercus*, *Pinus*, *Liriodendron*, 450 m, 30 Sept 2017, R.A. Swenie RAS239 (TENN 073067). Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail, with *Tsuga*, *Pinus*, *Quercus*, 936 m, 25 Aug 2004, E.B. Lickey TFB12039 (TENN 060288). Tennessee: Great Smoky Mountains National Park, Maddron Bald Trail, scattered on soil and hardwood leaf litter with *Tsuga*, *Quercus*, *Fagus*, *Pinus*, 575 m, 29 Aug 2013, S.A. Trudell SAT1324109 (TENN 068871). Great Smoky Mountains National Park, Greenbrier, Injun Creek Trail, under *Tsuga*, 450 m, 18 Nov 2004, E.B. Lickey TFB12369 (TENN 060445). Great Smoky Mountains National Park, Cosby, Gabes Mountain Trail, with *Tsuga* and mixed hardwoods, 685 m, 16 Oct 2006, E.B. Lickey TFB13482 (TENN 061745). Big South Fork National River & Recreation Area, John Litton Farm Trail, scattered at base of dead *Tsuga*, in mixed woods with *Tsuga*, *Quercus*, *Pinus*, 425 m, 29 Oct 2017, R.A. Swenie RAS247 (TENN 073181).

Discussion: *Hydnum umbilicatum* is widespread in eastern North America at low and high elevations, mostly in conifer-dominated forests or mixed woods including conifers. The macromorphology can vary dramatically among basidiomes with some specimens displaying the namesake umbilicate pileus while others do not. The presence of an umbilicus is not a unifying taxonomic feature as its presence has been observed in several distantly related clades of *Hydnum*. Peck (1901) included a color plate illustration with his description depicting basidiomes with thin, convex, umbilicate pilei and slender stipes that are slightly longer than the diameter of the pileus. Unfortunately, we were unable to obtain DNA sequences from the type collection. However, in comparison to other closely related clades, specimens of *H. umbilicatum* have slightly larger globose to subglobose basidiospores averaging 8.4 × 8 μm with average Q values below 1.08, which closely matches our spore measurements of the holotype. In addition, Peck mentioned that "sometimes a definite line separates the paler margin from the more highly colored center of the pileus", a trait that has been observed in several of the specimens of this species that cluster in a single ITS lineage.

Species from eastern North America – Incertae sedis

Hydnum geminum **Swenie & Matheny, nom. nov.**
MycoBank MB825498
Figure 7

≡ *Hydnum caespitosum* Banning ex Peck, Rep. N.Y. St. Mus. 44: 74 (1891), non Valenti (1868)

Type: UNITED STATES. Maryland: Carroll County, in clusters at the roots of trees and near old stumps, Aug–Sep, ca. 1880, M.E. Banning (holotype: NYS-F-3506).

Etymology: *geminum* (L.), twin, in reference to the clustered habit

Description: Pileus up to 40 mm wide, subconfluent, convex to expanded or sub-plane, subregular; surface appressed-fibrous, pale ochre, yellow, or dark flesh-colored.

Spines short (<3 mm long), conical, acute, decurrent, pale ochre or light flesh color. Stipe up to 60 × 10 mm, united at the base, subcylindrical, subflexuous, floccose above, subglabrous below, whitish, staining yellow where bruised, solid. Context fleshy, white, turning yellow where cut. Taste mild.

Basidiospores 6–6.4–7 μm × 4.5–5.2–6 μm, Q=1.09–1.24–1.36 (n=12/1), broadly elliptic to subglobose, smooth, thin-walled, hyaline in KOH. Basidia not reviving, with 4–5 sterigmata. Pileipellis not observed. Clamp connections present.

Distribution: Eastern U.S. – Maryland (type).

Ecology: In clusters at the roots of trees and near old stumps, August to September.

Discussion: The new binomial *H. geminum* is introduced to replace the illegitimate name *H. caespitosum* Banning ex Peck, which is a later homonym of *H. caespitosum* Valenti. The gross morphological description here is reproduced from Banker (1906) after reformatting for style, and measurements appear to be based on dried specimens. Peck's protologue, Banning's painting, and Banker's notes depict a species best characterized by the overall yellowish color, short decurrent spines, flavescent or yellowing flesh, mild taste, and broadly elliptic to subglobose basidiospores that are mostly 6.5 × 5 μm in size. Although specimens of *H. subconnatum* (described above) may share the similar caespitose or clustered habit, it differs from *H. geminum* by the peach-orange to dark orange-brown pileus, longer non-decurrent spines, and a stipe that bruises orange-brown, not yellow. In addition, the basidiospores of *H. subconnatum* are larger than in *H. geminum* – 8.5–9.5 × 7.5–9 μm. Basidiomes of *H. subtilior* sometimes have an overall pale yellow tone and stain when bruised or cut in half, but basidiospores are smaller in *H. geminum,* and the overall basidiome stature of the holotype appears much stouter than in the generally slender *H. subtilior*. Upon examining the holotype of *H. caespitosum*, we found that basidiomes had very short spines (<1 mm) with very few spores and basidia. This aligns with Peck's notes indicating he did not obtain spores and suggests the holotype consists of immature basidiomes. We have not yet recorded *H. geminum* in eastern North America. Banker (1906) refers to a collection made by Earle from Connecticut now housed at NCU (NCU- F-0012251). We have not re-examined this collection, but Banker describes it as somewhat darker than the type.

Key to species of *Hydnum* in eastern North America

Note that the five species in couplet 17 are most reliably distinguished by phylogenetic analysis of ITS sequences.

1. Pileus white, cream, yellow, peach, pale orange, or light tan before handling.....2
1'. Pileus darker than above, orange to tawny brown before handling.....11

2. Pileus mostly pale white to off-white or cream 3
2'. Pileus mostly with tones of yellow, peach, pale orange, or light tan 7

3. Pileus small to medium-sized, <60 mm wide at maturity.....4
3'. Pileus larger than above, >60 mm wide at maturity.....5

4. Basidiomes staining bright orange within two minutes of handling.....*H. alboaurantiacum*
4.' Basidiomes remaining white where handled or slowly staining orange-brown to ochre-brown after several minutes to hours.....*H. albidum*

5. Pileus with adhering litter debris.....*H. albomagnum*
5'. Pileus free of adhering debris.....6

6. Basidiomes staining bright orange where handled, spores <7 µm long.....*H. alboaurantiacum*
6'. Basidiomes not staining bright orange where handled, spores mostly >7 µm long.....*H. vagabundum*

7. Stipe >20 mm wide.....*H. subolympicum*
7'. Stipe <20 mm wide.....8

8. Basidiospores mostly 4–6 × 3–5 µm.....*H. alboaurantiacum*
8'. Basidiospores larger than above, mostly 6–9 × 5–7.5 µm.....9

9. Basidiospores mostly 6–7 × 5–6 µm.....*H. geminum*
9'. Basidiospores mostly 7–9 × 5.5–7.5 µm.....10

10. Pileus light cream yellow to cream orange buff, known only from the southeastern U.S. and Mexico in deciduous or mixed woods.....*H. subtilior*
10'. Pileus pale orange, known only from eastern Canada and the western US in coniferous or mixed woods.....*H. washingtonianum*

11. Basidiomes caespitose.....*H. subconnatum*
11'. Basidiomes solitary or scattered.....12

12. Spines decurrent.....*H. mulsicolor*
12'. Spines subdecurrent or adnate.....13

13. Basidiospores mostly 5.5–7.5 × 5–7.5 µm.....*H. ferruginescens*
13'. Basidiospores larger than above, 6.5–10 × 6–9.5(10) µm.....14

14. In *Sphagnum* in conifer-dominated woods.....15
14'. On soil in deciduous, mixed, or coniferous woods.....16

15. Basidiospores 6.5–8 × 6–7.5 µm.....*H.* sp. AS30
15'. Basidiospores larger than above, 8–9.5 × 7–9 µm ..... *H. quebecense*

16. Basidiospores 8.5–10 × 7.5–9.5 µm, known only from the southeastern U.S. in mixed hardwoods under 900 m elevation .....*H. subconnatum*
16'. Basidiospores smaller than above, 7–9.5(10) × 6–9 µm, widespread or known only from northeastern North America in coniferous or mixed woods at various elevations ...17

17. Known from mixed and hardwoods at all elevations, common and widespread in eastern U.S. (Gulf coast, southeast, midwest) ..... *H. aerostatisporum*
17'. Known only from coniferous woods at low elevation in Québec ....*H. submulsicolor*
17″. Known only from coniferous woods at low elevation in northeastern Canada and high elevation in southeastern U.S.....*H. canadense*
17‴. Known from coniferous, mixed forests, and hardwoods at various elevations in midwest and southeastern U.S. ..... H. cuspidatum
17⁗. Known from coniferous and mixed woods at all elevations, common and widespread in eastern North America (southeast, northeast, Midwest).....*H. umbilicatum*

Acknowledgements

We thank TENN, NYS, NY, WTU, and CSU herbaria and staff for collection loans. We are grateful to Lorinda Leonardi at NYS for providing the image of *Hydnum caespitosum*. We thank Clark Ovrebo, Roy Halling, Mike Hopping, Michael Kuo, Ron Pastorino, Brian Looney, Steve Trudell, Matthew Smith and Laurel Kaminsky for sharing specimen photographs and information. We also thank Mary Berbee and Anna Bazzicalupo for sharing sequences of *Hydnum washingtonianum*. We are grateful for the assistance provided by the UT Genomics Core staff Joe May and Veronica Brown. We thank the members of the North American Mycological Association and Cumberland Mycological Society, as well as summer research interns at Tremont Institute who collected specimens. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 1452154. Additional research funding was provided by the Hesler Endowment Fund at the University of Tennessee. Field work was supported by an Appalachian Highlands Research Grant from the National Park Service.

REFERENCES

Agerer R, Kraigher H, Javornik B (1996) Identification of ectomycorrhizae of *Hydnum rufescens* on Norway spruce and the variability if the ITS region of *H. rufescens* and *H. repandum* (Basidiomycetes). Nova Hedwigia 63(1–2): 183–194.

Ammirati JF, Parker AD, Matheny PB (2007) *Cleistocybe*, a new genus of Agaricales. Mycoscience 48(5): 282–289. https://doi.org/10.1007/S10267-007-0365-5

Banker HJ (1906) A contribution to a revision of the North American Hydnaceae. Memoirs of the Torrey Botanical Club 12: 99–194. https://doi.org/10.5962/bhl.title.97394

Buyck B, Duhem B, Das K, Jayawardena RS, Niveiro N, Pereira OL, Prasher IB, Adhikari S, Albertó EO, Bulgakov TS, Castañeda-Ruiz RF, Hembrom ME, Hyde KD, Lewis DP, Michlig A, Nuytinck J, Parihar A, Popoff OF, Ramirez NA, Silva MD, Verma RK, Hofstetter V (2017) Fungal Biodiversity Profiles 21–30. Cryptogamie, Mycologie 38(1): 101–146. https://doi.org/10.7872/crym/v38.iss1.2017.101

Coker WC, Beers AH (1951) The stipitate hydnums of the eastern United States. The University of North Carolina Press, 15–21.

Donk MA (1933) Revision der Niederlandischen Homobasidiomycetae-Aphyllophoraceae II. Mededelingen van de de Nederlandse Mycologische Vereeniging 22: 1–278.

Donk MA (1956) The Generic Names Proposed for Hymenomycetes V “Hydnaceae” (continuation). Taxon 5(4): 69–80. https://doi.org/10.2307/1217317

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Feng B, Wang X-H, Ratkowsky D, Gates G, Lee SS, Grebenc T, Yang ZL (2016) Multilocus phylogenetic analyses reveal unexpected abundant diversity and significant disjunct distribution pattern of the Hedgehog Mushrooms (*Hydnum* L.). Scientific Reports 6(4): 1:11. https://doi.org/10.1038/srep25586

Fries EM (1821) Systema Mycologicum I, 397.

García-Guzmán OM, Garibay-Orijel R, Hernández E, Arellano-Torres E, Oyama K (2017) Worldwide meta-analysis of *Quercus* forests ectomycorrhizal fungal diversity reveals southwestern Mexico as a hotspot. Mycorrhiza 27: 811. https://doi.org/10.1007/s00572-017-0793-9

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Garibay-Orijel R, Cifuentes J, Estrada-Torres A, Caballero J (2006) People using macro-fungal diversity in Oaxaca, Mexico. Fungal Diversity 21: 41–67.

Grebenc T, Martín MP, Kraigher H (2009) Ribosomal ITS diversity among the European species of the genus *Hydnum* (Hydnaceae). Anales Del Jardín Botánico de Madrid 66S1: 121–132. https://doi.org/10.3989/ajbm.2221

Hall D, Stuntz DE (1971) Pileate Hydnaceae of the Puget Sound area. I. White-spored genera: *Auriscalpium*, *Hericium*, *Dentinum* and *Phellodon*. Mycologia 63: 937–1098. https://doi. org/10.1080/00275514.1971.12019214

Harrison KA, Grund DW (1987) Preliminary keys to the terrestrial stipitate Hydnums of North America. Mycotaxon 28: 419–426.

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proceedings of the National Academy of Sciences 94(22): 12002–12006. https://doi.org/10.1073/pnas.94.22.12002

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30(22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lee SS, Watling R, Noraini-Sikin Y (2002) Ectomycorrhizal basidiomata fruiting in lowland forests of Peninsular Malaysia. Bois et Forêts des Tropiques 274: 33–43.

Maas Geesteranus RA (1964) Notes on Hydnums II. Persoonia 3(2): 155–192.

Maas Geesteranus RA (1971) Hydnaceous fungi of the eastern old world. Verhandelingen Der Koninklijke Nederlandse Akademie Van Wetenschappen, 1–75.

McNabb RFR (1971) Some new and revised taxa of New Zealand Basidiomycetes (Fungi). New Zealand Journal of Botany 9(2): 355–370. https://doi.org/10.1080/0028825X.1971.10429148

Niskanen T, Liimatainen K, Nuytinck J, Kirk P, Olariaga Ibarguren I, Garibay-Orijel R, Norvell L, Huhtinen S, Kytövuori I, Ruotsalainen J, Niemelä T, Ammirati JF, Tedersoo L (2018) Identifying and naming the currently known diversity of the genus *Hydnum* with an emphasis on European and North American taxa. Mycologia 110(5): 890–918. https://doi.org/10.1080/00 275514.2018.1477004

Olariaga I, Grebenc T, Salcedo I, Martín MP (2012) Two new species of *Hydnum* with ovoid basidiospores: *H. ovoideisporum* and *H. vesterholtii*. Mycologia 104(6): 1443–55. https:// doi.org/10.3852/11-378

Peck CH (1887) Descriptions of new species of New York fungi. Bulletin of the New York State Museum 1(2): 5–24.

Peck CH (1897) Report of the State Botanist (1896). Annual Report on the New York State Museum of Natural History 50: 77–159.

Peck, CH (1902) Report of the State Botanist (1901). Annual Report on the New York State Museum of Natural History, 54: 929–984.

Pine EM, Hibbett DS, Donoghue MJ (1999) Phylogenetic relationships of cantharelloid and clavaroid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. Mycologia 91: 944–963. https://doi.org/10.2307/3761626

Restivo JH, Petersen RH (1976) Studies on Nuclear Division and Behavior within Basidia in *Hydnum umbilicatum*. Mycologia 68(3): 666–672. https://doi.org/10.1080/00275514.1976.12019951

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Roody WC (2001) Mushrooms of West Virginia and the Central Appalachians. University Press of Kentucky, 398.

Sarmiento E, Fontecha G (2013) Conocimiento tradicional de los hongos en el occidente de Honduras. Revista Ciencia Y Tecnología 13: 19–29. https://doi.org/10.5377/rct.v0i13.1710

Smith AH, Smith HV, Weber NS (1981) How to Know the Non-Gilled Mushrooms. Wm. C. Brown Publishers, 1-324.

Stamatakis A (2014) RAxML 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Vizzini A, Picillo B, Ercole E, Voyron S, Contu M (2013) Detecting the variability of *Hydnum ovoideisporum* (Agaricomycetes, Cantharellales) on the basis of Italian collections, and *H. magnorufescens* sp. nov. Mycosphere 4(1): 32–44. https://doi.org/10.5943/mycosphere/4/1/2

White TJ, Bruns TD, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: A guide to the methods and applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

APPENDIX

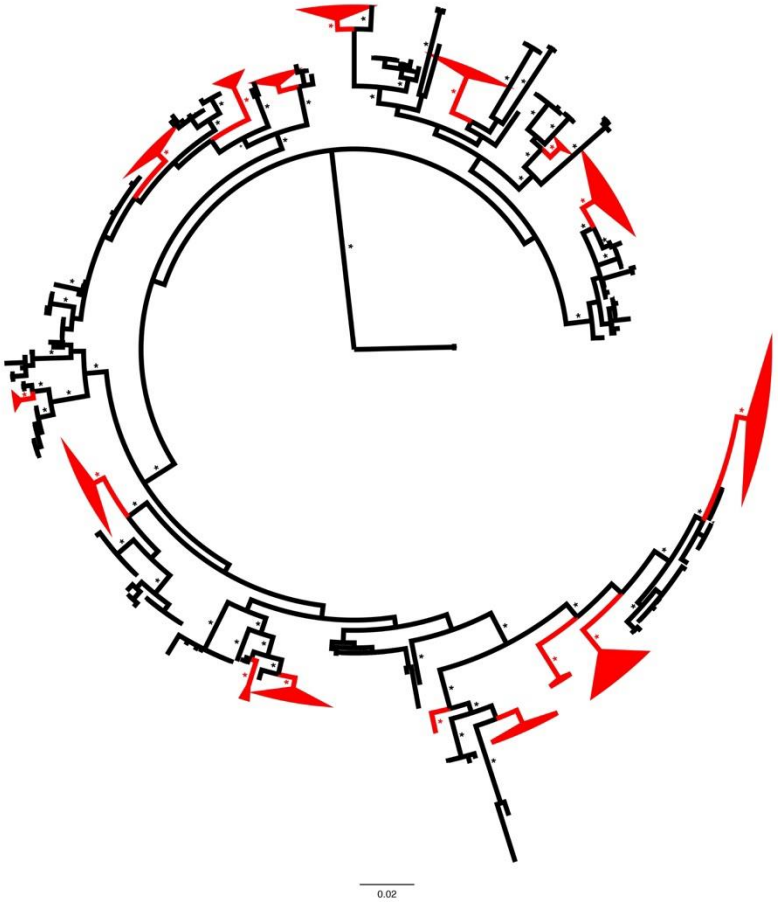

Figure 1. Global BI phylogeny of *Hydnum*. Species-level clades containing sequences from eastern United States and Canada are colored red. Posterior probabilities ≥0.95 are denoted with asterisks.

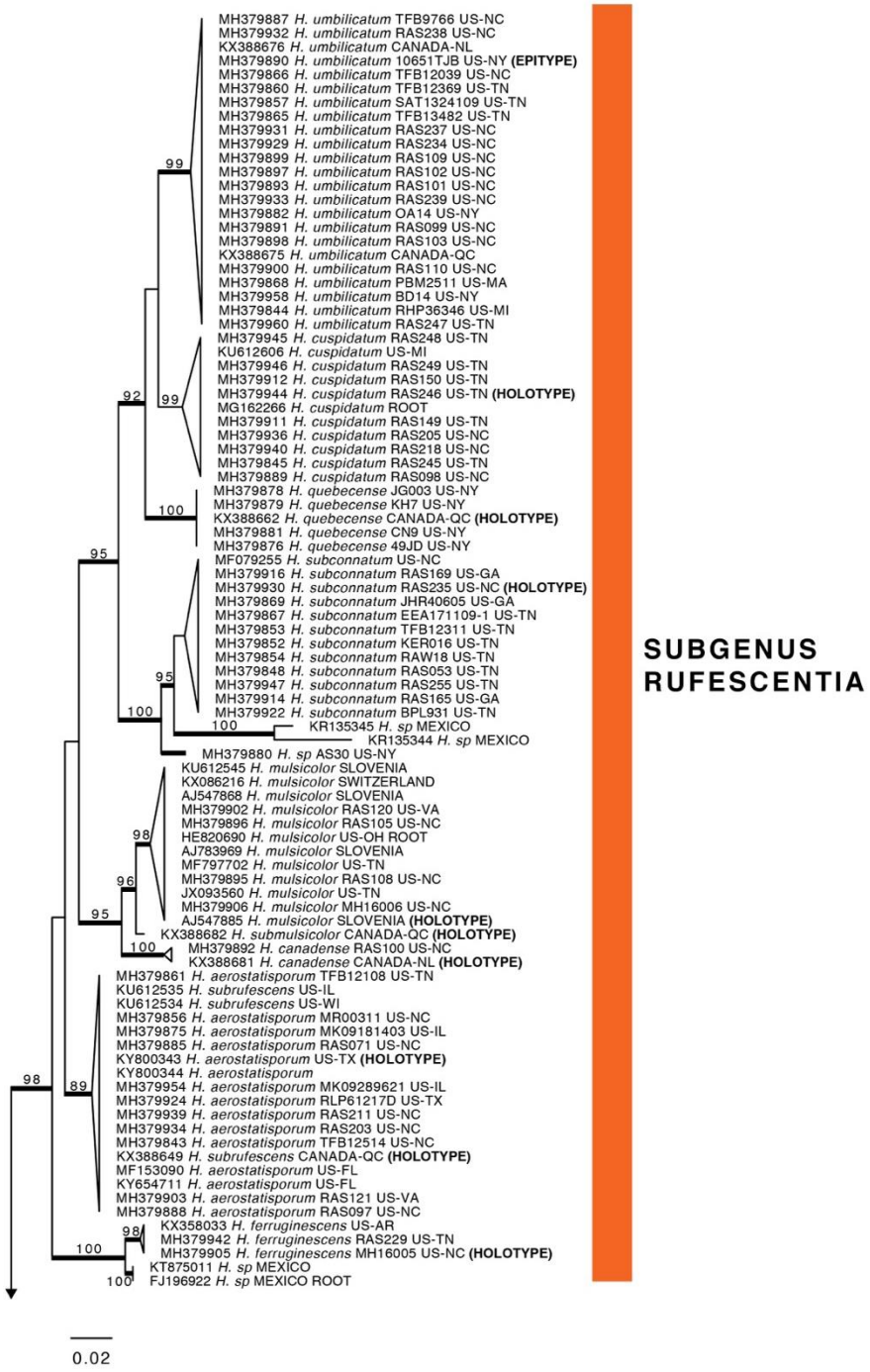

Figure 2. ML pruned phylogeny of North American species of *Hydnum*. Bootstrap support values ≥70% are shown above branches. Posterior probabilities ≥0.95 are shown with branches in bold.

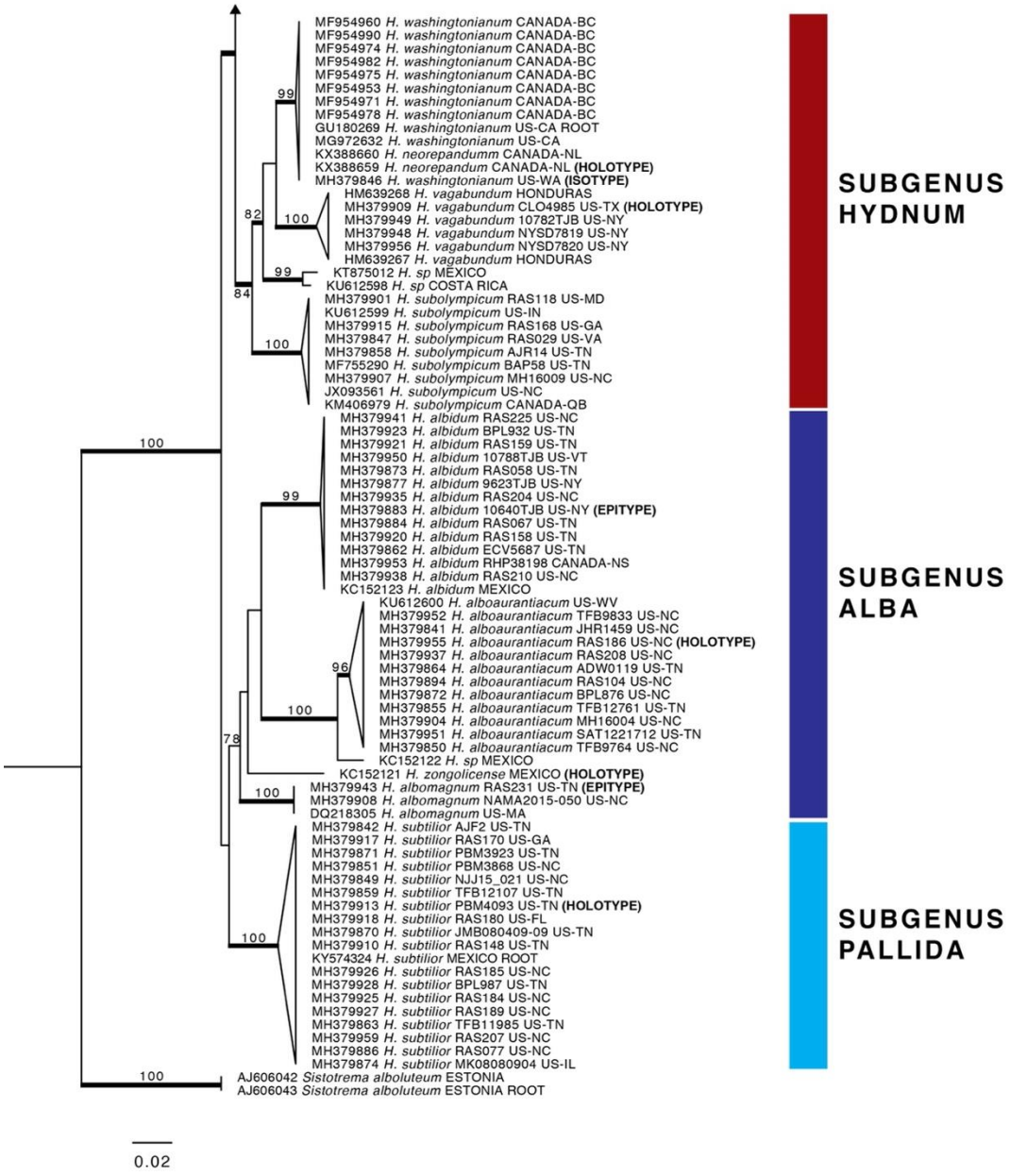

Figure 2. Continued.

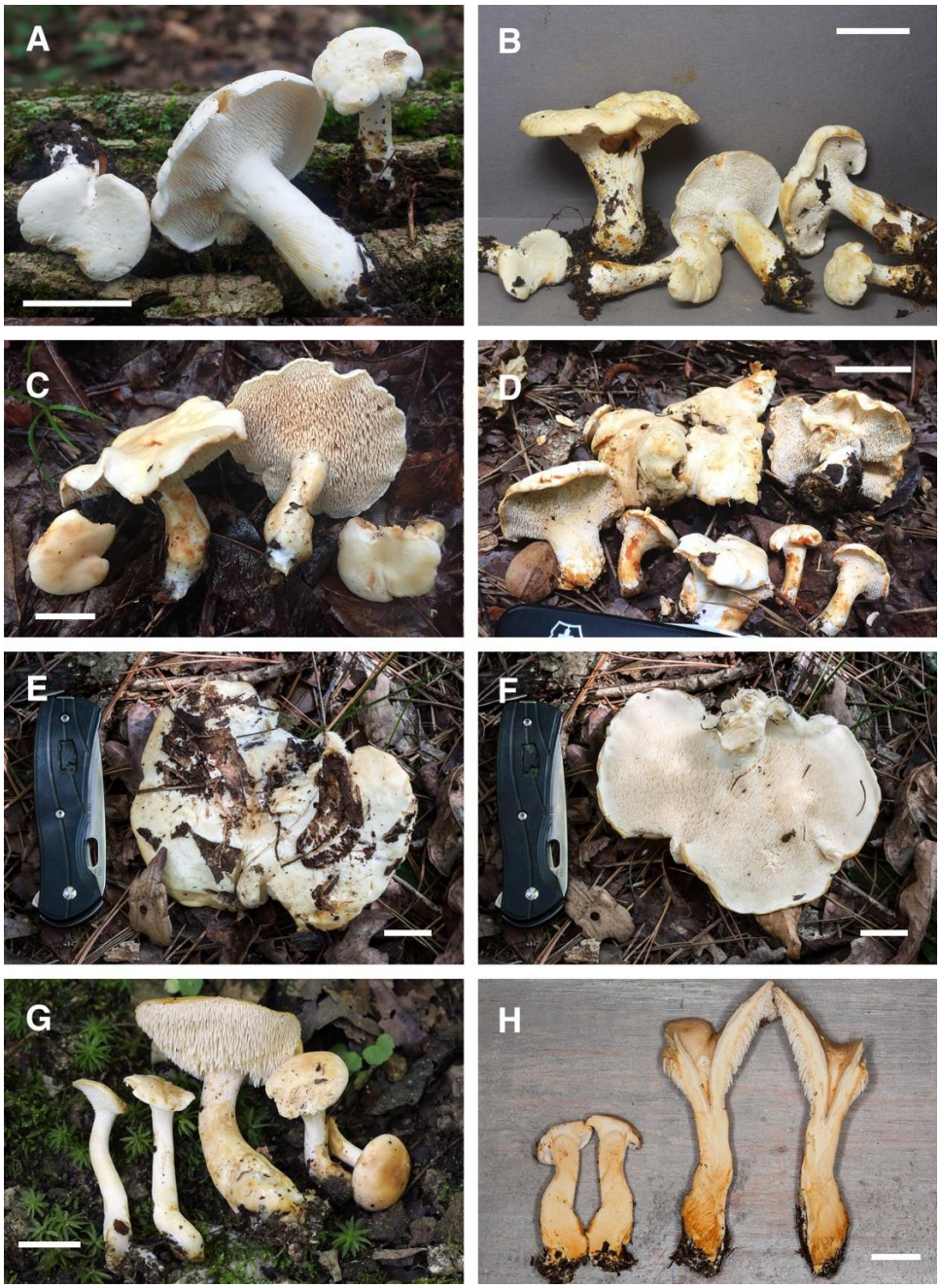

Figure 3. Basidiomes of Hydnum species. A, B H. albidum 10640TJB (CORT 012029, epitype, photo T.J. Baroni) C H. alboaurantiacum RAS186 (TENN 073053, holotype, photo R.A. Swenie) D H. alboaurantiacum BPL876 (TENN 073003, photo B.P. Looney) E, F H. albomagnum RAS231 (TENN 073062, epitype, photo R.A. Swenie) G H. subtilior PBM4093 (TENN 073034, holotype, photo P.B. Matheny) H H. subtilior showing characteristic staining of context RAS180 (TENN 073050, photo R.A. Swenie). Scale bar: 20 mm.

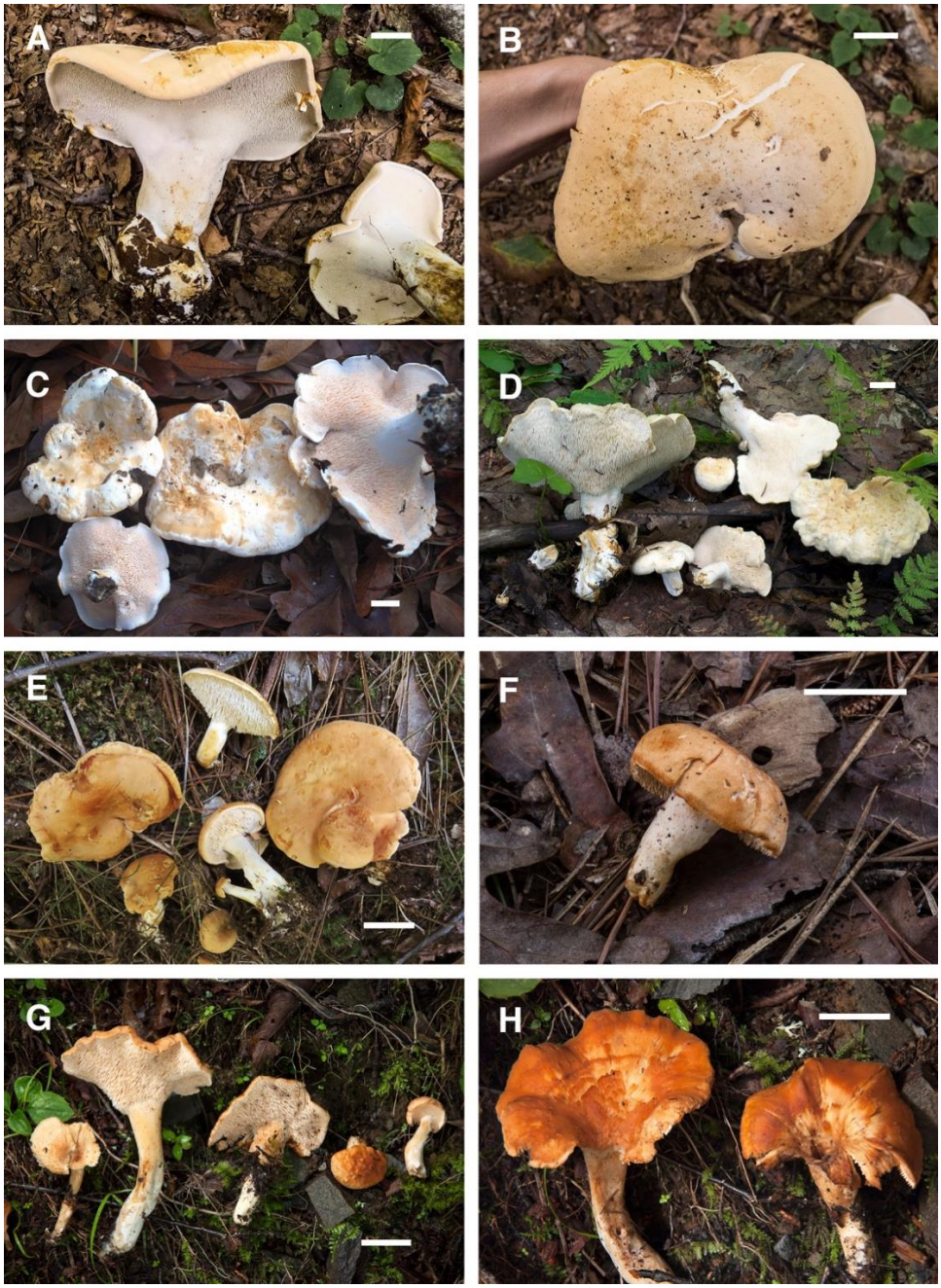

Figure 4. Basidiomes of *Hydnum* species. A, B *H. subolympicum* RAS029 (TENN 070845, photo R.A. Swenie) C *H. vagabundum* CLO4985 (CSU 01477, holotype, photo C.L. Ovrebo) D *H. vagabundum* 10782TJB (CORT 014461, photo T. J. Baroni) E *H. ferruginescens* MH16005 (TENN 073549, holotype, photo M. Hopping) F *H. ferruginescens* RAS229 (TENN 073061, photo R.A. Swenie) G, H *H. aerostatisporum* RAS071 (TENN 073001, photo R.A. Swenie). Scale bar: 20 mm.

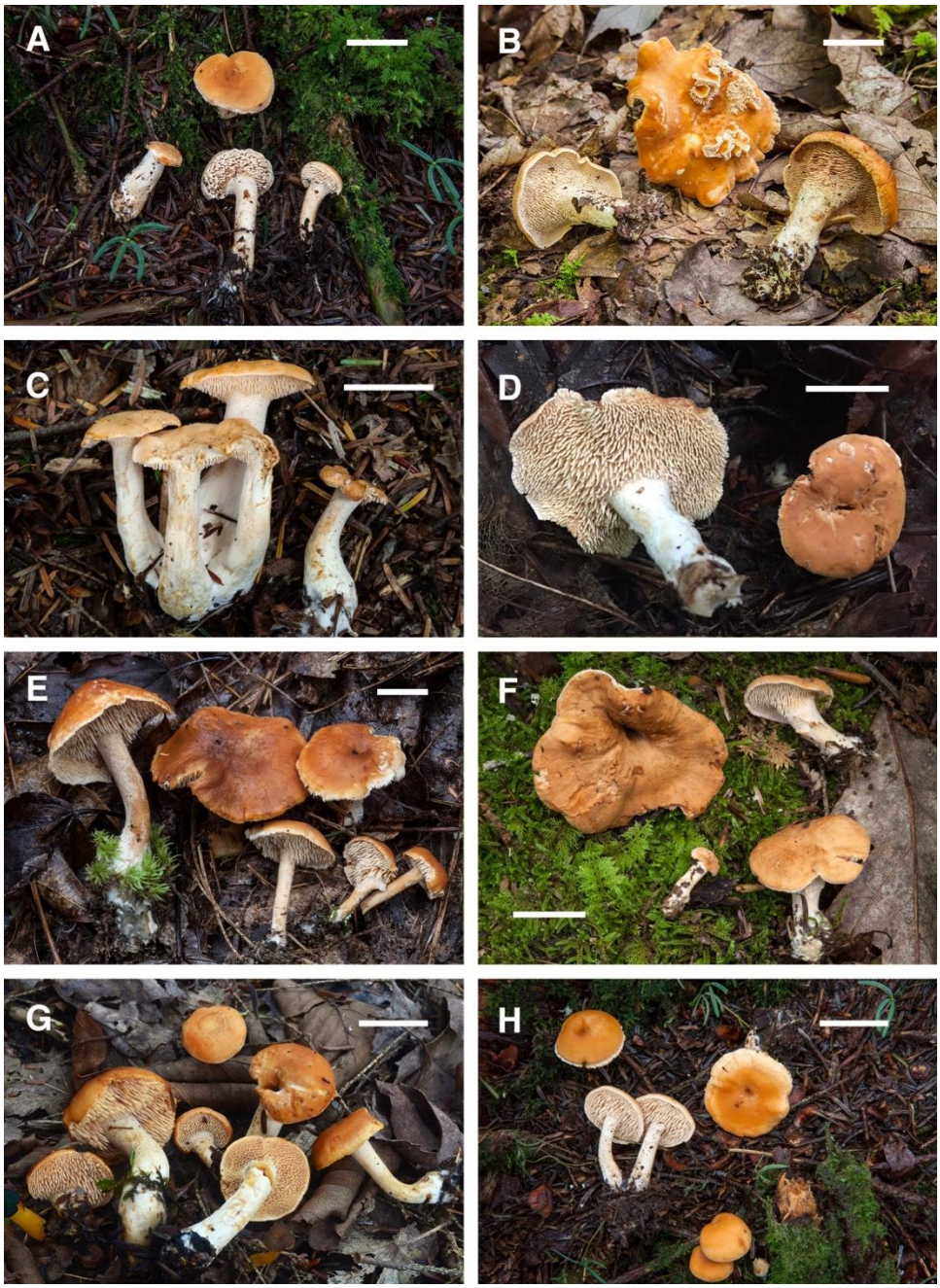

Figure 5. Basidiomes of *Hydnum* species. A *H. canadense* RAS100 (TENN 073010) B *H. mulsicolor* RAS023 (TENN 070321) C *H. subconnatum* RAS235 (TENN 073064, holotype) D *H. subconnatum* RAS169 (TENN 073048) E *H. cuspidatum* RAS246 (TENN 073086, holotype) F *H. cuspidatum* RAS150 (TENN 073037) G *H. umbilicatum* 10651TJB (CORT 012241, epitype) H *H. umbilicatum* RAS101 (TENN 073011). Photos A–F, H by R.A. Swenie; Photo G by T.J. Baroni. Scale bar: 20 mm.

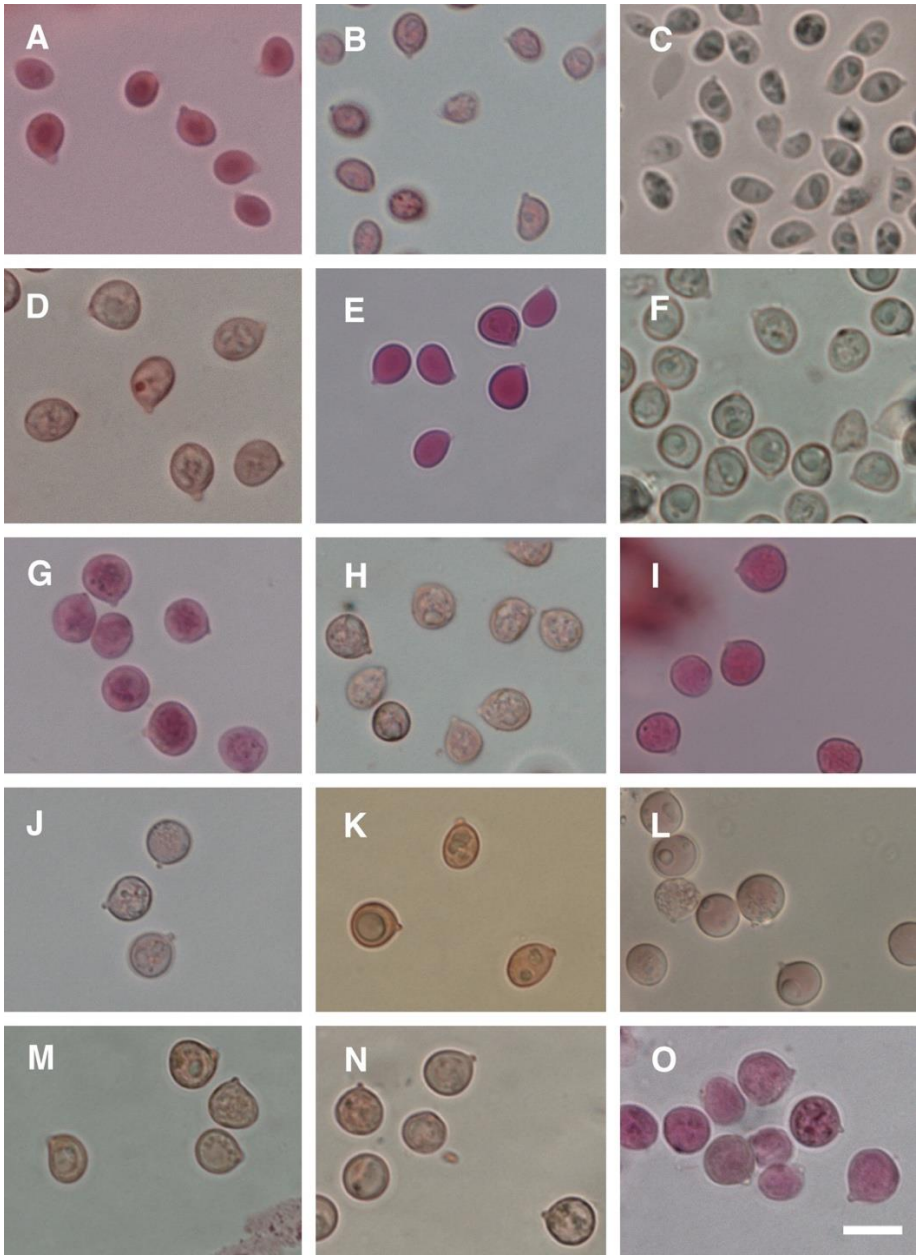

Figure 6. Basidiospores of *Hydnum* species. A *H. albidum* 9623TJB (CORT 014489) B *H. alboaurantiacum* TFB9833 (TENN 058812) C *H. albomagnum* PBM2512 (TENN 066858) D *H. subtilior* PBM3868 (TENN 067482) E *H. subolympicum* AJR14 (TENN 073004) F *H. vagabundum* 10782TJB (CORT 014461) G *H. ferruginescens* MH16005 (TENN 073549, holotype) H *H. aerostatisporum* MK9181403 I *H. canadense* RAS100 (TENN 073010) J *H. mulsicolor* MH16006 (TENN 073550) K *H. quebecense* JG003 (CORT 007330) L *H. subconnatum* RAS053 (TENN 070846) M *H. cuspidatum* BPL989 (TENN 073033) N *H. umbilicatum* 10651TJB (CORT 012241, epitype) O *H. umbilicatum* Peck (NYS-F-3258, holotype). Scale bar: 10 µm.

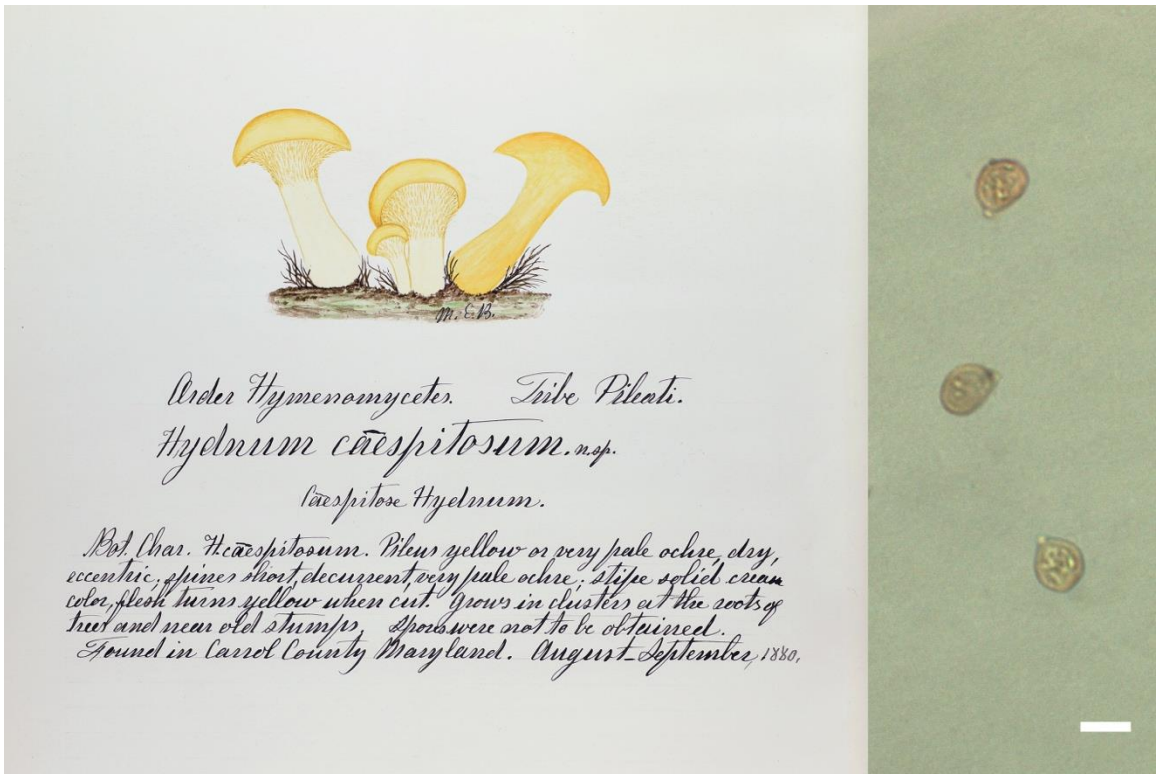

Figure 7. Holotype and basidiospores of *Hydnum caespitosum*. Scale bar: 5 µm.

Table 1. Specimen data for sequences produced in this study.

GenBank no.	Species	Original specimen	Voucher ID	Herbarium ID	Location	Date	Ecology
MH379862	H. albidum	H. repandum	ECV5687	TENN:068699	USA: Tennessee, Sevier County, University of Tennessee Biology Field Station	1-Sep-2013	
MH379953	H. albidum	H. albidum	RHP38198	TENN:038198	CANADA: Nova Scotia, Victoria County, Cape North, Grey Glen Brook	8-Sep-1973	under Picea, Betula
MH379873	H. albidum	H. sp.	RAS058	TENN:072000	USA: Tennessee, Great Smoky Mountains National Park, Cherokee Orchard, Ogle Place	5-Jun-2016	in soil among leaf litter under Tsuga, Betula
MH379877	H. albidum	H. albidum	9623TJB	CORT:014489	USA: New York, Cortland County, Kennedy State Forest, Scutt Hill Road	10-Aug-2003	on humus under Quercus, Fagus, Acer
MH379883	H. albidum	H. albidum	10640TJB	CORT:012029 (epitype)	USA: New York, Cortland County, Kennedy State Forest, Scutt Road	30-Jul-2014	on humus under Quercus, Fagus, Acer
MH379884	H. albidum	H. albidum	RAS067	TENN:073000	USA: Tennessee, Rock Island State Park	2-Jul-2016	
MH379920	H. albidum	H. albidum	RAS158	TENN:073041	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove, Schoolhouse Gap Trail	8-Jul-2017	under Quercus, Betula, Pinus
MH379921	H. albidum	H. albidum	RAS159	TENN:073042	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove, Schoolhouse Gap Trail	8-Jul-2017	under Quercus, Betula, Pinus
MH379923	H. albidum	H. cf. albidum	BPL932	TENN:73029	USA: Tennessee, Great Smoky Mountains National Park, Trillium Gap Trail	9-Jul-2017	in mixed woods
MH379935	H. albidum	H. albidum	RAS204	TENN:071752	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Picea
MH379938	H. albidum	H. sp.	RAS210	TENN:073173	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Quercus, Tsuga
MH379941	H. albidum	H. sp.	RAS225	TENN:073060	USA: North Carolina, Great Smoky Mountains National Park, Flat Creek Trail	9-Sep-2017	under Betula, Abies
MH379950	H. albidum	H. albidum	10788TJB	CORT:014475	USA: Vermont, Windham County, Stratton Mountain Resort area	28-Jul-2017	
MH379850	H. alboaurantiacum	H. albidum	TFB9764	TENN:058665	USA: North Carolina, Pisgah National Forest, Yellow Gap Road	18-Jul-2000	
MH379951	H. alboaurantiacum	H. sp.	SAT1221712	TENN:067355	USA: Tennessee, Great Smoky Mountains National Park, Maddron Bald Trail	4-Aug-2012	scattered on mossy soil and leaf litter in mixed woods with Tsuga, Quercus, Fagus, Pinus
MH379841	H. alboaurantiacum	H. crocidens	JHR1459	TENN:040599	USA: North Carolina, Great Smoky Mountains National Park, Deep Creek, Indian Creek	9-Jul-1974	on soil in mixed woods
MH379952	H. alboaurantiacum	H. albidum	TFB9833	TENN:058812	USA: North Carolina, Macon County, Highlands, Glen Falls Trail	14-Jul-2000	
MH379855	H. alboaurantiacum	H. albidum	TFB12761	TENN:061328	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove, Gregory Ridge Trail	18-Aug-2005	
MH379864	H. alboaurantiacum	H. repandum	ADW0119	TENN:064272	USA: Tennessee, Sevier County, University of Tennessee Biology Field Station	27-Jul-2009	on soil under Quercus, Tsuga, Pinus and mixed hardwoods
MH379872	H. alboaurantiacum	H. sp.	BPL876	TENN:073003	USA: North Carolina, Durham, Duke Experimental Forest	25-May-2016	scattered in mixed duff under Quercus, Pinus
MH379894	H. alboaurantiacum	H. sp.	RAS104	TENN:073014	USA: North Carolina, Blue Ridge Parkway near mile 342	19-Aug-2016	deciduous woodlot
MH379904	H. alboaurantiacum	H. sp.	MH16004	TENN:073548	USA: North Carolina, Buncombe County, Bent Creek Experimental Forest, near Boyd Branch Road	22-Aug-2016	on side of ridge in mossy acidic forest with Quercus, Liriodendron, Betula, Tsuga, Acer
MH379955	H. alboaurantiacum	H. sp.	RAS186	TENN:073053 (holotype)	USA: North Carolina, Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail	28-Jul-2017	scattered under Betula, Fagus with Tsuga nearby
MH379937	H. alboaurantiacum	H. cf. albidum	RAS208	TENN:071751	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Quercus, Betula, Tsuga
MH379908	H. albomagnum	H. sp.	NAMA2015-050	F:C0305359F	USA: North Carolina, Buncombe County, Black Mountain YMCA Blue Ridge Assembly, Wolfpit Loop	24-Sep-2015	on soil in leaf litter in broadleaf forest with Quercus, Acer
MH379943	H. albomagnum	H. albomagnum	RAS231	TENN:073062 (epitype)	USA: North Carolina, Big South Fork National River and Recreation Area, Bandy Creek area	23-Sep-2017	solitary under Quercus, Pinus, possibly Carya
MH379849	H. subtilior	H. sp.	NJJ15_021	TENN:070350	USA: North Carolina, Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail	7-Jul-2015	
MH379851	H. subtilior	H. repandum	PBM3868	TENN:067482	USA: North Carolina, Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail	9-Aug-2012	on soil under Tsuga
MH379859	H. subtilior	H. albidum	TFB12107	TENN:060045	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove Road	31-Jul-2004	
MH379842	H. subtilior	H. repandum	AJF2	TENN:069607	USA: Tennessee, Anderson County, Norris Dam State Park, Clear Creek Trail	31-Aug-2009	solitary in leaf litter on wooded slope under Carya, Fagus, Quercus

Table 1. Continued.

GenBank no.	Species	Original specimen	Voucher ID	Herbarium ID	Location	Date	Ecology
MH379863	H. subtilior	H. albidum	TFB11985	TENN:059988	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove	14-Jul-2004	
MH379870	H. subtilior	H. repandum	JMB080409-09	TENN:064273	USA: Tennessee, Great Smoky Mountains National Park, Elkmont	4-Aug-2009	
MH379871	H. subtilior	H. repandum var. album	PBM3923	TENN:071999	USA: Tennessee, Great Smoky Mountains National Park, Tremont	14-Jul-2013	scattered singly on soil in mixed woods next to river under Tsuga, Betula
MH379874	H. subtilior	H. repandum	MK08080904		USA: Illinois, Coles County, Lakeview Park	8-Aug-2009	scattered under Quercus, Carya
MH379886	H. subtilior	H. albidum	RAS077	TENN:073002	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	24-Jul-2016	on mossy bank with Betula, Tsuga
MH379910	H. subtilior	H. sp.	RAS148	TENN:073035	USA: Tennessee, Great Smoky Mountains National Park, Tremont Institute, Lagoon Trail	23-Jun-2017	solitary under Tsuga, Carpinus, Betula
MH379913	H. subtilior	H. sp.	PBM4093	TENN:073034 (holotype)	USA: Tennessee, Anderson County, Norris Dam State Park, Clear Creek Trail	24-Jun-2017	on soil under Fagus, Carya, Quercus
MH379917	H. subtilior	H. cf. vesterholtii	RAS170	TENN:073049	USA: Georgia, Putnam County, Eatonton, Rock Eagle 4H Center	20-Jul-2017	in mixed woods under Quercus, Pinus, Carpinus
MH379918	H. subtilior	H. cf. vesterholtii	RAS180	TENN:073050	USA: Florida, Alachua County, San Felasco Hammock Preserve State Park, Moonshine Sink Trail	23-Jul-2017	in deep leaf litter under Carya
MH379925	H. subtilior	H. cf. vesterholtii	RAS184	TENN:073051	USA: North Carolina, Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail	28-Jul-2017	under Betula, Fagus, Quercus, Tsuga
MH379926	H. subtilior	H. cf. vesterholtii	RAS185	TENN:073052	USA: North Carolina, Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail	28-Jul-2017	under Betula, Fagus, Tsuga, Quercus
MH379927	H. subtilior	H. cf. vesterholtii	RAS189	TENN:073054	USA: North Carolina, Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail	28-Jul-2017	under Betula, Fagus, Tsuga, Quercus
MH379928	H. subtilior	H. sp.	BPL987	TENN:073032	USA: Tennessee, Great Smoky Mountains National Park, Greenbrier picnic area	28-Jul-2017	in riparian forest under Tsuga, Betula
MH379959	H. subtilior	H. cf. vesterholtii	RAS207	TENN:073057	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Picea
MH379847	H. subolympicum	H. repandum	RAS029	TENN:070845	USA: Virginia, Mount Rogers National Recreation Area, Mount Rogers Trail	17-Aug-2015	on soil in mixed hardwood forest under Betula and Quercus
MH379858	H. subolympicum	H. repandum	AJR14	TENN:073004	USA: Tennessee, Great Smoky Mountains National Park, Schoolhouse Gap Trail	23-Oct-2013	under Quercus in mixed hardwood/conifer forest
MH379901	H. subolympicum	H. rufescens	RAS118	TENN:073021	USA: Maryland, Susquehanna State Park	9-Sep-2016	
MH379907	H. subolympicum	H. sp.	MH16009	TENN:073551	USA: North Carolina, Buncombe County, Bent Creek Experimental Forest, Boyd Branch	19-Sep-2016	solitary in a mature bottomland forest near a boggy spot under Liriodendron tulipifera
MH379915	H. subolympicum	H. cf. albidum	RAS168	TENN:073047	USA: Georgia, White County, Unicoi State Park, Unicoi/Helen Trail	16-Jul-2017	
MH379909	H. vagabundum	H. sp.	CLO4985	TENN:074443 (holotype)	USA: Texas, Newton County, State Hwy 87 & Co. Rd. 3062	29-Dec-2011	on soil in forest of Fagus, Pinus, Quercus
MH379948	H. vagabundum	H. albidum		NYS:D-007819	USA: New York, Bolton County, Sand Lake	Aug	
MH379956	H. vagabundum	H. albidum		NYS:D-007820	USA: New York, Rensselaer County, Burden Lake	1-Sep	
MH379949	H. vagabundum	H. repandum	10782TJB	CORT:014475	USA: Vermont, Windham County, Stratton Mountain Resort area	28-Jul-2017	
MH379846	H. washingtonianum	H. washingtonianum	214	WTU:014341 (isotype)	USA: Washington, Kitsap County, Tracyton	27-Dec-1893	On ground in deep conifer woods
MH379905	H. ferruginescens	H. sp.	MH16005	TENN:073549 (holotype)	USA: North Carolina, Buncombe County, Tanbark Ridge	4-Sep-2016	growing singly or conjoined in moss at trailside with Kalmia latifolia, Pinus strobus, Quercus prinus
MH379942	H. ferruginescens	H. sp.	RAS229	TENN:073061	USA: North Carolina, Big South Fork National River and Recreation Area, West Bandy Trail	23-Sep-2017	under Quercus, Carya, Pinus, Tsuga
MH379856	H. aerostatisporum	H. rufescens	MR00311	TENN:067153	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	1-Aug-2012	
MH379861	H. aerostatisporum	H. rufescens	TFB12108	TENN:060046	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove Road	31-Jul-2004	
MH379843	H. aerostatisporum	H. repandum	TFB12514	TENN:060681	USA: North Carolina, Great Smoky Mountains National Park, Cataloochee, Big Fork Ridge Trail	18-Jun-2005	
MH379954	H. aerostatisporum	H. repandum	MK09289621		USA: Illinois, Coles County, Fox Ridge State Park	28-Sep-1996	
MH379875	H. aerostatisporum	H. repandum	MK09181403		USA: Illinois, Dewitt County, Weldon Springs State Recreation Area	18-Aug-2014	gregarious under Carya with Quercus, Ulmus nearby

Table 1. Continued.

GenBank no.	Species	Original specimen	Voucher ID	Herbarium ID	Location	Date	Ecology
MH379885	H. aerostatisporum	H. rufescens	RAS071	TENN:073001	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	24-Jul-2016	scattered on mossy bank with Picea, Betula, and other hardwoods
MH379888	H. aerostatisporum	H. sp.	RAS097	TENN:073007	USA: North Carolina, approx. 20 miles west of Little Switzerland	19-Aug-2016	
MH379903	H. aerostatisporum	H. rufescens	RAS121	TENN:073024	USA: Virginia, Shenandoah National Park, Hogback Mountain	9-Sep-2016	
MH379919	H. aerostatisporum	H. rufescens	RAS157	TENN:073040	USA: Tennessee, Great Smoky Mountains National Park, Tremont Institute, Tremont River Trail	4-Jul-2017	mixed woods under Tsuga
MH379924	H. aerostatisporum	H. rufescens	RLP61217D	TENN:073547	USA: Texas, Polk County, Big Thicket National Preserve, Beaverslide Trail	12-Jun-2017	on ground in mixed woods
MH379934	H. aerostatisporum	H. sp.	RAS203	TENN:073055	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Picea
MH379939	H. aerostatisporum	H. aerostatisporum	RAS211	TENN:073174	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Picea
MH379892	H. canadense	H. umbilicatum	RAS100	TENN:073010	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	19-Aug-2016	under Abies fraseri
MH379895	H. mulsicolor	H. rufescens	RAS108	TENN:073018	USA: North Carolina, near Little Switzerland	19-Aug-2016	in mixed woods
MH379896	H. mulsicolor	H. rufescens	RAS105	TENN:073015	USA: North Carolina, Blue Ridge Parkway near Little Switzerland	19-Aug-2016	deciduous woodlot with Quercus, Rhododendron
MH379902	H. mulsicolor	H. umbilicatum	RAS120	TENN:073023	USA: Virginia, Shenandoah National Park	9-Sep-2016	
MH379906	H. mulsicolor	H. umbilicatum	MH16006	TENN:073550	USA: North Carolina, Buncombe County, Tanbark Ridge	4-Sep-2016	growing singly in mature acidic cove forest with Quercus alba, Quercus rubra, Acer rubrum
MH379880	H. sp AS30	H. umbilicatum	AS30	CORT:007356	USA: New York, Hamilton County, Raquette Lake, Silver Beach Bog	28-Jul-1982	on sphagnum substrate with Tsuga, Larix
MH379848	H. subconnatum	H. repandum	RAS053	TENN:070846	USA: Tennessee, Union County, Big Ridge State Park, Ghost House Loop Trail	9-Nov-2015	scattered to cespitose on soil in mixed forest with Quercus, Fagus, Carya, Pinus virginiana
MH379852	H. subconnatum	H. umbilicatum	KER016	TENN:073006	USA: Tennessee, Great Smoky Mountains National Park, Schoolhouse Gap Trail	26-Oct-2013	scattered on ground beneath Pinus and Tsuga; Quercus and Betula nearby
MH379853	H. subconnatum	H. rufescens	TFB12311	TENN:060359	USA: Tennessee, Great Smoky Mountains National Park, Meigs Creek Trail	30-Sep-2004	under Pinus, Tsuga, Quercus, Acer
MH379854	H. subconnatum	H. repandum	RAW18	TENN:073005	USA: Tennessee, Great Smoky Mountains National Park, Cades Cove	23-Nov-2013	scattered in groups on ground under Fagus, Quercus, Carya, Pinus
MH379867	H. subconnatum	H. repandum	EEA171109-1	TENN:073744	USA: Tennessee, Great Smoky Mountains National Park, trail to Look Rock fire tower	17-Nov-2009	forming a ring among Quercus litter on soil beside trail
MH379869	H. subconnatum	H. umbilicatum	JHR40605	TENN:040605	USA: Georgia, Chatham County, Wormsloe Plantation	26-Dec-1976	in duff, growing under Pinus and Juniperus virginiana
MH379914	H. subconnatum	H. cf. magnorufescens	RAS165	TENN:073026	USA: Georgia, White County, Unicoi State Park, Unicoi/Helen Trail	16-Jul-2017	under Pinus and mixed hardwoods
MH379916	H. subconnatum	H. cf. umbilicatum	RAS169	TENN:073048	USA: Georgia, White County, Unicoi State Park, Unicoi/Helen Trail	16-Jul-2017	under Pinus and mixed hardwoods
MH379922	H. subconnatum	H. sp.	BPL931	TENN:073028	USA: Tennessee, Great Smoky Mountains National Park, Trillium Gap Trail	9-Jul-2017	
MH379930	H. subconnatum	H. sp.	RAS235	TENN:073064 (holotype)	USA: North Carolina, Yancey County, Carolina Hemlocks Campground, Picnic area	29-Sep-2017	cespitose under Tsuga carolinensis, Quercus, Liriodendron
MH379947	H. subconnatum	H. cf. repandum	RAS255	TENN:073070	USA: Tennessee, Union County, Big Ridge State Park, Ghost House Loop Trail	30-Oct-2017	
MH379876	H. quebecense	H. umbilicatum	49JD	CORT:007367	USA: New York, Hamilton County, Raquette Lake, Long Point, Blue Mountain Trail	25-Jul-2001	muscicolous with Fagus grandifolia
MH379878	H. quebecense	H. umbilicatum	JG003	CORT:007330	USA: New York, Hamilton County, Raquette Lake, Silver Beach Bog	22-Jul-1997	on sphagnum substrate
MH379879	H. quebecense	H. umbilicatum	KH7	CORT:007322	USA: New York, Hamilton County, Raquette Lake, Silver Beach Bog	26-Jul-1993	on sphagnum substrate with Picea
MH379881	H. quebecense	H. umbilicatum	CN9	CORT:007365	USA: New York, Hamilton County, Raquette Lake, Silver Beach Bog	23-Jul-1988	on sphagnum substrate
MH379889	H. cuspidatum	H. sp.	RAS098	TENN:073008	USA: North Carolina, McDowell County, Armstrong Creek	19-Aug-2016	in mixed woods
MH379911	H. cuspidatum	H. umbilicatum	RAS149	TENN:073036	USA: Tennessee, Great Smoky Mountains National Park, Tremont Institute, Lagoon Trail	23-Jun-2017	in soil with Tsuga, Betula

Table 1. Continued.

GenBank no.	Species	Original specimen	Voucher ID	Herbarium ID	Location	Date	Ecology
MH379912	H. cuspidatum	H. rufescens	RAS150	TENN:073037	USA: Tennessee, Great Smoky Mountains National Park, Tremont, Lumber Ridge Trail	23-Jun-2017	in moss with Quercus, Tsuga, Fagus
MH379936	H. cuspidatum	H. umbilicatum	RAS205	TENN:073056	USA: North Carolina, Great Smoky Mountains National Park, Heintooga Round Bottom Road	17-Aug-2017	on embankment under Betula, Picea
MH379940	H. cuspidatum	H. sp.	RAS218	TENN:073059	USA: North Carolina, Great Smoky Mountains National Park, Appalachian Highlands Science Learning Center	9-Sep-2017	under Quercus, Betula, Tsuga
MH379845	H. cuspidatum	H. cf. umbilicatum	RAS245	TENN:073175	USA: North Carolina, Big South Fork National River and Recreation Area, John Litton Farm Trail	29-Oct-2017	under Quercus, Tsuga, Liriodendron
MH379944	H. cuspidatum	H. cf. rufescens	RAS246	TENN:073068 (holotype)	USA: North Carolina, Big South Fork National River and Recreation Area, John Litton Farm Trail	29-Oct-2017	under Quercus, Tsuga, Pinus
MH379945	H. cuspidatum	H. sp.	RAS248	TENN:073176	USA: North Carolina, Big South Fork National River and Recreation Area, John Litton Farm Trail	29-Oct-2017	under Quercus, Pinus, Tsuga
MH379946	H. cuspidatum	H. sp.	RAS249	TENN:073069	USA: North Carolina, Big South Fork National River and Recreation Area, Grand Gap Loop	29-Oct-2017	under Quercus, Pinus
MH379857	H. umbilicatum	H. umbilicatum	SAT1324109	TENN:068871	USA: Tennessee, Great Smoky Mountains National Park, Maddron Bald Trail	29-Aug-2013	on soil in mixed woods with Tsuga, Quercus, Fagus, Pinus
MH379860	H. umbilicatum	H. umbilicatum	TFB12369	TENN:060445	USA: Tennessee, Great Smoky Mountains National Park, Greenbrier, Injun Creek Trail	18-Nov-2004	under Tsuga
MH379865	H. umbilicatum	H. umbilicatum	TFB13482	TENN:061745	USA: Tennessee, Great Smoky Mountains National Park, Gabes Mountain Trail	16-Oct-2006	under Tsuga and mixed hardwoods
MH379866	H. umbilicatum	H. rufescens	TFB12039	TENN:060288	USA: North Carolina, Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail	25-Aug-2004	under Tsuga, Pinus, Quercus, Rhododendron, Acer
MH379868	H. umbilicatum	H. umbilicatum	PBM2511	TENN:066875	USA: Massachusetts, Worchester County, Rutland State Park	1-Nov-2003	on soil in forest of Quercus and Pinus strobus
MH379844	H. umbilicatum	H. albidum	RHP36346	TENN:036346	USA: Michigan, Marquette County, Big Bay, Alder Creek	2-Sep-1971	
MH379882	H. umbilicatum	H. umbilicatum	OA14	CORT:008175	USA: New York, Tompkins County, Ridgewood Reserve	15-Sep-2007	
MH379958	H. umbilicatum	H. umbilicatum	BD14	CORT:007319	USA: New York, Tompkins County, Eames Bog	22-Sep-2011	
MH379887	H. umbilicatum	H. repandum	TFB9766	TENN:058667	USA: North Carolina, Pisgah National Forest, Yellow Gap Road	18-Jul-2000	under Quercus
MH379890	H. umbilicatum	H. umbilicatum	16051TJB	CORT:012241 (epitype)	USA: New York, Cortland County, Lime Hollow Nature Center Tunison Aquatic Lab	27-Aug-2014	on humus in wet, boggy area with Tsuga, Betula
MH379891	H. umbilicatum	H. umbilicatum	RAS099	TENN:073009	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	19-Aug-2016	under Abies fraseri
MH379893	H. umbilicatum	H. umbilicatum	RAS101	TENN:073011	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	19-Aug-2016	under Abies fraseri
MH379897	H. umbilicatum	H. umbilicatum	RAS102	TENN:073012	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	19-Aug-2016	under Abies fraseri
MH379898	H. umbilicatum	H. umbilicatum	RAS103	TENN:073013	USA: North Carolina, vicinity of Little Switzerland	19-Aug-2016	coniferous woodlot
MH379899	H. umbilicatum	H. umbilicatum	RAS109	TENN:073019	USA: North Carolina, Yancey County, Mount Mitchell State Park	19-Aug-2016	high-elevation conifer forest
MH379900	H. umbilicatum	H. umbilicatum	RAS110	TENN:073020	USA: North Carolina, Yancey County, Mount Mitchell State Park	19-Aug-2016	high-elevation conifer forest
MH379929	H. umbilicatum	H. sp.	RAS234	TENN:073063	USA: North Carolina, Yancey County, Carolina Hemlocks Campground, Picnic area	29-Sep-2017	under Tsuga carolinensis, Quercus, possibly Betula or Carpinus
MH379931	H. umbilicatum	H. sp.	RAS237	TENN:073065	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	29-Sep-2017	under Abies fraseri
MH379932	H. umbilicatum	H. sp.	RAS238	TENN:073066	USA: North Carolina, Yancey County, Mount Mitchell State Park, Balsam Nature Trail	29-Sep-2017	under Abies fraseri
MH379933	H. umbilicatum	H. cf. umbilicatum	RAS239	TENN:073067	USA: North Carolina, McDowell County, Armstrong Creek	30-Sep-2017	under Quercus, Pinus, Liriodendron
MH379960	H. umbilicatum	H. cf. umbilicatum	RAS247	TENN:073181	USA: North Carolina, Big South Fork National River and Recreation Area, John Litton Farm Trail	29-Oct-2017	under Tsuga, Quercus, Pinus

CHAPTER II
NEW REPORTS, NEW SPECIES, AND HIGH DIVERSITY OF *CANTHARELLUS* IN THE SOUTHERN APPALACHIANS

A version of this chapter was originally published by Rachel A. Swenie and P. Brandon Matheny:

Rachel A. Swenie, P. Brandon Matheny. “New reports, new species, and high diversity of *Cantharellus* in the southern Appalachians.” *Mycologia* (2023) 115(1):44–68.

R.A.S. and P.B.M. designed the study and conducted field work. R.A.S. conducted laboratory work and phylogenetic analyses. R.A.S. wrote the manuscript and P.B.M. provided manuscript edits.

Abstract

Chanterelles (genus *Cantharellus*) are among the most popular wild edible mushrooms worldwide. Efforts to understand chanterelle diversity have yielded numerous new species in recent years, particularly in eastern North America. We constructed a multilocus phylogeny including all described temperate species of *Cantharellus* and newly collected specimens from the eastern United States with an emphasis on southern Appalachia. We describe a new species, *Cantharellus vicinus*, an oak-associated chanterelle known only from lower-elevation areas in east Tennessee, based on phylogenetic and morphological data. *Cantharellus vicinus* is characterized by a compact stature, bright yellow hymenophore that turns salmon when mature, white stipe, and pale yellow pileus with a whitish bloom. The southeastern *Cantharellus minor* f. *intensissimus* is elevated to species level based on morphological and molecular evidence. The taxon is epitypified due to the sterile state of the holotype and ambiguity concerning application of the name. Evaluation of genetic diversity and gene conflict within *Cantharellus camphoratus* shows that it is a widespread species with populations in Atlantic Canada, the southeastern United States, and Japan. Similarly, *C. cibarius* and *C. tenuithrix* form complexes and may be more geographically widespread than previously thought. Additionally, we report the first known instances of *Cantharellus betularum*, *C. corallinus*, and *C. altipes* from the southern Appalachian Mountains.

Introduction

Chanterelles (genus *Cantharellus* Adans. ex Fr.) are among the most popular wild edible mushrooms in North America (Redhead et al. 1997) and likely account for over a billion dollars in commercial value each year worldwide (Pilz et al. 2003). For many years, European species of chanterelles such as *Cantharellus cibarius* Fr. were assumed to occur in North America. Researchers made efforts to distinguish additional North American taxa within *C. cibarius*, but the lack of diagnostic morphological characteristics made this a difficult task (Buyck and Hofstetter 2011; Petersen 1976, 1979). More recently, DNA sequencing has greatly expanded the number of recognized North American *Cantharellus* species by uncovering morphologically cryptic species that resemble European taxa but that are molecularly divergent (Arora and Dunham 2008;

Buyck and Hofstetter 2011; Dunham et al. 2003). However, other studies have shown that some eastern North American chanterelle species are highly morphologically variable (e.g., *C. velutinus* Buyck & V. Hofst.), whereas other species are morphologically indistinguishable from related species (i.e., *C. lateritius* (Berk.) Singer and *C. flavolateritius* Buyck & V. Hofst.; see Buyck et al. 2016a), which can confound efforts to identify species reliably without sequence data.

At present, 25 *Cantharellus* species are recognized in eastern North America and four additional species are known from Mexico (Table 2; all Tables and Figures are located in the Appendix). Most of these were described over the past decade based on a combination of DNA sequence analysis and microscopic characteristics. Many species appear to be regional endemics, and previous molecular systematic studies revealed no *Cantharellus* species that are shared between Europe and southeastern North America (Buyck and Hofstetter 2011; Buyck et al. 2016b). The use of the standard internal transcribed spacer (ITS) fungal DNA barcode is discouraged for *Cantharellus* due to high levels of heterogeneity and length variation, particularly the ITS1 region, which ranges from 820 to 1100 bp in length (Feibelman et al. 1994). In its place, other gene regions such as the D1–D2 domains of nuc 28S rDNA (28S), a portion of translation elongation factor 1-alpha (*tef1*), and the most variable region of the second largest subunit of RNA polymerase II (*rpb2*) have been employed to identify and contribute to species recognition. Due to accelerated rates of molecular evolution at rDNA loci in *Cantharellus*, Moncalvo et al. (2006) recommended use of *rpb2* and other protein- coding genes to resolve phylogenetic relationships among cantharelloid fungi.

Despite these recent advances in *Cantharellus* taxonomy and systematics, it is not clear how many species occur in the southern Appalachian region encompassing the southern Appalachian Mountains and surrounding areas of eastern Tennessee, western North Carolina, western Virginia, northwest South Carolina, and north- ern Georgia. We collected chanterelles over several field seasons in the eastern United States, with an emphasis on the southern Appalachians, to assess the taxonomic diversity in the region. To test hypotheses about species identities, we compiled the first multilocus phylogeny of all described *Cantharellus* species from the north temperate zone with molecular data. For clades with strongly supported gene conflict, we employed network analyses to measure genetic distances between closely related species at multiple loci. Network analyses have been used to visualize genetic conflict and delimit species in a variety of fungi from agarics to truffles, rusts, and *Cordyceps*-like fungi (Aime and McTaggart 2021; Cripps et al. 2019; Gazis et al. 2014; Orihara et al. 2021).

Here, we recognize *Cantharellus* species based on a combination of differences in morphology, ecology, phylogeny, and network analyses. As a result, we describe a new oak-associated chanterelle species discovered in Tennessee and provide taxonomic notes on additional *Cantharellus* species, including *C. camphoratus* R.H. Petersen, *C. betularum* Voitk & Thorn, *C. altipes* Buyck & V. Hofst., and *C. corallinus* Buyck & V. Hofst., reported for the first time from the southern Appalachians. We also recognize *C. minor* f. *intensissimus* R.H. Petersen at species rank based on morphological and phylogenetic data and compare this species with its well-known relative *C. cinnabarinus*

(Schwein.) Schwein. Additionally, we discuss genetic diversity within *C. cibarius* and provide the first report of *C. cibarius* from eastern North America.

Materials and Methods

Field collection

A total of 45 specimens were collected from localities in Connecticut, New York, North Carolina, South Carolina, Tennessee, and Virginia from 2015 to 2021. Observations of gross morphology and spore prints were obtained from fresh specimens. Color notes were made using Munsell Soil Color Charts (1954, 2009) (i.e., 7.5 YR 4/2). Specimens were preserved using a food dehydrator and accessioned into TENN. Additional specimens were obtained from UWO. Herbarium codes follow Thiers (continuously updated).

Microscopy

Microscopic examinations were per- formed on sections of dried tissues rehydrated in 5% KOH and stained with Congo red (Largent et al. 1977). Measurements and photographs were taken using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan), a Nikon DS-Fi1 camera, and Nikon NIS Elements 3.1 software. Basidiospores were measured from spore prints and reported with mean values in italics and measurements in excess of two standard deviations in parentheses. Q-values were calculated as basidiospore length divided by width. The number of spores measured for each species (n) is reported as total number of spores/number of specimens (e.g., n = 20/3).

DNA extraction and sequencing

Tissue samples were collected from specimens for DNA extraction using the Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, Missouri). Primers were used in the following combinations to amplify and sequence the 28S, *tef1, rpb2*, and the second internal transcribed spacer region of nuc rDNA ITS1-5.8S-ITS2 (ITS2), respectively: LR0R/LR5 or LR0R/LR7 using LR5 as an internal sequence primer (Cubeta et al. 1991; Vilgalys and Hester 1990), tef1F/tef1R or 983 F/2218 R (Morehouse et al. 2003; Rehner and Buckley 2005), b6F/b7.1 R (Matheny 2005), and 5.8SR/ITS4 (White et al. 1990). Sequencing was performed on an Applied Biosystems 3730 Genetic Analyzer at the University of Tennessee Genomics Core, and sequence reads were assembled in Sequencher 5.0.1 (Gene Codes, Ann Arbor, Michigan).

Taxon sampling and phylogenetic analyses

Additional *Cantharellus* sequences for each locus were downloaded from GenBank, including all described species from subgenera *Cantharellus, Parvocantharellus* Eyssart. & Buyck, and *Cinnabarinus* Buyck & V. Hofst. These subgenera contain all described species from north temperate areas of the globe. Tropical species *C. decolorans* Eyssart. & Buyck and *C. conspicuus* Eyssart., Buyck & Verbeken in subgenus *Cinnabarinus* were omitted due to highly divergent sequences and long branch lengths. Type sequences were

included for each species where available; for species with no available sequences from type material, vouchers with the most gene coverage were chosen to represent each species. All specimens and GenBank accession numbers are listed in Table 3. Sequences were aligned separately for each locus using MUSCLE in AliView 1.27 (Larsson 2014). Minor adjustments were made manually to each alignment, and, where applicable, primers were trimmed from the beginning and ends of sequences.

For phylogenetic analysis, we used partial sequences of 28S, *tef1,* and *rpb2* following other multilocus studies in this genus (Buyck et al. 2013; Olariaga et al. 2017; Zhang et al. 2021). *Cantharellus guyanensis* Mont. from subgenus *Afrocantharellus* Buyck. & V. Hofst. was chosen as an outgroup since *Afrocantharellus* is sister to other subgenera of *Cantharellus* (Wilson et al. 2012). Four phylogenies were generated: a phylogeny for each separate locus plus a concatenated data set of all three loci after inspection for strongly supported intergene conflicts. Maximum likelihood (ML) analysis was conducted with RAxML 8.2.9 (Stamatakis 2014) with 1000 bootstrap replicates under the model GTR+GAMMA as recommended by the user manual. Bayesian inference (BI) analysis of the multilocus data set was run in MrBayes 3.2.7 (Ronquist et al. 2012) for 25 million generations, sampling trees and parameters every 2500 generations. Data were partitioned into seven subsets using the best-fit partition scheme found by PartitionFinder 2.1 (Lanfear et al. 2017) as follows: *tef1* codon 1, GTR+I+G; *rpb2* codon 1, GTR+I+G; *rpb2* codon 2 and *tef1* codon 2, GTR+I+G; *rpb2* codon 3 and *tef1* codon 3, GTR+G; *tef1* intron, K80 +I+G; *rpb2* intron, HKY+G; and 28S, GTR+I+G. To visualize the phylogenies, we used FigTree 1.4.4.

Network analysis

Phylogenetic network analysis was conducted to compare sequence divergence among clades with strongly supported gene conflict using SplitsTree 5.0.3 (Huson and Bryant 2006) with the options "NeighborNet" and "Uncorrected P." The NeighborNet analysis is a distance-based method that represents genetic separation between nodes in the network. The split network models ambiguous signals within a data set, wherein parallel edges represent splits between taxa. The lengths of these edges correspond to branch lengths in a phylogenetic tree (Huson and Bryant 2006). For each clade, three networks were constructed, one for each locus (28S, *tef1, rpb2*).

Taxonomy

A typification section was included for each species with commentary to clarify type specimen details, which may be incomplete in the literature or lacking in associated GenBank accessions. Many GenBank accessions lack type designation status and/or are insufficiently labeled.

Results

A total of 100 new sequences were produced for this study (25 28S, 31 *tef1,* 40 *rpb2,* 4 ITS2; Table 3). For the BI analysis, after 10 million generations the standard deviation of

split frequencies fell below 0.01; thus, the first 40% of the posterior distribution was discarded as burn-in, for a total of 12,002 trees sampled. Average effective sample size (ESS) values were >4000, and Potential Scale Reduction Factor (PSRF) was 1.0 for each parameter, indicating satisfactory mixing behavior and sampling from the posterior distribution. No strongly supported topological conflicts were found between the ML and BI analyses, and the ML topology is shown in Figure 8. The 28S data set contained 121 taxa and 1355 sites; the *tef1* data set contained 120 taxa and 945 sites; the *rpb2* data set contained 96 taxa and 1171 sites; and the 28S + *tef1* + *rpb2* data set (hereafter referred to as the combined data set) contained 152 taxa and 3471 sites.

Sequences of our collections mainly from the southern Appalachians were distributed in 11 different clades (Figure 8): the *Cantharellus tenuithrix* complex containing *C. deceptivus* Buyck, Justice & V. Hofst., C. flavus Foltz & T. J. Volk, C. phasmatis Foltz & T.J. Volk, and *C. tenuithrix* Buyck & V. Hofst.; the *C. cibarius* complex containing *C. cibarius, C. enelensis* Voitk et al., and *C. roseocanus* (Redhead, Norvell & Danell) Redhead, Norvell & Moncalvo; *C. velutinus; C. altipes; C. camphoratus; Cantharellus* sp. (described as new below); *C. betularum; C. persicinus* R.H. Petersen; *C. flavolateritius; C. minor* f. *intensissimus* R.H. Petersen (elevated to species rank below); and *C. corallinus*. However, strongly supported gene conflict was detected within the following clades: *C. camphoratus, C. cibarius*, and *C. tenuithrix*. The results of phylogenetic and network analyses for each of these clades are discussed separately below.

Specimens of *Cantharellus* "JPN sp. 2" from Japan differed from North American *C. camphoratus* by fewer than 1% of base pairs at each locus. The *tef1* sequences from Japanese samples closely matched U.S. and Canadian samples (Figure 9). Shared polymorphisms among *tef1* sequences showed that the Canadian sample 120922av02 from Newfoundland and Labrador had the highest genetic diversity. This result may be due to shared ancestral polymorphisms, but data from addi- tional loci would be necessary to test this hypothesis, as only 28S and *tef1* sequences were available from the Japanese specimens. The results of our network analyses displayed these conflicting results at different loci (Figure 10). Because samples from Canada, USA, and Japan were closely allied at the *tef1* and/or *rpb2* loci and did not vary in their morphology and ecology, we considered these samples to be different populations of *C. camphoratus*.

Very low levels of genetic variation (<1%) were observed among specimens of *C. cibarius, C. enelensis*, and *C. roseocanus* (Figure 11). Apart from 28S, there was no clear geographic clustering of samples; for example, the *tef1* sample RAS895 from New York differed from Canadian *C. enelensis* (VilneffE5) and German *C. cibarius* (AFTOL-607) by less than 1% similarity. The *rpb2* sequence for RAS859 differed by one base pair from European *C. cibarius* specimens. The white U.S. *cibarius*-like specimen, CLC2018-2, differed from *C. roseocanus* DAOM220723 by a single base pair at the *rpb2* locus. Gene histories revealed a similar pattern. Based on these results, *C. cibarius* appears to be a widespread species across the temperate Northern Hemisphere.

The *C. tenuithrix* clade included several poorly supported subclades. Most species within this complex lack multilocus sequence data from their holotype specimens. We assigned our field names using morphological species descriptions, but sequence results

largely failed to support these morphological concepts. Whereas specimens were largely undifferentiated by 28S data, gene conflict was observed between *tef1* and *rpb2* gene networks (Figure 12) and phylogenies. Genetic differentiation was detected between European *C. pallens* and North American samples in the *C. tenuithrix* complex, and the ML analysis showed weak support for three North American clades: (i) *C. deceptivus*, (ii) *C. tenuithrix*, and (iii) *C. flavus* plus *C. phasmatis*. We assigned provisional species names to our samples based on their phylogenetic affinity to these taxa.

Taxonomy

Cantharellus subg. *Cantharellus*

Cantharellus altipes Buyck & V. Hofstetter, Fungal Diversity 49:39. 2011.
Figure 13A–B
Typification: USA. TEXAS: Montgomery County, Conroe (30.3, −95.45), 60 m, in *Quercus-Pinus* woods, 28 Jul 2007, *Buyck 07.112* (holotype PC 0084080).
GenBank: *tef1* = GQ914941.

Ecology and distribution: On soil in *Quercus-Pinus* forests in Gulf Coast states (Texas (type), Louisiana) and in northern hardwood forests with *Fagus, Quercus*, and *Betula* in the southern Appalachians (Tennessee, Virginia) at elevations reaching 1200–1300 m. Jul to Dec.

Specimens examined: USA. TENNESSEE: Sevier County, Great Smoky Mountains National Park, Trillium Gap Trail (35.670837, −83.438888), 1332 m, scattered under *Betula alleghaniensis, Fagus*, and *Tsuga*, 9 Aug 2020, *R.A. Swenie RAS639* (TENN- F-075384); VIRGINIA: Grayson County, Mt. Rogers National Recreation Area, Mt. Rogers Trail (36.65942, −81.54085), 1220 m, scattered under *Fagus* and *Quercus*, 14 Aug 2016, *R.A. Swenie RAS084* (TENN-F-071461).

Commentary: In addition to Texas oak-pine woods where the species was described, *Cantharellus altipes* also occurs in northern hardwood forests dominated by *Fagus, Betula*, and/or *Quercus* at moderate elevations in the southern Appalachians. Basidiomata of *C. altipes* generally have stipe lengths exceeding the pileus diameter, but pileus coloration is highly variable ranging from bright yellow to pale brownish yellow with a brown disc (Figure 13A–B). Stipe coloration also varies from pale cream to bright egg yellow, although the hymenophore is consistently pale cream-yellow. The large spore size (7.3–*9.4*–12 × 4.4–*4.9*–5.7 µm) combined with brown pileus tones can lead this species to be confused with *C. betularum*; however, *C. altipes* has somewhat smaller spores than *C. betularum* and is found in northern hardwood forests around 1200– 1300 m in elevation in the southern Appalachian Mountains, as opposed to *C. betularum*, which has spores averaging 10.4 × 5.6 µm and is known from high-elevation birch-spruce-fir habitat at 1800 m elevation.

There has been some confusion about the holotype designation for *C. altipes*. Collection BB 07.112, denoted as the holotype in the protolog, is represented only by a *tef1* sequence (GQ914941). However, Buyck et al. (2013) later referred to BB 07.019 as the holotype of *C. altipes*. BB 07.019 is represented by 28S, *tef1*, and *rpb2* sequences,

and the *tef1* sequence matches that of BB 07.112. Phylogenetic analysis showed that our specimens form a clade with the holotypes of *C. altipes* and *C. septentrionalis* A.H. Sm. Buyck et al. (2016b) discussed the possibility that these two taxa are synonymous. The binomial *C. septentrionalis*, which is the older name, has not been widely applied, and the holotype is represented by 28S sequence data only preventing a more thorough phylogenetic comparison between the two species. However, given what we know from contemporary collections of *C. altipes*, the phylogenetic affinity to *C. septentrionalis*, and variable macromorphology of *C. altipes* (Buyck and Hofstetter 2011; Figure 13A–B), sug- gests that the two species may indeed be synonymous. Additional sequence data from the type or collections from the type locality of *C. septentrionalis* in Michigan would help confirm or refute this.

Cantharellus betularum Voitk & Thorn, Persoonia 45:331. 2020.
Figure 13C
Typification: CANADA. NEWFOUNDLAND AND LABRADOR: Humber Village, trail to Barry's Lookout (48.988, −057.792), 159 m, in leaf litter under *Betula papyrifera, B. cordifolia,* and *B. alleghaniensis*, 14 Sep 2013, *Andrus Voitk 13.09.14.av01* (holotype DAOM 721702; isotype DAOM 734027). GenBank: 28S = KX592700.

Ecology and distribution: On soil under *Betula*, southeast Canada (type) and at high elevation (1800 m) in the southern Appalachians (North Carolina). Jul to Sep.
Specimens examined: CANADA. NEWFOUNDLAND AND LABRADOR: Humber Village, trail to Barry's Lookout (48.988, −057.792), 159 m, in leaf litter under *Betula papyrifera, B. cordifolia,* and *B. alleghaniensis*, 24 Aug 2008, *A. Voitk 11.08.12.av01* (DAOM 734022); ibid., 24 Aug 2008, *M. Voitk 17.09.30.av01* (DAOM 984767). USA. NORTH CAROLINA: Swain County, Great Smoky Mountains National Park, Appalachian Trail (35.67990, −83.27344), 1800 m, scattered under *Betula alleghaniensis, Abies fraseri*, and *Picea rubens*, 25 Jul 2020, *R.A. Swenie RAS631* (TENN-F-075393).
Commentary: Cantharellus betularum is a recently described North American species closely related to the European *C. amethysteus* (Quél.) Sacc. We collected *C. betularum* from a high-elevation (1768 m) spruce-fir-birch forest in the Great Smoky Mountains National Park in North Carolina and confirmed the identification using multiple loci (28S, *tef1, rpb2*). In addition, we generated *rpb2* sequences from Canadian specimens cited in Thorn et al. (2020). Although *rpb2* and *tef1* sequences of *C. betularum* closely match Italian collections of *C. amethysteus* (99% sequence similarity), the two species form monophyletic sister clades strongly supported by the multilocus ML analysis. Thorn et al. (2020) reported that, unlike *C. amethysteus*, basidiomata of *C. betularum* do not always have violet scales on the pileus, and although the pileus of the young basidiomata we collected were minutely scaly and yellow-brown, no violet tones were noted. When young, *C. betularum* resembles *C. camphoratus*, a similarly brown-capped species that also occurs in high-elevation spruce-fir forests in the southern Appalachians. However, the spores of *C. betularum* are consistently larger than those of *C. camphoratus*, averaging 10.2 × 6.1 µm in *C. betularum* versus 9.1 × 5.2 µm in *C. camphoratus* based on measurements of sequenced specimens. Furthermore, *C. betularum* is found under *Betula*, whereas *C. camphoratus* occurs under *Abies* and *Picea* (Thorn et al. 2017).

Another brownish to lilac-colored species, *C. lewisii* Buyck & V. Hofst., has much smaller spores and occurs in floodplain hardwood forests typically in Gulf Coast states. In our experience, *C. betularum* most closely resembles *C. altipes* in the field since *C. altipes* can occur in forests mixed with *Betula* and has brown tones at the center of the pileus. However, in the southeast, *C. altipes* is only known from northern hardwood habitat below 1400 m in elevation, and not the high-elevation spruce-fir-birch habitat at 1800 m where *C. betularum* was found. Additionally, *C. betularum* can be distinguished from *C. altipes* by the larger spore size (*C. betularum*: (7.7)8.7–*10.4*–14.3 × (3.9)4.6–*5.6*–7.1(7.7) µm; *C. altipes*: 7.3–*9.4*–12 × 4.4–*4.9*–5.7 µm). Thorn et al. (2020) also noted that *C. betularum* has distinctly shallow, blunted hymenial folds, which may be a useful identifying feature in the field. Still, genetic confirmation may be necessary to reliably distinguish *C. betularum* from other species.

Cantharellus camphoratus R.H. Petersen [as *'comphoratus'*], Nova Hedwigia 31:3. 1979. Figure 13D–E

Typification: CANADA. NOVA SCOTIA: Kings County, Cape Split, Scots Bay (45.32, −64.45), 75 m, *Picea* and *Abies* with occasional *Betula*, only occasional parklands, mostly foggy and continually wet, 29 Aug 1973, *R.H. Petersen* (holotype TENN-F-038025 [!]). GenBank: 28S = KX896788.

Ecology and distribution: On acidic soils in coniferous woods under *Abies* and *Picea* or these trees mixed with *Tsuga* and *Betula*. Canada (holotype, Nova Scotia; Newfoundland and Labrador), northeastern United States (New York), southeastern United States (North Carolina), also Japan (Nagano, Gifu). Jul to Sep.

Specimens examined: USA. NEW YORK: Essex County, Northville Placid Trail (43.24733, −74.3118), 425 m, on acidic soil under *Picea* and *Abies*, 14 Aug 2021, *R.A. Swenie RAS913* (TENN-F-075907); NORTH CAROLINA: Yancey County, Mount Mitchell State Park, Old Mitchell Trail (35.75143, −82.27356), 1890 m, scattered on acidic soil under *Abies fraseri*, *Picea rubens*, and *Tsuga canadensis*, 12 Jul 2019, *P.B. Matheny PBM4274* (TENN-F-074836); ibid., 17 Jul 2020, *R.A. Swenie RAS614* (TENN-F-075387); ibid., 21 Aug 2020, *R.A. Swenie RAS659* (TENN-F-075388) and *RAS661* (TENN-F-075389); ibid., 19 Sep 2020, *P.B. Matheny PBM4501* (TENN-F-075373). CANADA. NEWFOUNDLAND AND LABRADOR: Newfoundland, Pasadena Ski Trails (48.996, −57.586), 90 m, on soil in mixed forest (*Abies balsamea*, *Betula*, *Picea*), 3 Sep 2012, *A. Voitk 12.09.03.av17* (DAOM 734036); Newfoundland, Deer Lake (49.17, −57.43), 26 m, 13 Sep 2018, *M. Voitk 18.09.13.av07* (UWO).

Commentary: Cantharellus camphoratus, originally described from Nova Scotia, has now been confirmed from the Adirondack Mountains in New York and high-elevation conifer forests in North Carolina. However, all basidiomata lacked the camphor odor and scaly pileus originally attributed to *C. camphoratus* (Petersen 1979). Petersen (1979) noted that a similar taxon without the camphor odor and scaly pileus also occurred in the southern Appalachians.

The *tef1* sequences from U.S. collections most closely matched specimens from coniferous forests in Japan (3 bp differences; Figures 9, 10B). However, 28S and *rpb2* sequences of our specimens closely matched the holotype and recent collections of *C.*

camphoratus from Newfoundland and Labrador (Figures 10A, C). We hypothesize that Newfoundland (island), samples of which contain the greatest genetic diversity at the *tef1* locus (Figure 9), may represent a refugium. Atlantic Canada is thought to have been a glacial refugium for some plant species (Brassard 1984; Tremblay and Schoen 1999). *Cantharellus camphoratus* features multiple *tef1* alleles, from which the southeastern U.S. and Japanese populations may be derived, and that the variable gene history of the *tef1* locus relative to 28S and *rpb2* may be due to incomplete lineage sorting. If so, *C. camphoratus* has a wider disjunct range than most other chanterelle species in eastern North America. We recognize the Japanese specimens ("JPN2"/"sp. 2" in Ogawa et al. 2018) within *C. camphoratus* due to very low 28S and *tef1* sequence divergence (<1%) from North American populations of *C. camphoratus* and due to similar ecology and morphology including spore size. Comparison of *rpb2* sequences from Japanese specimens of *C. camphoratus* is needed to confirm this relationship. Further sampling of the western North American *C. formosus* Corner is also desirable to clarify its relationship to *C. camphoratus*. Results of the multigene analysis (Figure 8A) and *tef1* phylogeny supported *C. formosus* as sister to *C. camphoratus*.

In contrast to the protologue, Thorn et al. (2017) noted that contemporary collections of *C. camphoratus* from Newfoundland and Labrador lacked the camphor odor and sometimes lacked scales on the pileus. However, two reliable field characteristics for *C. camphoratus* include the shallow blunt folds that may be merulioid when young and the golden pileus margin, both of which Petersen originally noted. In the field, *C. camphoratus* could be mistaken for *C. betularum*, which also has a brownish pileus and occurs in the southern Appalachian spruce-fir zone but associates with *Betula alleghaniensis*. The often merulioid hymenophore of *C. camphoratus* has also been noted in *C. enelensis*.

Our collections from North Carolina and New York are the first confirmed reports of *C. camphoratus* from the United States. In the southern Appalachians and in the Adirondacks, the species appears to prefer acidic soils under *Picea* and *Abies*.

Cantharellus cibarius Fr. complex
Figure 13F–G
*Typification (*Cantharellus cibarius): Bulliard, Herb. France 2:pl. 62, 1781–1782, lectotype (icon.) designated by Eyssartier and Buyck (2000). SWEDEN. UPPLAND: Uppsala, Nåsten, under *Picea abies*, with *Betula* and *Pinus*, on acidic ground, 23 Jul 2005, *A. Felipe & I. Olariaga* (epitype BIO-Fungi 10986; isoepitype UPS F-575623). GenBank: ITS2 = KR677501; 28S = KR677539; *tef1* = KX828823; *rpb2* = KX828742.

*Typification (*Cantharellus roseocanus): CANADA. BRITISH COLUMBIA: Vancouver Island, Pacific Rim National Park, Wickaninnish Bay, Long Beach (49.05, −125.716667), 45 m, solitary to gregarious, often in small clusters, on bare or mossy or grassy needle beds in second-growth *Picea stichensis* without other tree associates or under *Picea* with *Tsuga* and/or *Abies*, 15 Sep 1995, *S.A. Redhead* (holotype DAOM 220723). GenBank: 28S = KX828810; *tef1* = KX828837; *rpb2* = KX828758.

*Typification (*Cantharellus enelensis): CANADA. NEWFOUNDLAND AND LABRADOR: Sandy Point (48.449269, −58.521503), 0.4 m, in moss or fruticose lichens on moist sandy soil under *Picea glauca,* 21 Aug 2013*, Urve Voitk 13.08.21.av02* (holotype DAOM 721704; isotype DAOM 734041), GenBank: 28S = KX592712. USA. NEW YORK: Essex, Lake Pleasant, Northville Lake Placid Trail (43.73848, −74.46371), 640 m, on soil under *Picea* and *Abies,* 14 Aug 2021*, R. A. Swenie RAS913* (TENN-F-075907).

*Specimen examined (*Cantharellus cibarius): USA. CONNECTICUT: Litchfield, Torrington (41.8, −73.1), 165 m, white color variant among yellow forms in mixed woods under *Betula* and *Tsuga,* 16 Aug 2018*, C. LaChance CLC2018-2* (TENN-F-076274).

Commentary: In the past, most yellow chanterelle species in North America were referred to as *C. cibarius* before it was suggested, based on genetic analyses, that true *C. cibarius* did not occur in North America (Buyck and Hofstetter 2011). Indeed, both *C. roseocanus* and *C. enelensis* have been referred to as the western and eastern North American "*cibarius*," respectively (Redhead 2012; Thorn et al. 2017). More recently, *C. cibarius* has been reported from Japan and northeast China (Ogawa et al. 2018; Shao et al. 2021). We have yet to observe *C. cibarius* in the southern Appalachians; however, two specimens collected from the northeastern United States (Connecticut and New York) clustered with European *C. cibarius* within a strongly supported monophyletic group with minimal genetic differentiation (Figure 8A). Our 28S phylogeny distinguished two groups within the *C. cibarius* clade: (i) European specimens including the epitype and (ii) North American species *C. enelensis* and *C. roseocanus*, although the latter clade was not well supported. One specimen from New York (RAS859) was allied with other North American species in that phylogeny. We provisionally interpreted RAS859 and AFTOL-607 from Germany as *C. enelensis*. The *tef1* tree and network analysis (Figure 11B), however, showed that samples from Europe, Asia, and North America are poorly differentiated. Intergene conflicts were observed between the *tef1* and *rpb2* phylogenies and network analyses (SUPPLEMENTARY FIG. 3; FIG. 4C). Divergent gene histories could potentially be explained by low levels of ongoing gene flow among these populations. Alternatively, *C. cibarius* and *C. enelensis* may each be more widespread than previously thought with populations in both Europe and northern North America.

Olariaga et al. (2015, 2017) noted that basidiome color of *C. cibarius* is highly variable; a "typical" specimen has "an orange-yellow pileus, uniform-colored hymenophore and absence of marked staining upon bruising," but entirely orange, entirely white, citrine yellow, or pink-coated specimens occasionally occur. Our specimen CLC2018-2, a white chanterelle growing among yellow specimens presumed to be the same species (Figure 13G), matches this concept of a highly variable *C. cibarius*. Such variation in basidiome color was also identified in the eastern North American counterpart to *C. cibarius*: Thorn et al. (2021) described *C. enelensis* f. *acolodorus*, an albino variant lacking the β-carotene responsible for the golden color of the typical yellow form of *C. enelensis*. Our phylogenetic analyses showed low genetic divergence and conflicting phylogenetic affinities among samples of *C. cibarius, C. roseocanus*, and *C. enelensis*. Therefore, we refer to these species as members of the *C. cibarius* complex,

emphasizing a need for additional multilocus sampling from a broad geographic range to assess the extent of this iconic species. It is not clear from current data whether *C. cibarius* is a single widely distributed species, or whether the three currently described species in this complex can be reliably distinguished from one another with denser multilocus sampling.

Cantharellus flavolateritius Buyck & V. Hofst., Cryptog Mycol 37:372. 2016.
Figure 13H–I
Typification: USA. NORTH CAROLINA: Macon County, Highlands, south of Highlands Biological Station along Walking Stick Road (35.008283, −83.161300), 770 m, growing on side of dirt road, 21 Jun 2011, *Jay Justice NC Canth-4* (holotype PC 0713852). GenBank: *tef1* = KX857029.

Ecology and distribution: On soil in mixed woods including *Pinus, Tsuga, Fagus, Carya*, and *Quercus*, North Carolina (type), also Tennessee and Arkansas. May to Aug.

Specimens examined: USA. TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove near Elijah Oliver Place (35.602, −83.813), 366 m, on soil in mixed woods under *Pinus* and *Tsuga*, 7 Jul 2019, *R.A. Swenie RAS46*3 (TENN-F-074947); Abrams Creek, Little Bottoms Trail (35.611, −83.923), 436 m, on soil under *Pinus* and hardwoods, 9 Jul 2020, *R. A. Swenie RAS588* (TENN-F-076283); Abrams Creek, Little Bottoms Trail (35.613, −83.924), 436 m, on soil in mixed woods, 9 Jul 2020, *R.A. Swenie RAS590* (TENN-F-075271); Cumberland County, Ozone Falls State Natural Area (35.88, −84.81), 610 m, 5 Aug 2019, *A. Aromin RAS474* (TENN-F-074952); Union County, Big Ridge State Park, Lake Trail (36.2431, −83.9285), 335 m, on soil under *Fagus, Carya, Quercus*, and *Pinus virginiana*, 4 Jul 2019, *P.B. Matheny PBM4264* (TENN- F-074817).

Commentary: Cantharellus flavolateritius was described recently from the southern Appalachians as an addition to a group of "smooth chanterelles." Our sampling efforts suggest that this is a common species in oak-pine and oak-hickory forests of Tennessee and North Carolina but with a variable gross morphology. Basidiomata have a hymenophore that may be completely smooth or with shallow wrinkled folds and with hymenophore colors ranging from bright orange to yellow or nearly white. Similar variation is seen in the pileus color and texture ranging from orange to yellow and glabrous to velutinous or tomentose as in *C. velutinus* (e.g., PBM4264). However, *C. velutinus* has more distinctly and consistently lamellate folds, as well as a thicker pileus margin.

Cantharellus flavolateritius formed a strongly supported clade in our ML analysis but was poorly supported in the BI analysis (Figure 8A). Within-species sequence variation was largely confined to the 28S locus. *Cantharellus lateritius* is a closely related species, also described from North Carolina and recently epitypified from Texas (Buyck and Hofstetter 2011). According to Buyck et al. (2016a), *C. lateritius* is not morphologically distinguishable from *C. flavolateritius*, and the authors separated the two taxa based on *tef1* divergence. Despite the availability of multilocus sequence data generated from the epitype of *C. lateritius*, we have not confirmed any collections of *C. lateritius* from the southern Appalachians. Based on our multilocus phylogeny (Figure

8A), C. *lateritius* and *C. flavolateritius* formed a poorly supported monophyletic group most closely related to two oak-associated chanterelle species described from Mexico with a smooth to rugulose hymenophore, *C. furcatus* Bandala, Montoya & Ramos and *C. veraecrucis* Bandala, Montoya & M. Herrera (Montoya et al. 2021).

Cantharellus persicinus R.H. Petersen, Nova Hedwigia 42:158. 1986.
Figure 14A
= *Cantharellus spectaculus* Foltz & T.J. Volk, Mycologia 105:458. 2013.
*Typification (*C. persicinus): USA. TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove, vicinity of picnic area (35.605556, −83.770556), 594 m, 12 Aug 1975, *R.H. Petersen* (holotype TENN-F-026086).

*Typification (*C. spectaculus): USA. WISCONSIN: La Crosse County, Hixon Forest Park, along the path parallel to Bliss Road, approximately halfway up the path on right side, associated with *Quercus* in *Quercus-Carya* mixed deciduous forest (43.8157, −91.2091), 250 m, 18 Jul 2010, *Foltz C081, Collection CS001* (holotype C0171590F). GenBank: 28S = JX030421; *tef1* = JX030414.

Ecology and distribution: Widely distributed in eastern North America (sequenced specimens from Wisconsin (type of *C. spectaculus*), Missouri, Tennessee (type of *C. persicinus*), North Carolina), on karst topography in oak-hickory forests with *Quercus, Carya*, and *Fagus* and in mixed forests on acidic soils. Jun to Sep.

Specimens examined: USA. TENNESSEE: Anderson County, Norris Dam State Park (36.24, −84.12), 400 m, scattered singly deep in leaf litter on soil on karst topography in hardwood forest of *Quercus, Fagus*, and *Carya*, 28 Jul 2013, *P.B. Matheny PBM3933* (TENN- F-068446); Blount County, Great Smoky Mountains National Park, Schoolhouse Gap Trail (35.62696, −83.72645), 518 m, on soil in mixed woods, 17 Jul 2019, *R.A Swenie RAS468* (TENN-F-075071); NORTH CAROLINA: Haywood County, Great Smoky Mountains National Park, Big Creek, Chestnut Branch Trail (35.7595, −83.1068), 500 m, in leaf litter on acidic soil under mixture of *Pinus, Tsuga, Quercus, Betula*, and *Fagus*, 16 Jul 2015, *S.A. Trudell SAT1519702* (TENN- F-070322); Franklin County, Nantahala National Forest, Appalachian Trail above Rock Gap (35.093789, −83.522476), 1130 m, gregarious on acid soil in mixed forest with *Tsuga, Quercus, Fagus*, and *Rhododendron*, 30 Jul 2021, *R.A. Swenie RAS841* (TENN-F-075884).

Commentary: Cantharellus persicinus can be recognized in the field due to the pink lamellar folds and slender habit. This species has a contrasting ochraceous buff to yellow-orange pileus that distinguishes it from pink forms of *C. velutinus*, which feature a peach-pink colored pileus and pink folds. The stipe is usually longer and thinner than other chanterelle species and is often buried in deep layers of leaf litter. These features, plus the very large spore size (9.5–12(14) × 5–7 μm) and 4-spored basidia, distinguish *C. persicinus* readily (Buyck et al. 2016c; Foltz et al. 2013; Petersen 1986). Buyck et al. (2016c) noted *C. spectaculus* is a later synonym of *C. persicinus*, a result reinforced by our phylogenetic analysis (Figure 8A). Thus, *C. persicinus* has a wide range in the eastern United States from Wisconsin south to Missouri and eastward to Tennessee and North Carolina.

Cantharellus tenuithrix Buyck & V. Hofst. complex
Figure 14B–C
*Typification (*Cantharellus tenuithrix): USA. TEXAS: Newton County, Sandridge, Country Road 4045 near Lewis' Chapel (30.73, −93.766), 35 m, *Pinus-Quercus* woods on sandy soil, 28 Jul 2007, *Buyck 07.125* (holotype PC 0084084). GenBank: ITS = JN944017; 28S = JN940600; *tef1* = GQ914947; *rpb2* = KF294712.

*Typification (*Cantharellus flavus): USA. WISCONSIN: La Crosse County, Hixon Forest Park, along the path running parallel to Bliss Road, approxi- mately halfway up the path when heading uphill, on right side (43.8157, −91.2091), 250 m, associated with *Quercus* in *Quercus-Carya* mixed deciduous forest, 18 Jul 2011, *Foltz C066, Collection CF001* (holotype C0171585F). GenBank: 28S = JX030437.

*Typification (*Cantharellus phasmatis): USA. WISCONSIN: La Crosse County, Hixon Forest Park, along the path running parallel to Bliss Road (43.8157, −91.2091), 250 m, associated with *Quercus* in *Quercus- Carya* mixed deciduous forest, 18 Jul 2010, *Foltz C073, Collection CP002* (holotype C0171588F). GenBank: 28S = JX030426.

*Typification (*Cantharellus deceptivus): USA. WISCONSIN: Vilas County, Lost Lake (45.96, −89.477), 488 m, under hardwood trees including *Betula papyrifera* and *Populus grandidentata*), 24 Jul 2010, *1074/Jay Justice JJ13/WI-CANT-1* (holotype PC 0142430). GenBank: 28 = KX896779; *tef1* = KX857026.

Ecology and distribution: Widely distributed in east- ern North America (sequenced specimens from Wisconsin (types of *C. deceptivus, C. flavus*, and *C. phasmatis*), Missouri, North Carolina), Tennessee, Texas (type of C. *tenuithrix*), on karst topography and acidic soils in deciduous, coniferous, or mixed woods with *Quercus, Pinus, Tsuga, Carya, Fagus, Betula*, and *Populus*. Jun to Aug.

*Specimens examined (*Cantharellus deceptivus): USA. TENNESSEE: Sevier County, Great Smoky Mountains National Park, Elkmont, Little River Trail (35.6535, −83.579), 662 m, on soil in mixed woods, 3 Jun 2016, *R.A. Swenie RAS056* (TENN-F-071459); NORTH CAROLINA: Swain County, Great Smoky Mountains National Park, Cherokee, Straight Fork Road (35.624, −83.2113), 610 m, on soil embankment in mixed woods with *Tsuga* and *Quercus*, 7 Jul 2019, *R.A. Swenie RAS447* (TENN-F-074943).

*Specimens examined (*Cantharellus flavus): USA. TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove, Gregory Ridge Trail (35.561389, −83.844167), 610 m, on soil under *Pinus rigida* with *Tsuga* nearby, 17 Jul 2019, *R.A. Swenie RAS464* (TENN-F-074949); near Tipton Place (35.601944, −83.813611), 366 m, on soil in mixed woods under conifers (*Pinus, Tsuga*), 3 Aug 2019, *R.A. Swenie RAS473* (TENN-F-074951); Sevier County, Great Smoky Mountains National Park, Elkmont, first left off Elkmont Road by mulch pile (35.6638, −83.5889), 650 m, 2 Aug 2012, *M.G. Wood MGW1115* (TENN- F-067243).

*Specimens examined (*Cantharellus tenuithrix): USA. NORTH CAROLINA: Monroe County, Cherokee National Forest, Wildcat Road (35.30823, −84.23233), 639 m, on soil on xeric ridge under *Pinus*, 5 Jul 2020, *R. A. Swenie RAS584* (TENN-F-075272); TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove, Primitive Baptist Church (35.6020315, −83.8135083), 518 m, on soil in leaf litter under *Pinus, Tsuga*, and *Quercus*, 17 Jul 2019, *P.B. Matheny PBM4292* (TENN-F-074854);

Union County, Big Ridge State Park, Lake Trail (36.2431, −83.9285), 335 m, on soil under *Fagus, Carya, Quercus*, and *Pinus virginiana*, 4 Jul 2019, *P.B. Matheny PBM4268* (TENN- F-074821).

Commentary: Four very closely related eastern North American species formed a strongly supported monophyletic group sister to European *Cantharellus pallens* (Figure 8A). The group contains *C. deceptivus, C. flavus, C. phasmatis*, and *C. tenuithrix*, all of which were described recently. However, topological placement of each species conflicted across individual gene phylogenies. Each of the North American species was separated by previous researchers based on morphology and genetic differences among ITS, 28S, and/or *tef1* sequences (Buyck et al. 2016c; Foltz et al. 2013). However, based on our observations, the four species cannot be distinguished from one another reliably considering their ecology and morphology in the southern Appalachians. *Cantharellus flavus, C. phasmatis*, and *C. deceptivus* were all described from deciduous forests and *C. tenuithrix* from oak-pine forests. We failed to find any clear habitat preferences among the North American species. All were collected at lower elevations in the southern Appalachians below 700 m in coniferous or mixed conifer-hardwood forests on acidic soils and areas with karst topography. Efforts to distinguish the species based on morphology proved to be confounding. Hymenophore colors ranged from white to peach to yellow, sometimes within a single collection, and did not consistently correlate with different species in the complex.

Cantharellus pallens formed a distinct clade sister to the North American species in the multilocus phylogeny (Figure 8A). Phylogenetic and network analyses showed that European *C. pallens* is not distinguishable from members of the *C. tenuithrix* complex except at the *rpb2* locus (Figure 12). However, since *C. pallens* formed a phylogenetic clade that does not contain any North American specimens, we considered *C. pallens* autonomous from the North American *C. tenuithrix* complex.

Cantharellus tenuithrix was originally described from Texas as the eastern North American sister taxon of *C. cibarius*, distinguishable by *tef1* sequences and morphology (Buyck and Hofstetter 2011). The other three taxa in the *tenuithrix* complex, *C. flavus, C. phasmatis*, and *C. deceptivus*, were each described from the northern United States (Wisconsin) in 2013 and 2016 based on evaluation of very few collections and sequenced materials. The authors of *C. flavus* and *C. phasmatis* conducted phylogenetic analysis of the ITS, 28S, and *tef1* gene regions. Hymenophore color was used to distinguish between *C. flavus* and *C. phasmatis*. It is now clear that within-species morphological variation in *Cantharellus* can be quite broad (see *C. cibarius* complex and *C. flavolateritius* discussed here, and Buyck et al. 2016a for examples in other *Cantharellus* species), and specimens within the *tenuithrix* complex appear to be no exception. Hymenophore color can change dramatically with age of the basidiome (Figure 14B). *Cantharellus deceptivus*, on the other hand, was described as a morphologically cryptic look-alike of *C. phasmatis* based on *tef1* sequence divergence and a "less bright colored hymenophore" than *C. tenuithrix* or *C. flavus* (Buyck et al. 2016c). The holotype of *C. deceptivus* and another cited specimen formed a polytomy with other members of the *tenuithrix* complex in the *tef1* phylogeny in Buyck et al. (2016c), and the authors noted that sequence difference between the species is minimal with >99.5% genetic similarity. Indeed, Buyck et al.

(2016c) referred to these four species as a "complex." In our BI multigene analysis, *C. deceptivus*, *C. flavus*, *C. phasmatis*, and *C. tenuithrix* also formed a polytomy, with poor support for each as an autonomous taxon. By contrast, we found that (i) *C. tenuithrix*, (ii) *C. deceptivus*, and (iii) *C. flavus* plus *C. phasmatis* formed three distinctive but weakly supported clades in our multilocus ML analysis (Figure 8A). Each formed strongly supported monophyletic clades in our *rpb2*-only ML analysis; however, *rpb2* data are lacking for all holotypes except *C. tenuithrix*. Given these results, we assigned our specimens species names based on phyloge- netic affinity. However, since it is not possible to reliably distinguish these species in the field given their morphological plasticity and poorly supported phylogenetic placements, we are in agreement with Buyck et al. (2016c) who referred to this cluster of species as a complex.

Cantharellus velutinus Buyck & V. Hofst., Cryptog Mycol 37:372. 2016.
Figure 14D
Typification: USA. TEXAS: Tyler County, Big Thicket National Preserve, Beech Creek unit, along path running from the beginning of the trail near parking lot inside the Unit (30.72265, −94.224930), 55–70 m, 25 Jun 2014, *Buyck, Lewis & Hofstetter, Buyck 1321/BB14.038* (holotype PC 0142227). GenBank: ITS2 = KX896774; 28S = KX896789; *tef1* = KX857049.

Ecology and distribution: On soil on karst topography in hardwood forests under *Quercus*, *Fagus*, and *Carya*, and on acidic soils in *Pinus*-*Quercus* forests with sequenced specimens confirmed from southeastern states (Texas, Arkansas, Tennessee, Georgia, South Carolina, and West Virginia). Jun to Aug.

Specimens examined: USA. TENNESSEE: Anderson County, Norris Dam State Park, up slope between Clear Creek and Dyer Hollow Trails (36.2123, −84.0738), 305 m, in hardwood leaf litter on soil in oak-hickory forest under *Fagus*, *Quercus*, and *Carya* on karst topo- graphy, 14 Jul 2019, *P.B. Matheny PBM4291* (TENN- F-074853); Warren County, Rock Island State Park (35.8085, −85.6415), 225 m, cespitose on soil, 13 Jul 2017, *R.A. Swenie RAS456* (TENN-F-075070); SOUTH CAROLINA: Oconee County, Oconee State Park, Chestnut Branch Trail (34.872, −83.106), 549 m, scattered singly on acidic soil in *Pinus*-*Quercus* forest, 28 Jul 2021, *P.B. Matheny PBM4602* (TENN-F-075769).

Commentary: We collected both yellow-orange and pink forms (Figure 14D) of *C. velutinus* in hardwood and oak- pine forests in the southern Appalachians. Both color variants have a whitish stipe, weak staining reaction when handled, and medium stature with a stipe longer than or equal to the pileus width. The tomentose to velutinous pileus is distinctive, but this feature was also observed among collections of *C. flavolateritius*. Nevertheless, *C. velutinus* is usually distinguishable from *C. flavolateritius* by the pale orange-yellow to pale pink hymenophore with narrow, blunt, lamellar folds. Collections of *C. velutinus* formed a strongly supported monophyletic group in all phylogenetic analyses, and the multilocus phylogeny suggested that *C. velutinus* is sister to the western U.S. species *C. californicus*.

Cantharellus vicinus Swenie & Matheny, sp. nov.
Figures 14E–F, 15
MycoBank MB843295
Typification: USA. TENNESSEE: Knox County, Knoxville, Post Oak Drive, lawn of private residence (35.75143, −82.27356), 300 m, gregarious under *Quercus falcata* on soil on karst topography, 4 Jul 2019, *R.A. Swenie RAS426* (holotype TENN-F-074935). GenBank: ITS2 = OM654520; 28S = OM654523; *tef1* = OM751842; *rpb2* = OM687942.

Etymology: vicinus (Latin), neighbor, in reference to the occurrence in residential neighborhoods.

Diagnosis: A robust *Quercus*-associated species differing from *Cantharellus iuventateviridis* by sequence divergence, smaller basidiospore size, and pileus color. Differs from *Cantharellus chicagoensis* by sequence divergence and stipe color.

Pileus 20–100 mm wide, convex to plano-convex or shallowly depressed; surface glabrous, sometimes cracking with age; pale yellow (2.5 YR 8/6) to pale yellow-peach, often with a whitish bloom over the center; margin incurved to inrolled and irregularly lobate. Hymenophore with closely spaced folds that appear slightly wrinkled at first, becoming blunt but well-defined folds up to 3 mm deep, often forked and occasionally interconnected; vivid yellow to peach-yellow (10 YR 8/6–8/8), becoming salmon (7.5 YR 7/8), with pinkish or salmon tones upon drying. Stipe 20–60 × 15–50 mm, central, firm, stout, equal or tapering slightly toward the base; white to cream (10 YR 8/3), staining yellow-brown (10 YR 6/6) very slowly after handling or storage. Context white. Odor and taste mild.

Basidiospores (6)6.5–*7.6*–9 × 3.5–*4.3*–*5* µm, Q = 1.5–*1.8*–2.2(2.4), n = 78/4, smooth, elliptic, hyaline. Pileipellis a cutis, terminal hyphae 25–55 × 4– 6 µm, with thickened walls. Basidia 50–84 × 5–8 µm, clavate/undulate with (3)4–6 sterigmata. Cystidia absent. Clamp connections present throughout. Spore print cream-white.

Ecology and distribution: Solitary to gregarious on soil under *Quercus* on karst topography in residential areas and hardwood forests, known only from Tennessee. Jul to Aug.

Additional specimens examined: USA. TENNESSEE: Knox County, Knoxville, Sharp's Ridge Memorial Park (36.005, −83.940), 375 m, hardwood forest with *Quercus* on karst topography, 2 Jul 2020, *R.A. Swenie RAS577* (TENN-F-075382); Knox County, Knoxville, Post Oak Dr, lawn of private residence (35.75143, −82.27356), 300 m, solitary under *Quercus falcata* on karst topography, 9 Jul 2020, *R.A. Swenie RAS586* (TENN-F-075261); ibid., 30 Aug 2020, *R.A. Swenie RAS669* (TENN- F-075383).

Commentary: Cantharellus vicinus is known from residential areas and urban hardwood forests under oak trees in Knoxville, Tennessee. The species formed a well-supported monophyletic group that appeared to be most closely related to two other *Quercus*-associated species from the eastern United States: *C. chicagoensis* Leacock et al. and *C. iuventateviridis* Buyck et al. Neither of these species has been recorded from the southern Appalachian region. *Cantharellus chicagoensis* and *C. iuventateviridis* are known only from the Midwest and Gulf Coast states, respectively. Both species have basidiomata with greenish tones at the pileus margin when immature (Buyck et al. 2016c; Leacock et al. 2016), a feature lacking from *C. vicinus*. Basidiomata of *C. quercophilus*,

another *Quercus*-associated species known only from Gulf Coast states, are much smaller than those of *C. vicinus* and have a brown pileus (Buyck et al. 2010). *Cantharellus vicinus* is a relatively large, stout, fleshy chanterelle that grows slowly and persists through periods of dry weather, which may contribute to the sometimes cracked appearance of the pileus. Other distinctive features include the strongly inrolled pileus margin and hymenophore with crowded folds that turn orange or salmon-colored upon drying.

Cantharellus subg. *Cinnabarinus*

Cantharellus cinnabarinus (Schwein.) Schwein., Trans Am Phil Soc 4:153. 1834.

Typification: USA. TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove, Willie Myers (35.6, −83.8), 530 m, 14 Jul 2004, *Buyck 04.263* (neotype PC 084092). GenBank: *tef1* = GQ914983.

Ecology and distribution: On soil in mixed woods, possibly widespread throughout eastern North America, including reports from Mexico, the Bahamas, Puerto Rico, and observations as far south as Honduras. Jul to Aug.

Commentary: Samples of small red chanterelles analyzed in this study were collected from various localities within the Great Smoky Mountains National Park in North Carolina and Tennessee. *Cantharellus cinnabarinus*, originally described from Pennsylvania, was recently neotypified from Cades Cove on the Tennessee side of the Great Smoky Mountains National Park due to a lack of designated type material (Buyck et al. 2011). However, all samples of small red chanterelles sequenced during this study matched *C. corallinus*, not *C. cinnabarinus*. We therefore suspect that *C. corallinus* is more common than *C. cinnabarinus* in the southern Appalachians. The results of our phylogenetic analysis showed that samples from other studies originally labeled *C. cinnabarinus* (BB 07.053, GRSM77031) formed a clade sister to *C. minor* f. *intensissimus*, with the neotype of *C. cinnabarinus* sister to those two clades. As mentioned by Buyck et al. (2011), more specimens should be analyzed to determine the extent of genetic and species-level diversity within *C. cinnabarinus*. Other species of red chanterelles that may be confused for *C. cinnabarinus* include *C. texensis* Buyck & V. Hofst. in Texas and *C. coccolobae* Buyck, P.-A. Moreau & Courtec. *Cantharellus texensis* is known only from Texas and can be distinguished from *C. cinnabarinus* by longer spores and thin-walled pileipellis hyphae (Buyck et al. 2011). In contrast to *Cantharellus cinnabarinus, Cantharellus coccolobae* has larger spores and grows in association with *Coccoloba* in the Caribbean, Mexico, and southern Florida (Buyck et al. 2016d).

Cantharellus corallinus Buyck & V. Hofst., Cryptog Mycol 37:369. 2016.
Figure 14G

Typification: USA. MISSOURI: St. Louis County, Forest 44, conservation area (8.527267, −90.517017), 150 m, 16 Jul 2011, *1083/Jay Justice MO-Canth-2* (holotype PC 0713846). GenBank: 28S = KX896776; *tef1* = KX857031.

Ecology and distribution: Scattered to gregarious on soil in leaf litter or among mosses in mixed hardwood-conifer forests including *Quercus, Betula, Tsuga, Pinus,* and *Tilia* in the southern Appalachians (Tennessee and North Carolina), Missouri (type), and Florida, with observations from Indiana, Pennsylvania, and New York. Jun to Aug.

Specimens examined: USA. NORTH CAROLINA: Swain County, Great Smoky Mountains National Park, Heintooga Round Bottom Road (35.573, −83.180), 915 m, scattered on soil under *Betula, Quercus,* and *Tsuga*, 7 Jul 2019, *R.A. Swenie RAS445* (TENN- F-074944); Swain County, Great Smoky Mountains National Park, Hyatt Ridge Trail (35.5957, −83.2496), 1480 m, 24 Jul 2020, *R.A. Swenie RAS629* (TENN-F-075397); TENNESSEE: Blount County, Great Smoky Mountains National Park, Schoolhouse Gap Trail (35.636, −83.736), 550 m, on soil under *Tilia, Pinus, Quercus, Betula*, and *Tsuga*, 17 Jul 2019, *R.A. Swenie RAS466*(TENN-F-074950).

Commentary: We obtained sequences of red chanterelle specimens collected from Tennessee and North Carolina and found that several collections assumed to be *C. cinnabarinus* matched sequence data for the holotype of *C. corallinus*, known previously only from Missouri (Buyck et al. 2016a). Whereas the authors of *C. corallinus* noted a mild taste, our collections had a delayed but distinctly peppery taste much like *C. cinnabarinus*. Based on our field observations, the stipe of *C. corallinus* tends to have more white coloration than *C. cinnabarinus*, often with distinct white patches extending from the base of the stipe upward, and the hymenophore of *C. cinnabarinus* is lamellate, whereas *C. corallinus* has a somewhat shallower set of blunt lamellate ridges.

Cantharellus intensissimus (R.H. Petersen) Swenie & Matheny, stat. nov.
Figures 14H, 16
MycoBank MB843297, MBT10006505
Basionym: *Cantharellus minor* Peck f. *intensissima* R. H. Petersen, Nowa Hedwigia 31:21. 1979.

Epitypification: USA. NORTH CAROLINA: Nantahala National Forest, Standing Indian Campground (35.073, −83.529), 1034 m, gregarious to singly on acidic soil under *Tsuga, Pinus strobus, Quercus, Betula,* and *Rhododendron*, 30 Jul 2021, *P.B. Matheny PBM4615* (epitype TENN- F-075782), GenBank: ITS2 = OM964478, 28S = OM654545, *rpb2* = OM6877975; TENNESSEE: Blount County, Great Smoky Mountains National Park, Cades Cove (35.6, −83.82), 540 m, 12 Aug 1975, *R.H. Petersen* (holotype TENN-F-027451 [!]).

Pileus 10–30 mm wide, broadly convex to plane, not perforated but with a depressed center; margin decurved, faintly striate, raised and lobed or scalloped with age, thin; surface dry, dull, smooth; orange throughout or with an orange-yellow margin; flesh thin, salmon-orange, not staining where bruised. Hymenophore lamellate, up to 3 mm deep, decurrent, distant, somewhat thick, narrow, densely anastomosed with interveins at least with age, orange to light orange or yellowish orange. Stipe 25–30 × 3–5 mm, equal, terete or somewhat compressed; dry, smooth; orange or a shade lighter toward or at the base, without any characteristic bruising pattern; flesh very tough, salmon-orange. Odor mild. Taste peppery.

Basidiospores 7–*7.9*–9 × 4–*4.7*–5.5 µm, Q = 1.4–*1.7*– 2, n = 40/2, smooth, elliptic, hyaline. Pileipellis a cutis, hyphae mostly cylindric and 4–10 µm wide, terminal cells with slightly thickened walls or thin-walled. Basidia clavate/undulate, 60–76 × 7–10 µm, with 4– 6(7) sterigmata. Cystidia absent. Clamp connections present throughout. Spore print not observed.

Ecology and distribution: Scattered to gregarious on acidic soils in leaf litter or among mosses in mixed *Tsuga-Pinus* forests including *Quercus* and *Betula* at mid-elevations in the southern Appalachians (Tennessee, North Carolina, South Carolina), also Texas. Jul to Aug.

Additional specimen examined: USA. SOUTH CAROLINA: Oconee County, Cherry Hill Recreation Area (34.932, −83.088), 732 m, gregarious on acidic oil and leaf litter on edge of *Quercus-Pinus* forest, 28 Jul 2021, *P.B. Matheny PBM4601* (TENN-F-075768).

Commentary: We collected an unusual chanterelle resembling a bright orange *Cantharellus cinnabarinus* from acidic soils in mixed hardwood-conifer forests in North Carolina and South Carolina. The coloration of these specimens matched *Cantharellus minor* f. *intensissimus*, but the stature, deep and strongly anastomosing lamellar folds, upturned pileus margin with finely lobed edge, and peppery taste were all reminiscent of *C. cinnabarinus* and unlike *C. minor* Peck. Phylogenetic analysis (Figure 8B) also showed a close affinity with *C. cinnabarinus* rather than *C. minor*. One other sequence labeled *Cantharellus* f. *intensissimus* (BB 07.042) formed a clade with *Cantharellus tabernen- sis* Feib. & Cibula. As noted by the sequence authors, that specimen's identity was first determined based on field habit and color but subsequently was found to be genetically identical to *C. tabernensis* (Buyck et al. 2011). We have identified a variant of *C. tabernensis* with salmon-colored folds (TENN-F-076652; sequence data not shown) that may account for this discrepancy.

The holotype specimen of *C. minor* f. *intensissimus* comprises dozens of basidiomata, all of which are sterile as noted by Eyssartier (2001) and reconfirmed by our examination of the collection. Buyck et al. (2011) noted that the presence of thick-walled cells in the pileipellis of the type contrasted with the large, thin-walled, pileipellis hyphal ends typical of *C. minor*. Indeed, we found the often-thickened walls of the terminal cells of the pileipellis and the stature of the dried fruitbodies of our specimens and the holotype of *C. minor f. intensissimus* more closely matched *C. cinnabarinus* than *C. minor*. Sequences from our collections of *C. minor* f. *intensissimus* formed a well-supported monophyletic group closely related to, but separate from, the neotype of *C. cinnabarinus*. Here, we elevate the *forma intensissimus* to the rank of species, reflecting its unique distinct phylogenetic position and combination of morphological characters. We epitypify the taxon because of the sterile status of the holotype and because the protolog did not provide a complete morphological description, features that fail to allow an unambiguous interpretation of the taxon.

Cantharellus subg. *Parvocantharellus*

Cantharellus appalachiensis R.H. Petersen, Svensk Bot Tidskr 65:402. 1971.
Typification: USA. TENNESSEE: Sevier County, Great Smoky Mountains National Park, LeConte Creek Trail (35.66, −83.45), 22 Jul 1968, *RHP3433* (holotype TENN-F-033519).

Ecology and distribution: On soil under *Quercus* and *Pinus*, and in mixed woods, possibly widely distributed throughout eastern North America. Jun to Aug.

Specimens examined: USA. NORTH CAROLINA: Macon County, Nantahala National Forest, intersection of Glen Falls and Chinkapin Mountain trails (35.033123, −83.235678), 1063 m, gregarious on soil under *Pinus*, 29 Jul 2021, *R.A. Swenie RAS837* (TENN-F-075880); TENNESSEE: Cumberland County, I-40 rest area between exits 324 and 325 (35.9092, −84.878), 549 m, scattered singly on soil under *Quercus*, 3 Aug 2019, *P.B. Matheny PBM4310* (TENN-F-074872); Sevier County, Great Smoky Mountains National Park, Cherokee Orchard, Ogle Place nature trail (53.682852, −83.489795), 675 m, gregarious on soil in mixed forest near creek, 12 Jun 2017, *R.A. Swenie RAS146* (TENN-F-071629).

Commentary: Cantharellus appalachiensis R.H. Petersen appears to be a common and distinctive species easily recognizable in the field by the dull brown stipe and pileus and contrasting buffy yellow lamellate hymenophore. Petersen and Ryvarden (1971) also noted a dull reddish reaction to 15% $FeSO_4$ on the stipe and pileus flesh. The extent of this species' range outside of southern Appalachia remains to be confirmed. According to Lamoureux and Després (2004), the species occurs in Québec, but this has not been confirmed with sequence data. Shao et al. (2011) reported *C. appalachiensis* from China based on 28S sequence data. However, those 28S sequences more likely represent *C. austrosinensis*, a species recently described from China with 28S sequences identical to *C. appalachiensis*. Another brownish chanterelle that may be confused for *C. appalachiensis* in the southern United States is the closely related *C. tabernensis*, described from Mississippi, which has similarly sized spores to *C. appalachiensis* but differs by the brighter orange hymenophore and smaller stipe (Feibelman et al. 1996). *Cantharellus tabernensis* is more commonly encountered than *C. appalachiensis* in the southern Gulf Coast states (Buyck et al. 2010) and has not been reported from the southern Appalachians. The southern species *C. quercophilus*, which closely resembles *C. appalachiensis* morphologically, was described from oak habitat in Texas (Buyck et al. 2010) but has not been reported from the southern Appalachians. Sequences in our phylogeny obtained from GenBank include specimens from Tennessee, Missouri, and Texas; however, *tef1* and *rpb2* sequences across those specimens diverge up to 1.5%, indicating the need for closer examination of this species across its putative broad range.

Cantharellus minor Peck, Ann Rep Reg NY St Mus 23:122. 1872.
Typification: USA. NEW YORK: Greenbush (42.646, −74.444), 570 m, in open woods, Jul (holotype NYSf 1917).

Ecology and distribution: On soil in deciduous or mixed woods, widespread in eastern North America including observations from Mexico. Jun to Sep.

Specimens examined: USA. NORTH CAROLINA: Avery County, Linville, Tanawha Trail off Blue Ridge Parkway (36.1111, −81.8114), 1036 m, on soil in mixed

woods with *Tsuga* and hardwoods, 28 Sep 2020, *R.A. Swenie RAS718* (TENN-F-075543); Macon County, Highlands, Blue Valley Road (35.0064, −83.2156), 760 m, on sandy soil under *Pinus strobus* and *Quercus*, 8 Sep 2018, *P.B. Matheny & R.A. Swenie RAS328* (TENN-F-074544); TENNESSEE: Sevier County, Great Smoky Mountains National Park, Cherokee Orchard, Bullhead Trail (35.6725, −83.49306), 805 m, 16 Jul 2015, *P.B. Matheny PBM4044* (TENN-F-070648).

Commentary: Cantharellus minor is easily recognized in the field by the small slender stature, bright yellow to orange color, and distant lamellate folds. *Cantharellus minor* differs from *C. intensissimus* by the pileipellis with large, thin-walled, hyphal ends (Buyck et al. 2010) and lack of peppery taste. The species has not been studied in a molecular context across its putative broad geographic range, but Buyck et al. (2011) suggested one or more undescribed relatives in North America.

Discussion

This study fills previous gaps on the distribution and diversity of *Cantharellus* from eastern North America and in particular the southern Appalachians. Our multilocus phylogeny provides dense sampling of *Cantharellus* specimens from the southern Appalachians, a region rich in *Cantharellus* diversity. The genus has seen a flurry of recent taxonomic activity in eastern North America (Buyck et al. 2016a; Leacock et al. 2016; Thorn et al. 2020, 2017). Nevertheless, we found strong lines of evidence that support recognition of two new *Cantharellus* species, *C. vicinus*, sp. nov., and *C. intensissimus*, stat. nov. We also recorded range extensions for *C. betularum* and *C. camphoratus* in the United States and *C. altipes* and *C. corallinus* in the southern Appalachians. We recognize a total of 13 species, including one complex, in the southern Appalachians: *C. appalachiensis, C. altipes, C. betularum, C. camphoratus, C. cinnabarinus, C. corallinus, C. flavolateritius, C. intensissimus, C. minor, C. persicinus*, the *C. tenuithrix* complex (including *C. flavus, C. deceptivus*, and *C. phasmatis*), *C. velutinus*, and *C. vicinus*. Although some of these species appear to have broad geographic ranges spanning the eastern United States and southeastern Canada, we made no observations of four *Cantharellus* species described originally from Mexico (Table 2).

A combination of phylogenetic and network analyses show that *C. cibarius* and *C. tenuithrix* comprise complex mixtures of multiple species (referred to here as a "complex"), wherein multiple taxa previously described based on macromorphological differences and/or minor genetic differences at one or two loci comprise single well-supported monophyletic clades in our broader multilocus phylogenies and are not clearly differentiated at multiple loci in network analyses. Network analyses for these complexes (Figures 11, 12) show that these species are poorly distinguished from one another relative to outgroups. However, in the case of the *C. tenuithrix* complex, there is conflict between the topologies of the networks and clustering of taxa at the *tef1* and *rpb2* loci. It is also clear that there are large morphological differences across specimens within each of these complexes based on the constituent species' descriptions and our field observations, and relying on morphology alone can easily mislead species identification.

Our analyses show that a few *Cantharellus* species in eastern North America have broad intercontinental distributions, viz., *C. camphoratus* (North America and Asia) and *C. cibarius* (North America, Europe, and Asia). Although *C. cibarius* was previously reported from Asia (Shao et al. 2021), our results support an even broader Holarctic distribution for the species including eastern and western North America. Still, species comprising the *C. cibarius* complex and *C. tenuithrix* complex require additional multilocus sampling from a wide geographic range to better evaluate species boundaries. Many sampling gaps remain across the north temperate zone, even across northern North America. Multilocus sequence data of specimens from these areas will paint a more complete picture of *Cantharellus* distributions within and across continents.

Species-level recognition of *Cantharellus* species may be challenging in the field due to large intraspecific variation in macromorphology within some species (e.g., *C. velutinus* in Buyck et al. 2016a), as well as convergent evolution resulting in morphologically similar yet molecularly divergent taxa (e.g., *C. corallinus* and *C. cinnabarinus*). We observed that several other species found in the southern Appalachians, such as *C. altipes* and *C. flavolateritius*, also display these trends. There are, however, several easily recognizable species with a unique combination of macroscopic traits, such as *C. persicinus* with the contrasting pink hymenophore and slender stature; still others are recognizable by their distinct habitat preferences in the southeastern United States (*C. camphoratus, C. betularum*). Furthermore, *C. corallinus* differs slightly from *C. cinnabarinus* by macromorphology and with practice the two can usually be distinguished in the field. In our experience, the two members of subgenus *Parvocantharellus* found in the southern Appalachians, *C. minor* and *C. appalachiensis*, are also common and easily identified in the field by their stature and coloration, although Buyck et al. (2011) suggested that each species may have one or more undescribed relatives. Field identification of *Cantharellus* species requires close study of macroscopic and microscopic characteristics and habitat information to narrow the potential list of candidate species for identification, but often DNA barcoding of *tef1* and/or *rpb2* is required for reliable identification.

Furthermore, we emphasize the importance of producing DNA sequences from at least three unlinked loci for phylogenetic analysis and new species discovery in *Cantharellus*, as a single gene can provide less than adequate results at the species level. Here, we used 28S, *tef1*, and *rpb2* in our phylogenetic analyses since these loci are commonly employed in large-scale studies of *Cantharellus* (Buyck et al. 2013; Olariaga et al. 2017; Zhang et al. 2021). The 28S locus, however, generally lacks the necessary signal to distinguish *Cantharellus* species. Buyck and Hofstetter (2011) suggested that *tef1* is the most appropriate locus to differentiate terminal taxa in *Cantharellus*, but our results show that *tef1* alone does not reliably distinguish some species, such as those in the *C. cibarius* complex and *C. tenuithrix* complex (Figures 11, 12).

Due to reticulate gene histories and ancestral polymorphisms—exemplified here in the clades containing *C. camphoratus, C. cibarius* complex, and *C. tenuithrix* complex—a species may match samples of another species at one locus while diverging at other loci. Additionally, nucleotide polymorphisms occur regularly in both nuclear ribosomal and protein-coding loci, which can increase intraspecific sequence variability.

For these reasons, sequence divergence between specimens at a single protein-coding locus is often insufficient evidence for a new species.

Our phylogenies suggest that reevaluation of genetic relationships is necessary across many parts of the *Cantharellus* tree of life going forward. Unresolved potential synonymies (e.g., *C. altipes* and *C. septentrionalis* A.H. Sm. & Morse) can be ameliorated with further type sequencing and multilocus sequencing of additional collections. Relying on *tef1* as a barcode locus can mislead *Cantharellus* species identification, and a multilocus sequencing approach is necessary to help clarify species concepts and prevent future redescription of existing species.

Key to *Cantharellus* in the southern Appalachians

1. Basidiomata small, pileus ≤30 mm wide, stipe ≤5 mm thick; bright pink, red, or orange, taste peppery 2
1'. Basidiomata small or larger than above, some shade of yellow, yellow-orange, or brown; if small, then taste not peppery 4

2. Basidiomata orange ..... *C. intensissimus*
2'. Basidiomata pink or red 3

3. Stipe with patchy white areas, hymenophore some- what shallow with blunt lamellate ridges ..... *C. corallinus*
3'. Stipe lacking patchy white areas, hymenophore lamellate ..... *C. cinnabarinus*

4. Basidiomata small, pileus ≤30 mm wide, stipe ≤6 mm thick 5
4.' Basidiomata larger than above 6

5. Pileus yellow to yellow-orange, hymenophore light yellow to yellow, spores 7.5–10 × 4.5–6 μm ..... *C. minor*
5'. Pileus ochraceous buff to yellowish or yellow-orange, hymenophore pink or ochraceous buff, spores 9.5–12(14) × 5–7 μm ..... *C. persicinus*

6. Pileus with brown tones 7
6'. Pileus yellow to orange throughout, lacking brown tones 10

7. Stipe brown; average spore length <9 μm ..... *C. appalachiensis*
7'. Stipe cream, yellow, or orange; average spore length ≥ 9 μm 8

8. In conifer forests under *Abies* and *Picea*, spores 7.7– *9.1*–11.2(12.1) × 3.9–*5.0*–6.7 μm ..... *C. camphoratus*
8'. In hardwood or mixed forests under *Betula, Fagus,* and/or *Quercus,* average spore size longer than above 9

9. In northern hardwood forests under *Fagus, Betula*, and/or *Quercus*, stipe longer than pileus width at maturity, spores 7.3–*9.4*–12 × 4.4–*4.9*–5.7 µm..... *C. altipes*
9’. In northern hardwood or spruce-fir forests under *Betula*, stipe shorter or longer than pileus width at maturity, spores (7.7)8.7–*10.4*–14.3 × (3.9)4.6–*5.6*– 7.1(7.7) µm..... ..... *C. betularum*

10. Hymenophore mostly lacking distinct lamellar folds, partially to fully smooth .... *C. flavolateritius*
10’. Hymenophore with distinct lamellar folds 11

11. Lamellar folds pink or ochraceous buff when fresh 12
11’. Lamellar folds white or yellow when fresh 13

12. Pileus and stipe ochraceous buff to yellowish or yellow-orange, habit slender, stipe ≤5 mm wide ..... *C. persicinus*
12’. Pileus ochraceous salmon to pink, habit more robust than above, stipe >5 mm wide .....*C. velutinus* (pink form)

13. Lamellar folds turning dark orange or salmon-colored upon drying, pileus margin thick, basidiomata stout, in residential areas or urban forests under *Quercus*..... *C. vicinus*
13’. Lamellar folds not turning dark orange or salmon-colored upon drying, pileus margin thin, basidiomata stout or slender, under deciduous or coniferous trees 14

14. Pileus velutinous to hairy pubescent, yellow to orange-yellow, whitish bloom absent, lamellar folds pale orange-yellow, flesh staining weakly or strongly when handled, stipe white to buff, in deciduous forests.....*C. velutinus* (yellow-orange form)
14’. Pileus smooth, yellow, whitish bloom sometimes present, lamellar folds whitish or yellow, flesh staining or not when handled, stipe white to yellow, in deciduous or mixed forests..... *C. tenuithrix* complex

Acknowledgements

We thank Paul Super and the Great Smoky Mountains National Park for collection permit GRSM-2020-SCI-2452. We also thank Greg Thorn (UWO) and Carrie LaChance for providing specimens. We acknowledge Noah Walker and Django Grootmyers for laboratory assistance and Mary Grace Graddy for assisting with GenBank sequence submission. We thank Greg Thorn and two anonymous reviewers for their comments and suggestions on earlier versions of the manuscript.

This research was supported by the TENN Herbarium Hesler Fund Research Award and Breedlove, Dennis Fund Award to R.A.S., as well as a National Science Foundation Graduate Research Fellowship under grant no. 1452154 to R.A.S. and National Science Foundation DEB 2030779 to P.B.M.

REFERENCES

Aime MC, McTaggart AR. 2021. A higher-rank classification for rust fungi, with notes on genera. Fungal Systematics and Evolution. 7(1):21–47.

Arora D, Dunham SM. 2008. A new, commercially valuable chanterelle species, *Cantharellus californicus* sp. nov., associated with live oak in California, USA. Econ Bot. 62 (3):376–391.

Brassard GR. 1984. The Bryogeographical isolation of the Island of Newfoundland, Canada. Bryologist. 87(1):56–65.

Buyck B, Cruaud C, Couloux A, Hofstetter V. 2011. Cantharellus texensis sp. nov. from Texas, a southern lookalike of *C. cinnabarinus* revealed by tef-1 sequence data. Mycologia. 103(5):1037–1046.

Buyck B, Hofstetter V. 2011. The contribution of tef-1 sequences to species delimitation in the *Cantharellus cibarius* complex in the southeastern USA. Fungal Divers. 49(1):35–46.

Buyck B, Hofstetter V, Olariaga I. 2016b. Setting the record straight on North American *Cantharellus*. Cryptogam Mycol. 37(3):405–417.

Buyck B, Kauff F, Eyssartier G, Couloux A, Hofstetter V. 2013. A multilocus phylogeny for worldwide *Cantharellus* (Cantharellales, Agaricomycetidae). Fungal Divers. 64 (1):101–121.

Buyck B, Lewis DP, Eyssartier G, Hofstetter V. 2010. *Cantharellus quercophilus* sp. nov. and its comparison to other small, yellow or brown American chanterelles. Cryptogam Mycol. 31:17–33.

Buyck B, Moreau PA, Courtecuisse R, Kong A, Roy M, Hofstetter V. 2016d. *Cantharellus coccolobae* sp. nov. and *Cantharellus garnieri*, two tropical members of *Cantharellus* subg. *Cinnabarinus*. Cryptogamie Mycologie. 37(3):391–403.

Buyck B, Olariaga I, Justice J, Lewis D, Roody W, Hofstetter V. 2016a. The dilemma of species recognition in the field when sequence data are not in phase with phenotypic variability. Cryptogam Mycol. 37(3):367–389.

Buyck B, Olariaga I, Looney B, Justice J, Hofstetter V. 2016c. Wisconsin Chanterelles revisited and first indications for very wide distributions of *Cantharellus* species in the United States East of the Rocky Mountains. Cryptogam Mycol. 37(3):345–366.

Cripps CL, Eberhardt U, Schütz N, Beker HJ, Evenson VS, Horak E. 2019. The genus *Hebeloma* in the rocky mountain alpine zone. MycoKeys. 46:1–54.

Cubeta MA, Echandi E, Abernathy T, Vilgalys R. 1991. Characterization of Anastomosis groups of Binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA Gene. Phytopathology. 81(11):1395–1400.

Dunham SM, O'Dell TE, Molina R. 2003. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. Mycol Res. 107(10):1163–1177.

Eyssartier G 2001. Vers une monographie du genre *Cantharellus* Adans.Fr. [doctoral dissertation]. Paris (France): National History Museum.

Eyssartier G, Buyck B. 2000. Le genre *Cantharellus* en Europe. Nomenclature et taxonomie. Bull. Soc. Mycol. France. 116:91–137.

Feibelman T, Bayman P, Cibula WG. 1994. Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. Mycol Res. 98(6):614–618.

Feibelman TP, Bennett JW, Cibula WG. 1996. *Cantharellus tabernensis*: a new species from the southeastern United States. Mycologia. 88(2):295–301.

Foltz MJ, Perez KE, Volk TJ. 2013. Molecular phylogeny and morphology reveal three new species of *Cantharellus* within 20 m of one another in western Wisconsin, USA. Mycologia. 105(2):447–461.

Gazis R, Skaltsas D, Chaverri P. 2014. Novel endophytic lineages of Tolypocladium provide new insights into the ecology and evolution of *Cordyceps*-like fungi. Mycologia. 106(6):1090–1110.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol. 23(2):254–67.

Lamoureux Y, Després J 2004. Clé des chanterelles du Québec. Poster. Éd. Cercle des mycologues de Montréal.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting parti- tioned models of evolution for molecular and morphologi- cal phylogenetic analyses. Mol Biol Evol. 34(3):772–773.

Largent DL, Johnson D, Watling R. 1977. How to identify mushrooms to genus III: microscopic features. Eureka (CA): Mad River Press Inc.

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics. 30 (22):3276–3278.

Leacock PR, Riddell J, Wilson AW, Zhang R, Ning C, Mueller GM. 2016. *Cantharellus chicagoensis* sp. nov. is supported by molecular and morphological analysis as a new yellow chanterelle in midwestern United States. Mycologia. 108(4):765–772.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Mol Phylogenet Evol. 35(1):1–20.

Moncalvo J-M, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, Porter TM, Margaritescu S, Weiss M, Garnica S, et al. 2006. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia. 98(6):937–948.

Montoya L, Herrera M, Bandala VM, Ramos A. 2021. Two new species and a new record of yellow *Cantharellus* from tropical *Quercus* forests in eastern Mexico with the proposal of a new name for the replacement of *Craterellus confluens*. MycoKeys. 80:91–114.

Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, Longcore JE. 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol. 12(2):395–403.

Ogawa W, Endo N, Fukuda M, Yamada A. 2018. Phylogenetic analyses of Japanese golden chanterelles and a new species description, *Cantharellus anzutake* sp. nov. Mycoscience. 59(2):153–165.

Olariaga I, Buyck B, Esteve-Raventós F, Hofstetter V, Manjón JL, Moreno G, Salcedo I. 2015. Assessing the taxonomic identity of white and orange specimens of *Cantharellus*: occasional colour variants or independent species? Cryptogam Mycol. 36(3):287–300.

Olariaga I, Moreno G, Manjón JL, Salcedo I, Hofstetter V, Rodríguez D, Buyck B. 2017. *Cantharellus* (Cantharellales, Basidiomycota) revisited in Europe through a multigene phylogeny. Fungal Divers. 83(1):263–292.

Orihara T, Healy R, Corrales A, Smith ME. 2021. Multilocus phylogenies reveal three new truffle-like taxa and the traces of interspecific hybridization in *Octaviania* (Boletaceae, Boletales). *Boletaceae, Boletales* IMA Fungus. 12(1):1–22.

Petersen RH. 1976. Notes on cantharelloid fungi. VII. The taxa described by Charles H. Peck. Mycologia. 68(2):304–326.

Petersen RH. 1979. Notes on cantharelloid fungi. IX. Illustrations of new or poorly understood taxa. Nova Hedwigia. 31:1–23.

Petersen RH. 1986. Notes on clavarioid fungi. XIX. Color illustrations of selected taxa, with comments on *Cantharellus*. Nowa Hedwigia. 42:151–160.

Petersen RH, Ryvarden L. 1971. Notes on cantharelloid fungi. IV. Two new species of *Cantharellus*. Svensk Bot Tidskr. 65:399–405.

Pilz D, Norvell L, Danell E, Molina R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. Portland (OR): US Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Redhead S. 2012. Nomenclatural novelties. Index Fungorum. 5:1. 2049–2375.

Redhead SA, Norvell LL, Danell E. 1997. *Cantharellus formosus* and the Pacific golden chanterelle harvest in western North America. Mycotaxon. 65:285–322.

Rehner S, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia. 97(1):84–98.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61(3):539–542.

Shao SC, Liu PG, Wei TZ, Herrera M. 2021. New insights into the taxonomy of the genus *Cantharellus* in China: epityfication of *C. yunnanensis* W.F. Chiu and the first record of *C. cibarius* Fr. Cryptogam Mycol. 42(3):25–37.

Shao SC, Tian XF, Liu PG. 2011. *Cantharellus* in southwestern China: a new species and a new record. Mycotaxon. 116:437–446.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.

Thiers BM. [continuously updated]. Index Herbariorum. https://sweetgum.nybg.org/science/ih/

Thorn RG, Banwell A, Kim JI, Lebeuf R, Voitk A. 2020. *Cantharellus betularum*. Persoonia. 45:330–331.

Thorn RG, Banwell A, Pham TH, Vidal NP, Manful CF, Nadeem M, Ivanov AG, Szyszka Mroz B, Bonneville MB, Hüner NPA, et al. 2021. Identification and analyses of

the chemical composition of a naturally occurring albino mutant chanterelle. Sci Rep. 11(1):1–15.

Thorn RG, Kim JI, Lebeuf R, Voitk A. 2017. The golden chanterelles of Newfoundland and Labrador: a new species, a new record for North America, and a lost species rediscovered. Botany. 95(6):547–560.

Tremblay NO, Schoen DJ. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Mol Ecol. 8(7):1187–1198.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 172(8):4238–4246.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press, Inc; p. 315–322.

Wilson AW, Aime MC, Dierks J, Mueller GM, Henkel TW. 2012. Cantharellaceae of Guyana I: new species, combinations and distribution records of *Craterellus* and a synopsis of known taxa. Mycologia. 104(6):1466–1477.

Zhang M, Wang C, Buyck B, Deng W, Li T. 2021. Multigene phylogeny and morphology reveal unexpectedly high number of new species of *Cantharellus* subgenus *Parvocantharellus*. J Fungi. 7(11):919.

APPENDIX

Table 2. List of *Cantharellus* species within eastern North America, Mexico, and the Caribbean.

Species	Authority	Type locality	Eastern US and/or Canada	Southern Appalachians	Mexico	Caribbean
altipes	Buyck & V. Hofst.	USA-Texas	✓	✓		
appalachiensis	R.H. Petersen	USA-Tennessee	✓	✓		
betularum	Voitk & Thorn	Canada-Newfoundland and Labrador	✓	✓		
camphoratus	R.H. Petersen	Canada-Nova Scotia	✓	✓		
chicagoensis	Leacock, J. Riddell, Rui Zhang & G.M. Muell.	USA-Illinois	✓			
cibarius	Fr.	Sweden	✓			
cinnabarinus	(Schwein.) Schwein.	USA-Pennsylvania	✓	✓		
coccolobae	Buyck, P.-A. Moreau & Courtec.	Guadeloupe	✓			✓
corallinus	Buyck & V. Hofst.	USA-Missouri	✓	✓		
deceptivus	Buyck, Justice & V. Hofst.	USA-Wisconsin	✓			
enelensis	Voitk, Thorn, Lebeuf & J.I. Kim	Canada-Newfoundland and Labrador	✓			
flavolateritius	Buyck & V. Hofst.	USA-North Carolina	✓	✓		
flavus	Foltz & T.J. Volk	USA-Wisconsin	✓			
furcatus	Bandala, Montoya & Ramos	Mexico			✓	
intensissimus	(R.H. Petersen) Swenie & Matheny	USA-North Carolina	✓	✓		
iuventateviridis	Buyck, Looney, Harsch & V. Hofst.	USA-Mississippi	✓			
lateritius	(Berk.) Singer	USA-North Carolina	✓			
lewisii	Buyck & V. Hofst.	USA-Texas	✓			
minor	Peck	USA-New York	✓	✓		
parvoflavus	M. Herrera, Bandala & Montoya	Mexico			✓	
persicinus (=C. spectaculus)	R.H. Petersen	USA-Tennessee	✓	✓		
phasmatis	Foltz & T.J. Volk	USA-Wisconsin	✓			
quercophilus	Buyck, D.P. Lewis, Eyssart. & V. Hofst.	USA-Texas	✓			
septentrionalis	A.H. Sm.	USA-Michigan	✓			
tabernensis	Feib. & Cibula	USA-Mississippi	✓			
tenuithrix	Buyck & V. Hofst.	USA-Texas	✓	✓		
texensis	Buyck & V. Hofst.	USA-Texas	✓			
velutinus	Buyck & V. Hofst.	USA-Texas	✓	✓		
veraecrucis	Bandala, Montoya & M. Herrera	Mexico			✓	
vicinus	Swenie & Matheny	USA-Tennessee	✓	✓		
violaceovinosus	M. Herrera, V.M. Bandala & L. Montoya	Mexico			✓	

Table 3. Specimen data used in this study. New sequences produced for this study are in bold.

Species	Labeled name, if different	Type status	Field number	Locality	LSU	tef1	rpb2	ITS
afrocibarius		Holotype	BB96.235	Zambia	KF294668	JX192993	KF294746	
alborufescens			AH44783	France	KR677530	KX828817	KX828736	
alborufescens			BB12.075	Italy	KX929161	KX907243	KX907232	
albovenosus		Holotype	1690	South Korea		KY271942		
albus		Holotype	SPJian615	China	MT782540	MT776015	MT776012	
altipes			RAS084	USA-Virginia	MF797657		**OM687954**	
altipes	*amethysteus*		RAS639	USA-Tennessee		**OM751854**	**OM687955**	
altipes		Holotype	BB07.112	USA-Texas		GQ914941		

Table 3. Continued.

altipes			BB07.019	USA-Texas	KF294627	GQ914939	KF294702	
amethysteus		Neotype	AH44796	Spain	KR677550	KX828819	KX828738	
amethysteus			Estades10.454	France	KX907214	KX907237	KX907226	
amethysteus			BB07.284	Slovakia	KF294639	GQ914953	KF294716	
amethysteus			EC09.29	Italy	KX907220	KX907241	KX907230	
anzutake		Holotype	C84	Japan	LC085415	LC179800		
appalachiensis			JJ-MO3	USA-Missouri	KX857090	KX857032	KX856994	
appalachiensis			BB07.123	USA-Texas	KF294635	GQ914979	KF294711	
appalachiensis			GRSM77088	USA-Tennessee	DQ898690		DQ898748	
aurantinus		Holotype	GDGM46278	China	MZ766517	MZ766560		
austrosinensis		Holotype	GDGM81249	China	MZ605082	MZ613983	MZ614027	
betularum		Holotype	130914av01	Canada-Newfoundland and Labrador	KX592700			
betularum		Paratype	121003av01	Canada-Newfoundland and Labrador	KX592694	KX592693		
betularum		Paratype	110812av01	Canada-Newfoundland and Labrador		**OM751852**	**OM687978**	
betularum		Paratype	170930av01	Canada-Newfoundland and Labrador	**OM654531**	**OM751853**	**OM687979**	
betularum	*camphoratus*		RAS631	USA-North Carolina	**OM654532**		**OM687953**	**OM654522**
californicus		Holotype	OSC122878	USA-California	KX828795	KX828820	KX828739	
camphoratus			PBM4274	USA-North Carolina		**OM751845**	**OM687946**	
camphoratus			RAS614	USA-North Carolina	**OM654527**	**OM751846**	**OM687947**	**OM654521**
camphoratus			RAS659	USA-North Carolina	**OM654528**		**OM687948**	
camphoratus			RAS661	USA-North Carolina	**OM654529**		**OM687949**	
camphoratus			180913av07	Canada-Newfoundland and Labrador		**OM751848**	**OM687981**	
camphoratus			120906av01	Canada-Newfoundland and Labrador	KX592734	KX592735		
camphoratus			120903av17	Canada-Newfoundland and Labrador		**OM751847**	**OM687980**	
camphoratus			120922av02	Canada-Newfoundland and Labrador	KX592737	KX592738		

Table 3. Continued.

camphoratus		Holotype	TENN38025	Canada-Nova Scotia	KX896788			
camphoratus			RAS913	USA-New York			**OM687950**	
camphoratus "JPN2"			C106	Japan	LC085418	LC085473		
camphoratus "JPN2"			C88	Japan		LC085472		
cascadensis		Holotype	OSC75975	USA-Oregon	AY041163			
chicagoensis		Holotype	PRL8332	USA-Illinois	KP639214	KP639233		
cibarius			GE07.025	France	KF294658	GQ914949	KF294736	
cibarius			AH44780	Spain	KR677546	KX828821	KX828740	
cibarius			WXH2580	China		KM893847		
cibarius			CLC2018-2	USA-Connecticut	**OM654547**		**OM687977**	
cibarius		Epitype	BIOFungi10986	Sweden	KR677539	KX828823	KX828742	
cinnabarinus		Neotype	BB04.263	USA-Tennessee		GQ914983		
cinnabarinus			BB07.053	USA-Texas	KF294630	GQ914984	KF294705	
cinnabarinus			GRSM77031	USA-Tennessee	DQ898692		DQ898747	
citrinus		Holotype	1691	South Korea		MW124385		
coccolobae		Holotype	RC11-025	Guadeloupe	KX857089	KX857021	KX856993	
congolensis			ADK5214	Democratic Republic of Congo		KX834368	KX834424	
congolensis			BB98.058	Tanzania	KF294673	JX192996		
corallinus		Holotype	JJ-MO2	USA-Missouri	KX896776	KX857031		
corallinus	*cinnabarinus*		RAS445	USA-North Carolina		**OM751849**		
corallinus	*cinnabarinus*		RAS466	USA-Tennessee		**OM751850**	**OM687951**	
corallinus	*cinnabarinus*		RAS629	USA-North Carolina	**OM654530**	**OM751851**	**OM687952**	
curvatus		Holotype	1695	South Korea		MW124390		
cyphelloides		Holotype	TNS-F-61721	Japan	LC047741			
deceptivus		Holotype	JJ13-WI1	USA-Wisconsin	KX896779	KX857026		
deceptivus	*cibarius*		MGW1162	USA-Tennessee	MF797656			
deceptivus	*pallens*		RAS056	USA-Tennessee	MF797652	**OM751861**	**OM687963**	
deceptivus			RAS447	USA-Tennessee		**OM751855**	**OM687956**	
diminutivus			DS06.033	Malaysia	KF294661		KF294740	
enelensis			AFTOL-607	Germany		DQ059050	DQ366285	

Table 3. Continued.

enelensis	*sp.*		RAS859	USA-New York	**OM654546**	**OM751872**	**OM687976**	
enelensis		Isotype	130821av02	Canada-Newfoundland and Labrador	KX592712			
enelensis			VilneffE5	Canada-Newfoundland and Labrador	KX592719	KX592720		
ferruginascens			AH44782	France	KR677526	KX828826	KX828747	
ferruginascens			ICH453F	Iran	MH463326	MH463328	MH463327	
fibrillosus		Holotype	PUN3957	India	HM750917			
fistulosus		Isotype	DT43	Tanzania	KF294674	JX192997		
flavolateritius		Holotype	JJ-NC4	USA-North Carolina		KX857029		
flavolateritius			JJ-NC2	USA-North Carolina	KX896783	KX857027		
flavolateritius	*velutinus*		PBM4264	USA-Tennessee	**OM654540**			
flavolateritius			RAS463	USA-Tennessee	**OM654536**	**OM751864**	**OM687966**	
flavolateritius			RAS474	USA-Tennessee	**OM654537**	**OM751865**	**OM687967**	
flavolateritius			RAS588	USA-Tennessee	**OM654539**	**OM751867**		
flavolateritius			RAS590	USA-Tennessee	**OM654538**	**OM751866**	**OM687968**	
flavus		Holotype	C066	USA-Wisconsin	JX030437			
flavus			C067	USA-Wisconsin		JX030416		
flavus	*cibarius*		MGW1115	USA-Tennessee	MF797653	**OM751859**	**OM687960**	
flavus	*deceptivus*		RAS464	USA-Tennessee		**OM751857**	**OM687958**	
flavus	*deceptivus*		RAS473	USA-Tennessee	**OM654533**	**OM751858**	**OM687959**	
formosus			SAR220712	Canada-British Columbia	KR677553	KX828830	KX828751	
formosus			SAT1329820	USA-Oregon		KX592751		
friesii			GE07.077	France	KF294659		KF294737	
furcatus		Holotype	Botteri6	Mexico	MT371345			
galbanus		Holotype	GDGM86249	China	MZ766516	MZ766568	MZ766577	
guyanensis			1517	Guyana	KX857095	KX857061	KX856999	
hainanensis		Holotype	FHMU1931	China	KY407524	KY407536		
intensissimus stat. nov.			PBM4601	USA-South Carolina	**OM654544**		**OM687974**	
intensissimus stat. nov.		Epitype	PBM4615	USA-North Carolina	**OM654545**		**OM687975**	**OM964478**
intensissimus stat. nov.	*cinnabarinus*		BB07.001	USA-Texas	KF294624	GQ914985	KF294698	
iuventateviridis		Holotype	BPL523	USA-Mississippi	KX896780	KX857047		

Table 3. Continued.

koreanus		Holotype	1696	South Korea		MW124391		
laevihymenius		Holotype	Yuan13902	China	MW979521	MW999419	MW999454	
lateritius		Epitype	BB07.025	USA-Texas	KF294628	GQ914957	KF294703	
lewisii		Holotype	BB07.003	USA-Texas	JN940597		KF294700	
lilacinopruinatus			BB07.221	Slovakia	KF294637	GQ914951	KF294714	
luteolus		Holotype	GDGM60393	China	MZ766515	MZ766566	MZ766575	
luteovirens		Holotype	GDGM80672	China	MZ605090	MZ613992	MZ614035	
macrocarpus		Holotype	Zeng4050	China	MT986061	MT990634		
minioalbus		Holotype	GDGM78901	China	MZ605098	MZ613999	MZ614043	
minor			BB07.057	USA-Texas	KF294632	JX192979	KF294707	
minor			BB07.002	USA-Texas	KF294625	JX192978	KF294699	
pallens			AH39124	Morocco	KX828804	KX828833	KX828755	
pallens			BB09.409	Italy	KX907215	KX857014	KX929160	
parvoflavus		Holotype	Montoya5423	Mexico	MT371337	MT449706		
persicinus			JJ-MO4	USA-Missouri	KX896790	KX857033		
persicinus			MH15001	USA-North Carolina	KX896791	KX857080		
persicinus	*spectaculus*		PBM3933	USA-Tennessee	**OM654534**	**OM751860**	**OM687961**	
persicinus			RAS468	USA-Tennessee	**OM654543**	**OM751871**	**OM687972**	
persicinus			RAS841	USA-North Carolina			**OM687973**	
persicinus	*spectaculus*		SAT1519702	USA-North Carolina	MF797701		**OM687962**	
phasmatis		Holotype	C073	USA-Wisconsin	JX030426			
phasmatis			C074	USA-Wisconsin	JX030438	JX030418		
phloginus		Holotype	SSC98	South Korea	KF801100	KF801095		
pseudoformosus		Holotype	SMR2009a	India	GU237071			
quercophilus		Holotype	BB07.097	USA-Texas	KF294644	JX192981	KF294721	
romagnesianus			AH44218	Spain	KX828807			
romagnesianus			GE07.031	France			KF294738	
roseocanus		Holotype	DAOM220723	Canada-British Columbia	KX828810	KX828837	KX828758	
roseofagetorum			AH44789	Georgia	KX828812	KX828839	KX828760	

Table 3. Continued.

septentrionalis		Holotype	Smith67052	USA-Michigan	KX896785			
sinominor		Holotype	GDGM80842	China	MZ605107	MZ614006	MZ614050	
spectaculus		Holotype	C081	USA-Wisconsin	JX030421	JX030414		
subalbidus			OSC81782	USA-Oregon	KX828814	KX828841	KX828762	
subalbidus			BB13.014B	USA	KX896782	KX857038		
subamethysteus		Isotype	DS06.218	Malaysia	KF294664		KF294742	
subcyanoxanthus		Holotype	BB00.1137	Madagascar		JX192990	KF294734	
subminor		Holotype	Yuan13917	China	MW979522	MW999415	MW999455	
tabernensis			BB07.056	USA-Texas		GQ914974	KF294706	
tabernensis			BB07.119	USA-Texas	KF294634	GQ914976	KF294709	
tabernensis	*minor* f. *intensissimus*		BB07.042	USA-Texas		GQ914973		
tenuithrix		Holotype	BB07.125	USA-Texas	JN940600	GQ914947	KF294712	
tenuithrix	*deceptivus*		PBM4268	USA-Tennessee		**OM751862**	**OM687964**	
tenuithrix	*deceptivus*		PBM4292	USA-Tennessee		**OM751856**	**OM687957**	
tenuithrix			RAS584	USA-North Carolina	**OM654535**	**OM751863**	**OM687965**	
texensis		Holotype	BB07.018	USA-Texas	KF294626	GQ914988	KF294701	
vaginatus		Holotype	Hosaka07193	China	HM594681			
velutinus		Holotype	BB14.038	USA-Texas	KX896789	KX857049		
velutinus	*sp.*		RAS456	USA-Tennessee		**OM751868**	**OM687969**	
velutinus			PBM4291	USA-Tennessee	**OM654541**	**OM751869**	**OM687970**	
velutinus "pink"			PBM4602	USA-South Carolina	**OM654542**	**OM751870**	**OM687971**	
veraecrucis		Holotype	Bandala4505	Mexico	MT371344	MT449712		
versicolor			Tian160	China		KM893856		
vicinus sp. nov.	*deceptivus*	Holotype	RAS426	USA-Tennessee	**OM654523**	**OM751842**	**OM687942**	**OM654520**
vicinus sp. nov.			RAS577	USA-Tennessee	**OM654524**	**OM751843**	**OM687943**	
vicinus sp. nov.			RAS586	USA-Tennessee	**OM654525**	**OM751844**	**OM687944**	
vicinus sp. nov.			RAS669	USA-Tennessee	**OM654526**		**OM687945**	
violaceovinosus		Holotype	Corona648	Mexico	MF616525	MF616521		
yunnanensis		Epitype	XieXD174	China	KU720333	KU720337		
zangii			GDGM83171	China	MZ605113	MZ614012	MZ614056	
zangii			GDGM83228	China	MZ605119	MZ614018	MZ614062	

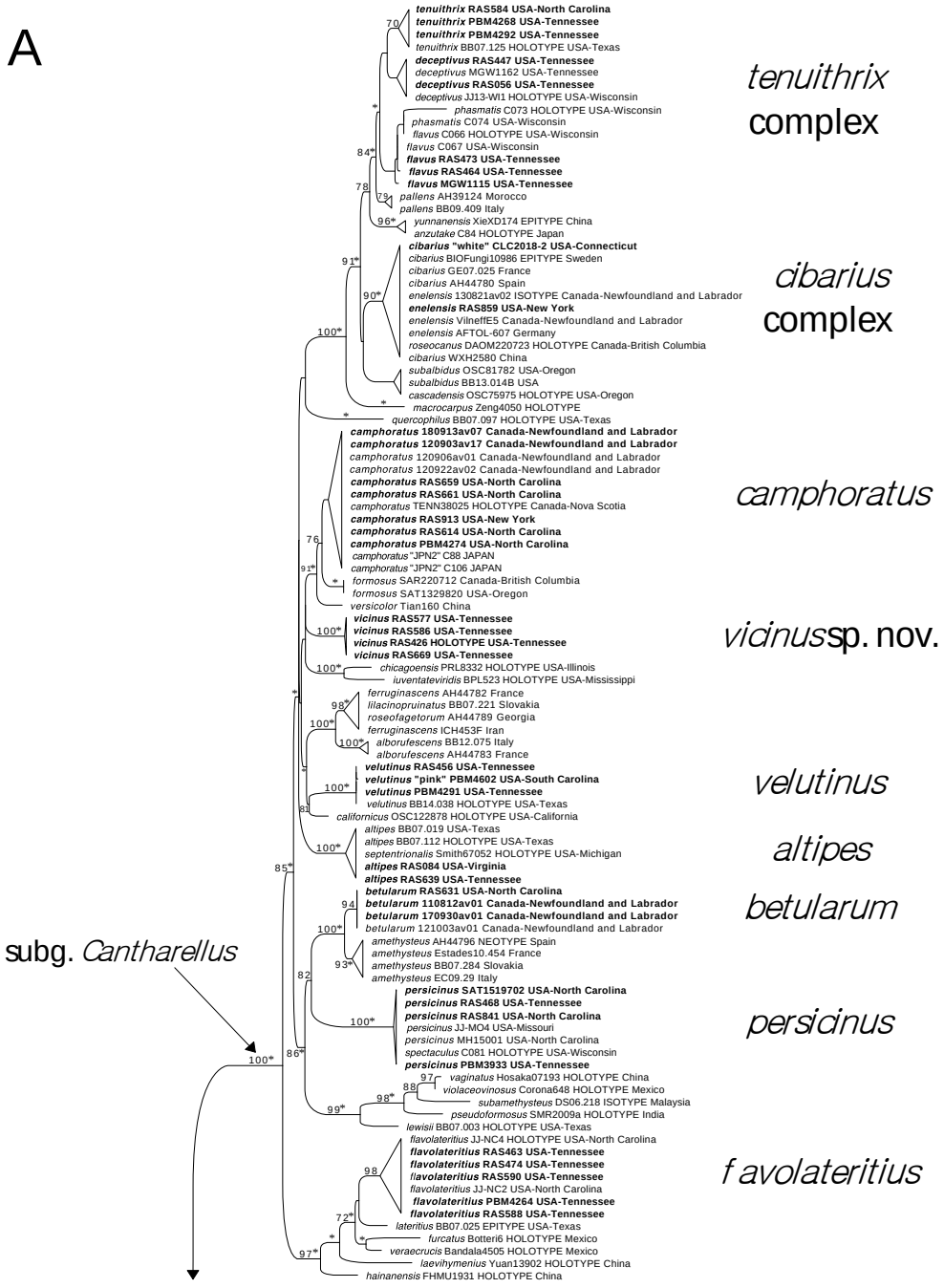

Figure 8. Multilocus (28S + *tef1* + *rpb2*) phylogeny of *Cantharellus* with pruned outgroup. A. Subgenus *Cantharellus*. B. Subgenera *Parvocantharellus* and *Cinnabarinus*. Sequences produced for this study are in bold. Bootstrap values ≥70 are shown on branches; an asterisk (*) indicates posterior probability ≥0.95.

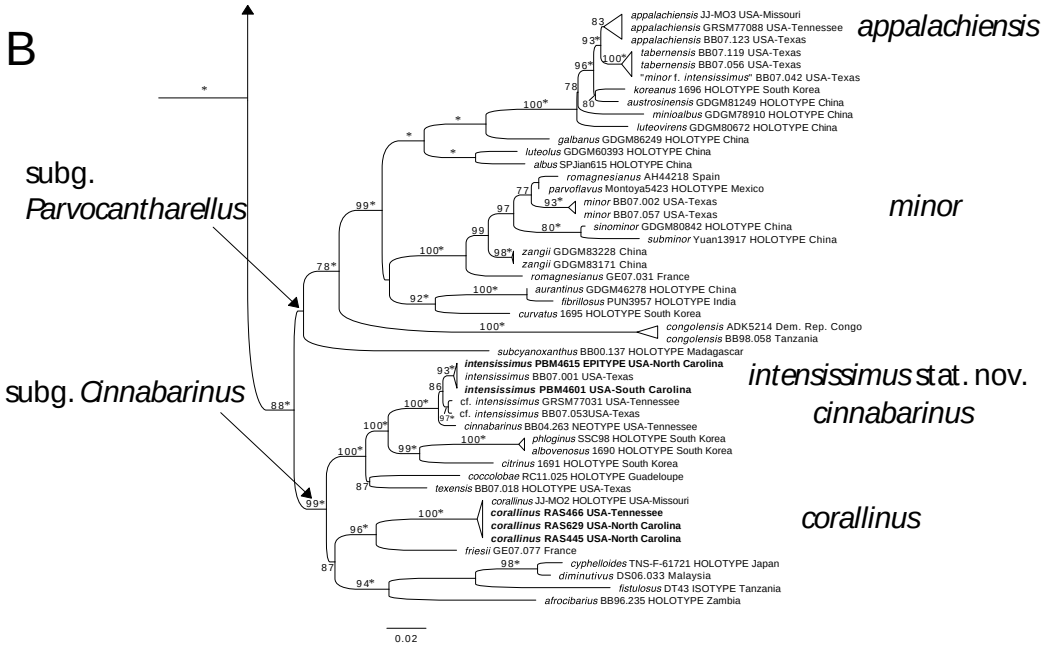

Figure 8. Continued.

Voucher #	Location	bp 414–416 (exon)			bp 515–517 (intron)			bp 573–575 (intron)			bp 766–769 (intron)			
TMI-5066	Japan	G	G	T	T	T	T	T	T	C	A	C	T	A
C106	Japan	G	G	T	T	T	T	T	T	C	A	C	T	A
C88	Japan	G	G	T	T	T	T	T	T	C	A	C	T	A
PBM4274	USA – NC	G	G	T	T	C	T	T	–	–	A	C	T	A
RAS614	USA – NC	G	G	T	T	C	T	T	–	–	A	C	T	A
120922av02 variant A	Canada – NL	G	G	C	T	Y	T	T	–	–	R	C	T	M
120922av02 variant B	Canada – NL	G	G	C	T	Y	T	T	T	C	R	C	T	M
180913av07	Canada – NL	G	G	C	T	T	T	T	T	C	G	C	T	C
120906av01	Canada – NL	G	G	C	T	T	T	T	T	C	G	C	T	C

Figure 9. Base map of *tef1* nucleotide polymorphisms and gaps (–) across populations of *Cantharellus camphoratus* from Japan, USA– North Carolina (NC), and Canada–Newfoundland and Labrador (NL). Numerical position of polymorphisms within the DNA sequence and coding status (intron or exon) are denoted below each set of base pairs (bp).

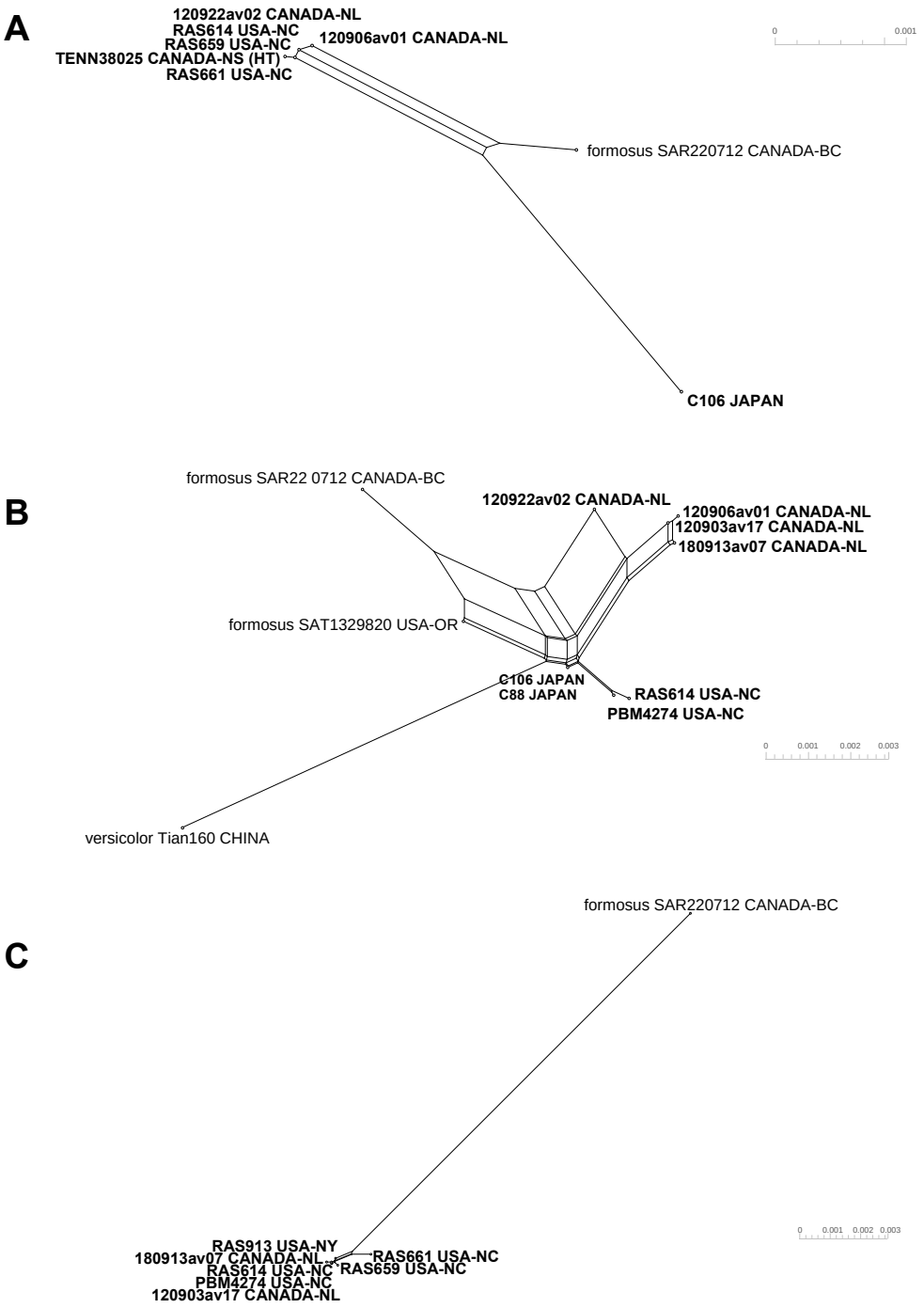

Figure 10. Network analysis of (A) 28S sequences, (B) *tef1* sequences, and (C) *rpb2* sequences across specimens of *Cantharellus camphoratus* (in bold). *Cantharellus formosus* and *C. versicolor* S.C. Shao & P.G. Liu are outgroups. HT denotes holotype.

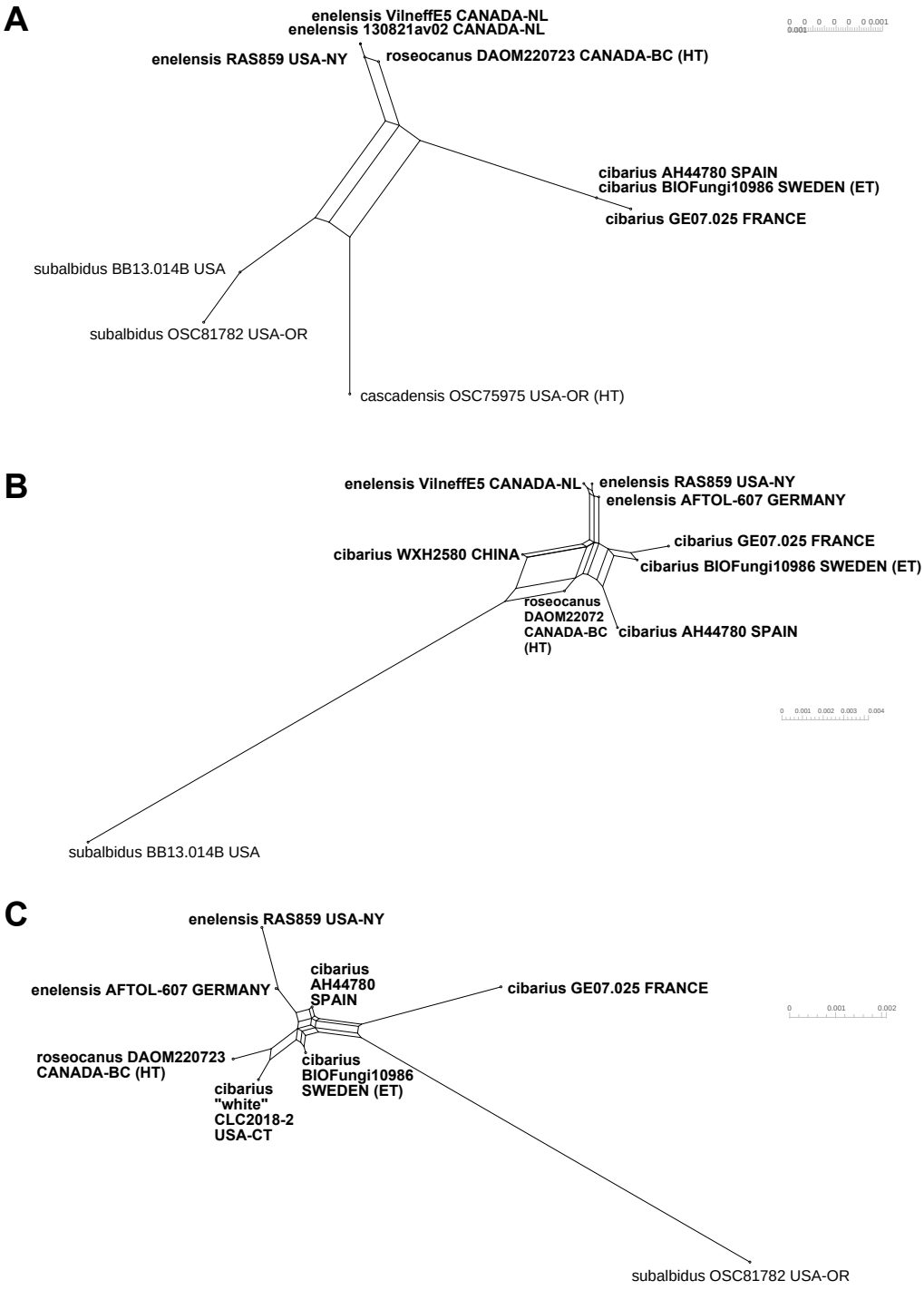

Figure 11. Network analysis of (A) 28S sequences, (B) *tef1* sequences, and (C) *rpb2* sequences across specimens in the *Cantharellus cibarius* complex (in bold). *Cantharellus cascadensis* and *C. subalbidus* are outgroups. HT denotes holotype; ET denotes epitype.

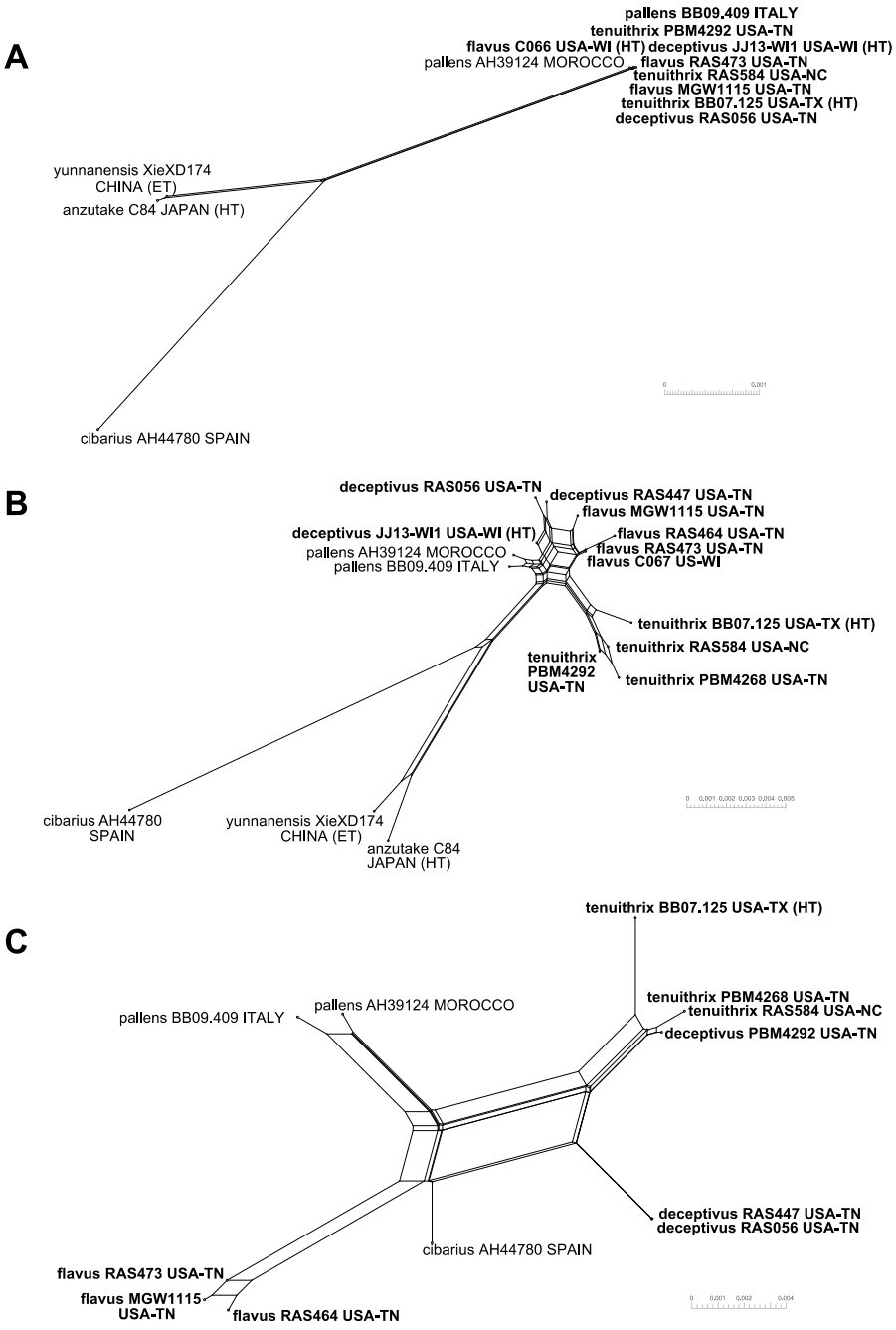

Figure 12. Network analysis of (A) 28S sequences, (B) *tef1* sequences, and (C) *rpb2* sequences, across specimens in the *Cantharellus tenuithrix* complex (in bold). *Cantharellus pallens, C. anzutake* W. Ogawa, N. Endo, M. Fukuda & A. Yamada, *C. yunnanensis* W.F. Chiu, and *C. cibarius* are outgroups. HT denotes holotype; ET denotes epitype.

Figure 13. Basidiomata of *Cantharellus*. A. *C. altipes* RAS084 (TENN-F-071461). B. *C. altipes* RAS639 (TENN-F-075384). C. *C. betularum* RAS631 (TENN-F-075393). D. *C. camphoratus* RAS614 (TENN-F-075387). E. *C. camphoratus* RAS661 (TENN-F-075389). F. *C. enelensis (C. cibarius* complex) RAS859 (TENN-F-075896). G. *C. cibarius* "white" CLC2018-2 (TENN-F-076274). H. *C. flavolateritius* RAS588 (TENN-F-076283). I. *C. flavolateritius* RAS590 (TENN-F-075271). Photos A–F, H, I by R. A. Swenie. Photo G by C. LaChance. Bars = 20 mm.

Figure 14. Basidiomata of *Cantharellus*. A. *C. persicinus* RAS841 (TENN-F-075884). B. *C. tenuithrix* PBM4292 (TENN-F-074854). C. *C. flavus (C. tenuithrix* complex) RAS464 (TENN-F-074949). D. *C. velutinus* (pink form) PBM4602 (TENN- F-075769). E. *C. vicinus* RAS426 (TENN-F-074935, holotype). F. *C. vicinus* RAS669 (TENN-F-075383). G. *C. corallinus* RAS629 (TENN- F-075397). H. *C. intensissimus* PBM4615 (TENN-F-075782, epitype). Photos A, C, E–G by R. A. Swenie; photos B, D, H by P. B. Matheny. Bars = 20 mm.

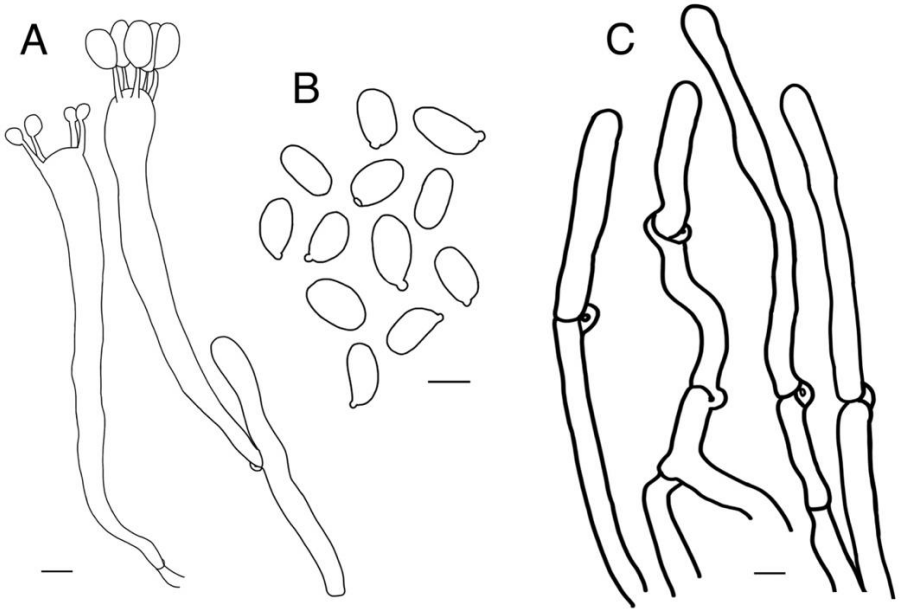

Figure 15. ***Cantharellus vicinus*** **(holotype). A. Basidia. B. Spores. C. Pileipellis terminal hyphae. Bars = 5 µm.**

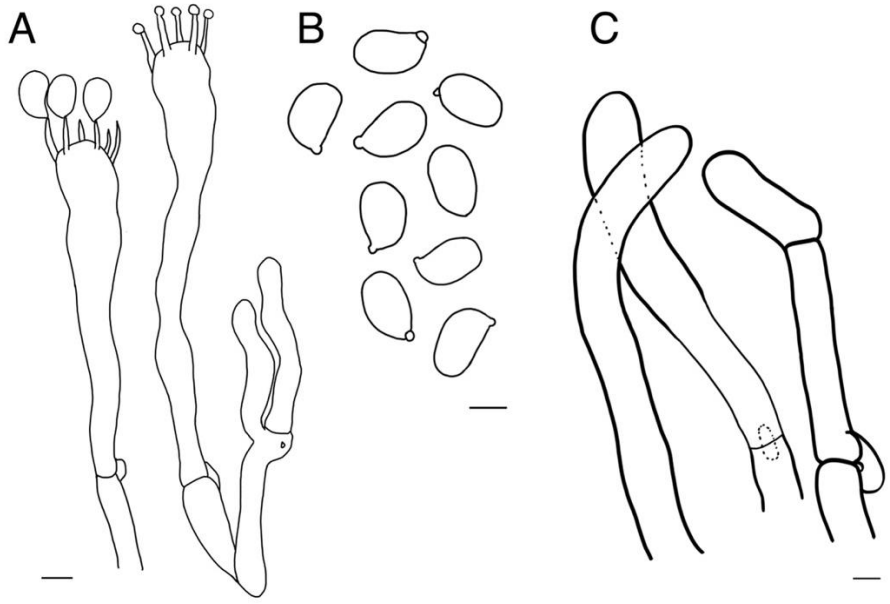

Figure 16. ***Cantharellus intensissimus*** **(epitype). A. Basidia. B. Spores. C. Pileipellis terminal hyphae. Bars = 5 µm.**

CHAPTER III
A PACBIO HIGH-THROUGHPUT MULTI-LOCUS SEQUENCING METHOD FOR FUNGAL SYSTEMATICS AND SPECIES DELIMITATION

A version of this chapter has been submitted to Molecular Ecology Resources:

Rachel A. Swenie, Brian P. Looney, Yi-Hong Ke, J. Alejandro Rojas, Marc A. Cubeta, Gitta Langer, Rytas Vilgalys, and P. Brandon Matheny. "A PacBio high-throughput multi-locus sequencing method for fungal systematics and species delimitation."

R.A.S. and P.B.M. designed the study; A.R., B.P.L., R.A.S., and R.V. developed the laboratory method; Y-H.K. and B.P.L. adapted and optimized the pipeline; R.A.S., M.A.C., G.E.L., and P.B.M. provided fungal specimens and culture isolates; R.A.S. and B.P.L. collected and analyzed data; R.A.S. wrote the manuscript and all authors provided edits.

Abstract

Multi-locus sequence data are widely used in fungal systematic and taxonomic studies to delimit species and infer evolutionary relationships. We developed and assessed the efficacy of a multi-locus pooled sequencing method using PacBio long-read high-throughput sequencing. Samples included fresh and dried voucher specimens, cultures, and archival DNA extracts of Agaricomycetes with an emphasis on the order Cantharellales. Of the 283 specimens sequenced, 93.6% successfully amplified at one or more loci with a mean of 3.3 loci amplified. Our method recovered multiple sequence variants representing alleles of rDNA loci and single copy protein coding genes *rpb*1, *rpb*2, and *tef*1. Within-sample genetic variation differed by locus and taxonomic group, with the greatest genetic divergence observed among sequence variants of *rpb*2 and *tef*1 from corticioid Cantharellales. Our method is a cost-effective approach for generating accurate multi-locus sequence data coupled with recovery of alleles from polymorphic samples and compound organisms. These results have important implications for understanding intra-individual genomic variation among genetic loci commonly used in species delimitation of fungi.

Introduction

High-throughput sequencing (HTS) methods have grown in popularity due to increased reliability and decreased cost. Single molecule sequencing, also referred to as "third generation" HTS, has been widely adopted in genome shotgun sequencing and transcriptome sequencing for generating accurate DNA sequences up to or exceeding 10 kb in length (Heather & Chain 2016). The PacBio Sequel third generation HTS platform has also shown promise for pooled barcoding of microbial soil samples and amplicon libraries (Bourne et al. 2018; Tedersoo et al. 2018; Runnel et al. 2022).

Multi-locus data from fungal specimens, conventionally produced by Sanger sequencing, are widely used in systematic and taxonomic studies. While Sanger sequencing remains popular due to accuracy and ease of use, it is a costly and time-consuming method for producing multi-locus data that is prone to generating heterogeneous reads when multiple alleles or contamination are present in a sample.

Sanger sequencing is also limited to reads one kb or less in length (Heather & Chain 2016). An alternative technique is desirable to overcome these limitations and obtain multi-locus sequence data more efficiently and accurately.

PacBio sequencing has been shown to meet or exceed the success and fidelity of Sanger sequencing at a similar cost, and samples with allele length polymorphisms or field contamination often succeed with PacBio sequencing when Sanger sequencing has failed (Runnel et al. 2022). Isolating a single allele to avoid heterogeneity issues with Sanger sequencing is possible with the additional step of cloning prior to PCR, however this extra step obviously limits efficiency. With PacBio long-read HTS, individual sequence reads are generated from a single strand of DNA, circumventing the need for cloning and mitigating cloning bias issues. With adequate read depth, sequence variants within a dikaryon or diploid genome can also be recovered, providing insight into within-sample genetic diversity. The ribosomal DNA (rDNA) loci commonly used for barcoding fungi– 18S, ITS, and 28S– occur in tens to thousands of copies per nucleus depending on the fungal taxon (Lofgren et al. 2019). Intraspecific and intraindividual rDNA sequence variation in fungi may be due to hybridization and/or a lack of concerted evolution, a process that homogenizes rDNA copies (Hughes et al. 2013; Tremble et al. 2020). In heterokaryotic fungi with multinucleate cells, the divergent genomes of different nuclei can also contribute to intraspecific or intraindividual genetic variation (Paloi et al. 2022).

The occurrence of intra-genome variation in rDNA makes delimiting species based on rDNA sequences problematic for some fungal groups. This is exemplified in the Agaricomycete order Cantharellales. Within the Cantharellales, some genera are prone to long stretches of sequence length heterogeneity (especially *Cantharellus* and *Tulasnella*; Moncalvo et al. 2006), making rDNA loci inappropriate barcode markers for these taxa. Other multinucleate groups of fungi such as the form genus *Rhizoctonia* in the Ceratobasidiaceae present challenges at species delimitation and phylogenetic inference, arising from discordance among multiple loci due to nuclear divergence within individual genomes of multinucleate hyphae, hybridization, and incomplete lineage sorting (Gónzalez et al. 2006; Gónzalez et al. 2016; Paloi et al. 2022). Single molecule HTS is favorable in these circumstances by recovering all alleles present in a sample to elucidate both intra- and intergenomic variation.

We have developed and tested a method for rapid DNA extraction and cost-effective multi-locus PacBio HTS of fungal voucher specimens and cultures, employing a software pipeline to infer intra-individual allelic diversity across nuclear rDNA loci as well as single-copy genes. This method is adaptable for use with a wide variety of organisms and target loci. We amplified six loci commonly used in phylogenetic studies of fungi and detected high levels of intraspecific and intraindividual genetic diversity. Here we demonstrate the utility of this method for generating high-quality multi-locus data from a variety of sources, including voucher specimens, cultures, multi-species samples, and contaminated fruiting bodies (basidiomes).

Materials and Methods

Molecular analyses

A total of 283 specimens were sequenced across two PacBio Sequel runs (96 in the first run and 187 in the second run). All specimens belonged to the phylum Basidiomycota with an emphasis on the order Cantharellales (264 Cantharellales and 19 non-Cantharellales). The 283 specimens comprised 168 vouchered specimens at the University of Tennessee Herbarium and 115 culture isolates provided by M.A. Cubeta, G.E. Langer, and R.A. Swenie, and the Center for Forest Mycology Research culture collection (Table 4; all Tables and Figures are in Appendix).

DNA from cultures and recent herbarium specimens (< 5 years old) were extracted using an Extract-N-Amp Plant Kit (Sigma-Aldrich, St. Louis, MO, USA). Approximately 2–5 mg of culture tissue or hymenophore tissue (such as a piece of lamella) from basidiomata was suspended in 40 µL Extraction Solution and preserved at room temperature until subsequent DNA extraction. An additional 26 archival genomic DNA samples extracted 2–12 years prior via a standard extraction method, i.e. using an E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Norcross, GA, USA) and stored at -20°C thereafter, were also included for subsequent PCR. In most cases, genomic DNA was diluted 1:10 with sterile double distilled H_2O before PCR amplification.

We targeted six loci commonly used in phylogenetic studies of fungi using the following locus-specific forward and reverse primers: NS1/SR2 for 18S (White et al. 1990; https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/); ITS1F/ITS4 for ITS (White et al. 1990; Gardes & Bruns 1993); LR0R/LR7 for 28S (Vilgalys & Hester 1990; https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/); tef1F/tef1R and 983F/2218R for *tef*1 (Morehouse et al. 2003; Rehner & Buckley 2005); RPB1-Afor/ RPB1-Crev for conserved domains A–C of *rpb*1 (Matheny et al. 2002); bRPB2-6F/ bRPB2-7.1R (Matheny 2005) and newly designed Cantharellales-specific *rpb*2 primers Canthb6F and Canth7.1R for domains 6–7 of *rpb*2. Primer sequences and thermocycler conditions are listed in Table 1. A 20nt universal adapter sequence was added at the 5' end of locus-specific primers (5′-CTGGAGCACGAGGACACTGA-3′ for forward primers; 5′-GCTGTCAACGATACGCTACG-3′ for reverse primers).

The first PCR step to amplify targeted loci was carried out in 25 µL volume reactions containing 5 µL 5X GoTaq Flexi Buffer, 1.5 µL $MgCl_2$, 0.5 µL for each 10 µM forward and reverse primer, 0.5 µL 10mM dNTPs, and 0.125–0.375 µL GoTaq G2 Hot Start Polymerase (with amount of GoTaq determined by the number of loci amplified in a reaction, 0.125 µL per locus), nuclease-free water to bring the volume to 24 µL, and 1 µL DNA. Up to 3 loci were simultaneously co-amplified. 18S, ITS, and 28S were co-amplified using ITS thermocycler conditions; *rpb*1 and *rpb*2 were co-amplified or amplified singly using RPB250 thermocycler conditions; and *tef*1 was amplified singly (Table 5). PCR product size was estimated by running 5 µL of PCR product for 40 minutes on a 2% agarose gel for 2- or 3-locus reactions, or a 1% agarose gel for 1-locus PCR reactions. Successful reactions were cleaned using 5 µL of PCR product and 2 µL ExoSAP-IT (Applied Biosystems, Waltham, MA, USA) in 0.2 mL PCR tubes. Cleaned PCR products were diluted 1:5 with nuclease-free water.

A second PCR barcoding step was carried out in 25 μL volume reactions containing 5 μL 5X GoTaq Flexi Buffer, 0.5 μL 10mM dNTPs, 0.25 μL GoTaq G2 Hot Start Polymerase, and 15.25 μL nuclease-free water, 1 μL of each 5 μM forward and reverse barcode primer, and 2 μL of the diluted cleaned PCR product using the following thermocycler conditions: an initial denaturation of 94°C for 30s, 35 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 90s, and a final extension of 72°C for 10 min. A unique dual barcode was assigned to each specimen for the amplicons of all loci. Barcode primers contained a 5nt padding sequence, followed by a 16nt barcode sequence, followed by the same 20nt universal adapter included in locus-specific primers. Second step PCR products were visualized by running 5 μL of PCR product on a 2% agarose gel for 40 min.

All second PCR barcoding products were scored visually on a scale of 1–3 based on gel band intensity with 1 for weak bands and 3 for strong bands. Products without a visible band were discarded. Multiple DNA pools were created, each containing second step PCR products from 12–26 specimens. For each PCR product, 10 to 20 μL of was used: 20 μL for weak bands (score 1), 15 μL for medium bands (score 2), and 10 μL for strong bands (score 3). Each pool was cleaned individually using Mag-Bind TotalPure NGS Kit (Omega BioTek, Norcross, GA, USA) with a bead ratio of 0.7x and quantified using a Nanodrop One Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Equimolar pooling was used to develop a final pooled library with a concentration between 1.7–1.9 μg. The final pooled library was sent to Duke University Center for Genomic and Computational Biology (GCB) for SMRTbell™ library preparation and PacBio Sequel sequencing on 1 SMRT cell.

Sequence data pipeline and analysis

Raw data in PacBio CCS fasta format was demultiplexed, quality filtered, corrected for PacBio sequencing error, and clustered by specimen and locus using a modified version of the purc pipeline (Rothfels et al. 2017). The purc pipeline (https://github.com/KeFungi/purc) output provides one fasta file per locus for each of the 283 specimens. Each fasta file contains sequence variants with a read depth of 4 or greater (labeled as “clusters” by the pipeline). Sequence variants were visualized in AliView 1.28 (Larsson 2014) and aligned using MUSCLE 3.8.31 (Edgar 2004). Locus-specific primers were trimmed from sequence ends as necessary, and identities were confirmed for all sequence variants using the BLASTn algorithm and comparison to the top 10 database matches on GenBank. For sequence variants from protein-coding loci with ambiguous BLASTn results, introns were removed, the sequence was translated into an amino acid sequence, and the BLASTp algorithm was used to compare the top 10 database matches on GenBank. The steps in the PacBio multi-locus long-read HTS workflow are summarized in Figure 1. Phylogenetic analysis for selected samples was conducted using RAxML 8.2.8 (Stamatakis 2014) under the GTRGAMMA model of nucleotide substitution for 1000 bootstraps.

Results

283 samples were subjected to PCR amplification across two PacBio runs (96 on run 1 and 187 on run 2). Of 283 samples, 265 (93.6%) amplified at one or more loci with 69.6% amplified at three or more loci (mean = 3.3 loci). 256 (90.5%) samples matched the expected taxonomic identity based on BLAST results; 42 samples contained sequences from the target taxon plus one or more non-target species; 13 samples yielded sequences that did not match the expected identity and were determined to be misidentified.

Overall, 886 sequence clusters (sequence variants) were recovered across all samples. Within each locus for each sample, the number of sequence variants ranged from 1 to 10 (mean = 1.69). 168 samples (63.4%) showed allelic diversity (> 1 sequence variant) at one or more loci. Sequence variant statistics by locus and taxonomic family are summarized in Table 2. Across 18S, ITS, and 28S rDNA regions, sequence variant diversity was consistently highest within the ITS with an average of 1.89 sequence variants per sample. Across the putatively single-copy protein-coding loci *rpb*1, *rpb*2, and *tef*1, intra-individual sample allelic diversity ranged from 0–5.5% (Table 7; Figures 18–19) and deleted or inserted exon positions within coding regions were observed (6.7% of *rpb*1 samples; 34.8% of *rpb*2 samples; 23.2% of *tef*1 samples).

A comparison of PacBio long-read HTS data to previously generated Sanger sequences from 170 samples for some of the same loci showed that the HTS sequences largely matched Sanger counterparts and often contained two complimentary sequence variants at sites that were polymorphic in the Sanger sequence. Occasionally HTS sequences contained other polymorphic sites not reflected in the Sanger sequence (Figure 20). PacBio long-read HTS produced high quality sequences for several samples where Sanger sequencing failed or produced unreadable sequences.

The 42 samples containing sequence data for more than two organisms (fungi or protists) were spread across taxonomic family and loci. In a few instances, it was not determined whether the additional organism was present in the DNA sample or whether it represented a PCR artifact. However, co-occurring organisms with a high sequence read depth or consistent presence across loci and/or samples were observed in 17 samples. For example, eight samples of *Cantharellus* extracted from fruiting bodies also yielded *tef*1, LSU, and/or ITS sequences of protists, while protist sequences were only detected in two other non-*Cantharellus* samples. Amplification of co-occurring organisms may be influenced by primer non-target specificity. An example of two corticioid samples containing multiple fungi recovered by HTS is presented in Figure 21.

Discussion

Our results demonstrate the utility of long-read HTS for generating multi-locus sequence data and understanding intra-individual and intraspecific genetic diversity. Of 283 samples in our study, 93.6% produced sequences from one or more loci, and 69.6% produced sequences from three or more loci. Sequence variation attributed to allelic

diversity was common within Cantharellales and other Agaricomycetes, both within multi-copy rDNA loci and putatively single-copy loci *rpb*1, *rpb*2, and *tef*1, which are commonly used in phylogenetic studies (Tables 6 & 7). The purc pipeline preserves sequence variants for each specimen and locus (denoted as "clusters" in the output files For 18S, ITS, and 28S sequences we interpreted these sequence variants as copy variants within the the rDNA operon, as allelic haplotype variants for single-copy genes, or as non-functional gene duplications where a frameshift is present in an exon.

Our long-read multi-locus HTS approach is compatible with quick and traditional DNA extraction methods and suitable for cultures, fresh and dried specimens, environmental samples, and archival DNA extracts. Using multiplex PCR to simultaneously amplify multiple loci and pooling samples for PacBio long-read HTS (Chen et al. 2015; Barge et al. 2022) is a cost-effective method compared to Sanger sequencing, allowing sequencing of up to six loci from multiple samples. Our pooled barcoding approach to DNA sequencing is more economical than Sanger sequencing, especially as PacBio sequencing has increased in reliability and fidelity (Tedersoo et al. 2021; Runnel et al. 2022). Major strengths of the PacBio platform include the ability to sequence longer reads than the 1000 bp limit of Sanger, as well as adaptability to other robotic methods suitable for HTS. Since PacBio sequence reads are produced from single molecules, individual alleles (as sequence variants) are recovered, and samples with length heterogeneity produce high quality reads without the need for cloning (Figure 20). The relative ease of this method, along with the high-quality data obtained from polymorphic or contaminated samples suggests PacBio long-read HTS is a viable and cost-effective replacement for multi-locus Sanger sequencing, particularly when sequencing many samples.

PacBio HTS sequencing is flexible to custom primer design and locus choice. Success rates for PCR amplification were very high overall but varied by taxonomic group and locus (see Table 6).). rDNA loci consistently amplified regardless of DNA source, while success in amplifying single-copy genes declined with age of specimens and DNA extracts. We did not detect issues of PacBio adapters or barcodes interfering with PCR amplification, but it is likely that some samples failed to amplify due to primer mismatch, particularly for *rpb*1, where fewer than 10% of attempted samples amplified. Optimization of PCR conditions is another important aspect of multiplex PCR. We hypothesize that lower read depth and fewer haplotypes recovered for 28S may be due in part to a non-optimal thermocycler DNA amplification protocol. Amplification of 28S separately from 18S and ITS using a more suitable thermocycler protocol is likely to yield greater numbers of reads. However, multiplexing several loci simultaneously produces savings in time and cost of PCR reagents, particularly dNTPs.

Understanding intra-allelic and intraspecific allele divergence is important for species delimitation and can also be informative for choosing OTU thresholds for analysis of environmental sequencing data. The purc pipeline corrects for PCR and PacBio sequence errors, removes chimeras, and groups reads from each allele into clusters (sequence variants) with a sequencing depth of four or more reads. The sequence variants are then grouped together by locus for each individual sample.

A particularly high level of intra-individual sequence variant diversity (up to 8.8%) was recovered in the Cantharellales families Botryobasidiaceae,

Ceratobasidiaceae, and Tulasnellaceae (Table 7). Species in these families have primarily binucleate or multinucleate hyphae and many have anamorphic states (Langer 1994; Vilgalys & Cubeta, 1994; Arifin et al. 2021). In the Ceratobasidiaceae, species diversity was traditionally recognized using anastomosis groups based on hyphal fusion criteria (Vilgalys & Cubeta, 1994). Phylogenetic analyses of the Ceratobasidiaceae have reported incongruence across multiple gene loci, with non-monophyletic genera and mixed support for anastomosis groups (Gónzalez et al. 2006; Veldre et al. 2013; Gónzalez et al. 2016). Our results suggest genomic divergence across nuclei within an individual is often higher than expected, particularly for protein-coding loci (Figure 19). Therefore, characterizing intra-individual genetic diversity may be an important aspect of species delimitation in these fungi. Utilizing PacBio long-read HTS is one such method to assess genomic divergence within samples.

Our long-read HTS method can also detect non-target species present in a single sample, and therefore is useful for identifying compound organisms such as lichens, corticioid fungi, and contaminated samples, as other studies utilizing PacBio HTS have shown (Gueidan et al. 2019; Runnel et al. 2022). Of the 42 samples containing sequence data from two or more organisms, slightly more than half of these were from corticioid fungi (e.g., Figure 21), which are ecologically diverse and notoriously difficult to classify taxonomically. Several corticioid fungi also contained non-target protist or Ascomycota sequences, however the presence of these non-target sequences rarely hindered sequencing of the target. Thus, the PacBio long-read HTS method is an efficient and accurate sequencing approach for uncovering within-sample genetic diversity, from alleles to compound organisms.

Data Accessibility

Sequence data from this study is deposited in the NCBI Sequence Read Archive, BioProject PRJNA945373. All specimens are accessioned in herbaria at the University of Tennessee. Pipeline is available at https://github.com/KeFungi/purc. Pipeline is available at https://github.com/KeFungi/purc. Phylogenetic trees and sequence alignments are available at FigShare (DOI: 10.6084/m9.figshare.22795817).

Acknowledgements

This research was supported by a University of Tennessee Student Faculty Research Award to R.A.S. and P.B.M, an award from the Herbarium at the University of Tennessee to R.A.S, an NSF Graduate Research Fellowship (No. 1452154) to R.A.S., and NSF ARTS award to P.B.M. (DEB-2030779). We thank the Center for Forest Mycology Research for providing fungal culture isolates and James Lawrey, Matt Smith, and Karen Hughes for providing additional DNA extracts. We also gratefully acknowledge the assistance of Graham Alexander and the Duke University Sequencing and Genomic Technologies core facility.

REFERENCES

Arifin, A. R., May, T. W., & Linde, C. C. (2021). New species of *Tulasnella* associated with Australian terrestrial orchids in the Cryptostylidinae and Drakaeinae. *Mycologia*, *113*(1), 212–230. https://doi.org/10.1080/00275514.2020.1813473

Barge, E. G., Leopold, D. R., Rojas, A., Vilgalys, R., & Busby, P. E. (2022). Phylogenetic conservatism of mycoparasitism and its contribution to pathogen antagonism. *Molecular Ecology*, *31*(10), 3018–3030. https://doi.org/10.1111/mec.16436

Bourne, E. C., Bunk, B., Monaghan, M. T., Heeger, F., Spröer, C., Overmann, J., … Baschien, C. (2018). Long-read DNA metabarcoding of ribosomal RNA in the analysis of fungi from aquatic environments. *Molecular Ecology Resources*, *18*(6), 1500–1514. https://doi.org/10.1111/1755-0998.12937

Chen, Y., Frazzitta, A. E., Litvintseva, A. P., Fang, C., Mitchell, T. G., Springer, D. J., ... & Perfect, J. R. (2015). Next generation multilocus sequence typing (NGMLST) and the analytical software program MLSTEZ enable efficient, cost-effective, high-throughput, multilocus sequencing typing. *Fungal Genetics and Biology*, *75*, 64–71.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*, *32*(5), 1792–1797.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113–118.

González, D., Cubeta, M. A., & Vilgalys, R. (2006). Phylogenetic utility of indels within ribosomal DNA and β-tubulin sequences from fungi in the Rhizoctonia solani species complex. *Molecular Phylogenetics and Evolution*, *40*(2), 459–470. https://doi.org/10.1016/j.ympev.2006.03.022

Gónzalez, D., Rodriguez-Carres, M., Boekhout, T., Stalpers, J., Kuramae, E. E., Nakatani, A. K., Vilgalys, R., Cubeta, M. A. (2016). Phylogenetic relationships of Rhizoctonia fungi within the Cantharellales. *Fungal Biology*, *120*(4), 603–619. https://doi.org/10.1016/j.funbio.2016.01.012

Gueidan, C., Elix, J.A., McCarthy, P.M., Roux, C., Mallen-Cooper, M., Kantvilas, G. (2019). PacBio amplicon sequencing for metabarcoding of mixed DNA samples from lichen herbarium specimens. *MycoKeys*, *53*, 73–91. https://doi.org/10.3897/mycokeys.53.34761

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics*, *107*(1), 1–8. https://doi.org/10.1016/j.ygeno.2015.11.003

Hughes, K. W., Petersen, R. H., Lodge, D. J., Bergemann, S. E., Baumgartner, K., Tulloss, R. E., … Cifuentes, J. (2013). Evolutionary consequences of putative intra-and interspecific hybridization in agaric fungi. *Mycologia*, *105*(6), 1577–1594. https://doi.org/10.3852/13-041

Langer, G. (1994). *Die gattung* Botryobasidium *Donk (Corticiaceae, Basidiomycetes)*. Gebrüder Borntraeger.

Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278.

Lofgren, L. A., Uehling, J. K., Branco, S., Bruns, T. D., Martin, F., & Kennedy, P. G. (2019). Genome-based estimates of fungal rDNA copy number variation across phylogenetic scales and ecological lifestyles. *Molecular Ecology*, *28*(4), 721–730.

Matheny, P. B., Liu, Y. J., Ammirati, J. F., & Hall, B. D. (2002). Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). *American Journal of Botany*, *89*(4), 688–698. https://doi.org/10.3732/ajb.89.4.688

Matheny, P. B. (2005). Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molecular Phylogenetics and Evolution*, *35*(1), 1–20. https://doi.org/10.1016/j.ympev.2004.11.014

Moncalvo, J.-M., Nilsson, R. H., Koster, B., Dunham, S. M., Bernauer, T., Matheny, P. B., … Vilgalys, R. (2006). The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia*, *98*(6), 937–948. https://doi.org/10.3852/mycologia.98.6.937

Morehouse, E. A., James, T. Y., Ganley, A. R., Vilgalys, R., Berger, L., Murphy, P. J., & Longcore, J. E. (2003). Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology*, *12*(2), 395–403. https://doi.org/10.1046/j.1365-294x.2003.01732.x

Paloi, S., Luangsa-ard, J. J., Mhuantong, W., Stadler, M., & Kobmoo, N. (2022). Intragenomic variation in nuclear ribosomal markers and its implication in species delimitation, identification and barcoding in fungi. *Fungal Biology Reviews*, *42*, 1–33. https://doi.org/10.1016/j.fbr.2022.04.002

Rehner, S. A., & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. *Mycologia*, *97*(1), 84–98. https://doi.org/10.3852/mycologia.97.1.84

Rothfels, C. J., Pryer, K. M., & Li, F. W. (2017). Next-generation polyploid phylogenetics: Rapid resolution of hybrid polyploid complexes using PacBio single-

molecule sequencing. *New Phytologist*, *213*(1), 413–429. https://doi.org/10.1111/nph.14111

Runnel, K., Abarenkov, K., Copoț, O., Mikryukov, V., Kõljalg, U., Saar, I., & Tedersoo, L. (2022). DNA barcoding of fungal specimens using PacBio long-read high-throughput sequencing. *Molecular Ecology Resources*, *22*(8), 2871–2879. https://doi.org/10.1111/1755-0998.13663

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313.

Tedersoo, L., Tooming-Klunderud, A., & Anslan, S. (2018). PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. *New Phytologist*, *217*(3), 1370–1385. https://doi.org/10.1111/nph.14776

Tedersoo, L., Albertsen, M., Anslan, S., & Callahan, B. (2021). Perspectives and benefits of high-throughput long-read sequencing in microbial ecology. *Applied and Environmental Microbiology*, *87*(17), e00626-21.

Tremble, K., Suz, L. M., & Dentinger, B. T. M. (2020). Lost in translation: Population genomics and long-read sequencing reveals relaxation of concerted evolution of the ribosomal DNA cistron. *Molecular Phylogenetics and Evolution*, *148*, 106804. https://doi.org/10.1016/j.ympev.2020.106804

Veldre, V., Abarenkov, K., Bahram, M., Martos, F., Selosse, M. A., Tamm, H., Kõljalg, U., & Tedersoo, L. (2013). Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecology*, *6*(4), 256–268. https://doi.org/10.1016/j.funeco.2013.03.004

Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology*, *172*(8), 4238–4246.

Vilgalys, R., & Cubeta, M. A. (1994). Molecular systematics and population biology of Rhizoctonia. *Annual Review of Phytopathology*, *32*(1), 135–155.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, *18*(1), 315–322.

APPENDIX

Table 4. Samples used in this study.

Organism	Family	Order	Voucher
Amanita	Amanitaceae	Agaricales	B69P16
Heterobasidion	Bondarzewiaceae	Russulales	JHG748
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS537
Botryobasidium asperulum	Botryobasidiaceae	Cantharellales	FP102150
Botryobasidium	Botryobasidiaceae	Cantharellales	FP102090
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	FD50
Botryobasidium asperulum	Botryobasidiaceae	Cantharellales	FP102150
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	FD50
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	FP101015
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	FP151108
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	GEL4232+2276-4
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	GEL2273
Botryobasidium	Botryobasidiaceae	Cantharellales	GEL2597-10
Botryobasidium vagum	Botryobasidiaceae	Cantharellales	GEL3330
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	GEL4673
Botryobasidium	Botryobasidiaceae	Cantharellales	GEL3304-1
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	GEL2160
Botryobasidium	Botryobasidiaceae	Cantharellales	GEL3416
Botryobasidium	Botryobasidiaceae	Cantharellales	GEL3335-1
Botryobasidium asperulum	Botryobasidiaceae	Cantharellales	RAS552
Botryobasidium vagum	Botryobasidiaceae	Cantharellales	RAS559
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS563
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS565
Botryobasidium aureum	Botryobasidiaceae	Cantharellales	RAS571
Botryobasidium asperulum	Botryobasidiaceae	Cantharellales	RAS578
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS583
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS594
Botryobasidium isabellinum	Botryobasidiaceae	Cantharellales	RAS579
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	RAS620
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS600
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	GEL2412
Botryobasidium vagum	Botryobasidiaceae	Cantharellales	GEL2136
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS736
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS737
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS712
Botryobasidium laeve	Botryobasidiaceae	Cantharellales	RAS762
Botryobasidium isabellinum	Botryobasidiaceae	Cantharellales	RAS769
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	RAS770
Botryobasidium rubiginosum	Botryobasidiaceae	Cantharellales	RAS776
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	RAS789
Botryobasidium conspersum	Botryobasidiaceae	Cantharellales	RAS259
Botryobasidium simile	Botryobasidiaceae	Cantharellales	RAS794
Botryobasidium simile	Botryobasidiaceae	Cantharellales	RAS793
Botryobasidium	Botryobasidiaceae	Cantharellales	MES2797
Botryobasidium	Botryobasidiaceae	Cantharellales	MES2884
Botryobasidium	Botryobasidiaceae	Cantharellales	VDZ15
Botryobasidium conspersum	Botryobasidiaceae	Cantharellales	MES1018
Botryobasidium	Botryobasidiaceae	Cantharellales	MES1763
Botryobasidium subcoronatum	Botryobasidiaceae	Cantharellales	RAS602
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS777
Botryobasidium	Botryobasidiaceae	Cantharellales	MES2058
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS773
Botryobasidium	Botryobasidiaceae	Cantharellales	RAS537

Table 4. Continued.

Ceratobasidium bulbillifaciens	Ceratobasidiaceae	Cantharellales	CBS132236
Ceratobasidium anceps	Ceratobasidiaceae	Cantharellales	CA1
Ceratobasidium	Ceratobasidiaceae	Cantharellales	C512
Ceratobasidium	Ceratobasidiaceae	Cantharellales	C517
Ceratobasidium	Ceratobasidiaceae	Cantharellales	C662
Ceratobasidium oryzae-sativae	Ceratobasidiaceae	Cantharellales	C511
Ceratobasidium oryzae-sativae	Ceratobasidiaceae	Cantharellales	PG1
Ceratobasidium	Ceratobasidiaceae	Cantharellales	A001C
Ceratobasidium	Ceratobasidiaceae	Cantharellales	70B
Ceratobasidium cereale	Ceratobasidiaceae	Cantharellales	BRGWP2
Ceratobasidium cereale	Ceratobasidiaceae	Cantharellales	KOUO41FW
Ceratobasidium	Ceratobasidiaceae	Cantharellales	SIR1
Ceratobasidium	Ceratobasidiaceae	Cantharellales	AH6
Ceratobasidium	Ceratobasidiaceae	Cantharellales	C653
Ceratobasidium	Ceratobasidiaceae	Cantharellales	FK022
Ceratobasidium	Ceratobasidiaceae	Cantharellales	C620
Ceratobasidium	Ceratobasidiaceae	Cantharellales	RH4
Ceratobasidium cereale	Ceratobasidiaceae	Cantharellales	RH1
Ceratobasidium	Ceratobasidiaceae	Cantharellales	BN37
Ceratobasidium	Ceratobasidiaceae	Cantharellales	RH7
Rhizoctonia calle	Ceratobasidiaceae	Cantharellales	RC1
Rhizoctonia endophytica	Ceratobasidiaceae	Cantharellales	RE1
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	56RS
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	RH08
Ceratobasidium filamentosum	Ceratobasidiaceae	Cantharellales	B1072
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	B1081
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	B195
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	RS29
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	RS293
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	RS44
Thanatephorus practicola	Ceratobasidiaceae	Cantharellales	RS359
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	10RS
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	RSBWB
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	RSIOEEA
Thanatephorus cucumeris	Ceratobasidiaceae	Cantharellales	C1
Ceratobasidium	Ceratobasidiaceae	Cantharellales	MES2155
Ceratobasidium	Ceratobasidiaceae	Cantharellales	MES2154
Ceratobasidium bulbillifaciens	Ceratobasidiaceae	Cantharellales	CBS129339
Ceratobasidium bulbillifaciens	Ceratobasidiaceae	Cantharellales	CBS132236
Dacrymyces chrysospermus	Dacrymycetaceae	Dacrymycetales	AMH013
Clavulina	Hydnaceae	Cantharellales	PBM4135
Sistotrema coronilla	Hydnaceae	Cantharellales	HHB10379
Craterellus tubaeformis	Hydnaceae	Cantharellales	RAS387
Hydnum subtilior	Hydnaceae	Cantharellales	PBM3868
Cantharellus flavolateritius	Hydnaceae	Cantharellales	RAS474
Sistotrema raduloides	Hydnaceae	Cantharellales	HHB4356
Multiclavula mucida	Hydnaceae	Cantharellales	RAS392
Clavulina rugosa	Hydnaceae	Cantharellales	RAS327
Sistotrema hirschii	Hydnaceae	Cantharellales	TCH8A
Sistotrema coronilla	Hydnaceae	Cantharellales	RAS538
Hydnum umbilicatum	Hydnaceae	Cantharellales	BD14
Hydnum alboaurantiacum	Hydnaceae	Cantharellales	RAS104
Hydnum mulsicolor	Hydnaceae	Cantharellales	RAS108
Clavulina rugosa grey	Hydnaceae	Cantharellales	RAS487
Clavulina cristata	Hydnaceae	Cantharellales	RAS323
Sistotrema resinicystidium	Hydnaceae	Cantharellales	HHB10232
Hydnum subolympicum	Hydnaceae	Cantharellales	RAS168
Hydnum subconnatum	Hydnaceae	Cantharellales	RAS169
Hydnum subtilior	Hydnaceae	Cantharellales	RAS180

Table 4. Continued.

Hydnum aerostatisporum	Hydnaceae	Cantharellales	RAS157
Hydnum albidum	Hydnaceae	Cantharellales	BPL932
Hydnum cuspidatum	Hydnaceae	Cantharellales	RAS162
Hydnum albidum	Hydnaceae	Cantharellales	RAS225
Hydnum ferruginescens	Hydnaceae	Cantharellales	RAS229
Sistotrema biggsiae	Hydnaceae	Cantharellales	HHB1663
Sistotrema brinkmanii	Hydnaceae	Cantharellales	WBC60142T
Sistotrema	Hydnaceae	Cantharellales	FP102155
Sistotrema farinaceum	Hydnaceae	Cantharellales	GB659
Craterellus ignicolor	Hydnaceae	Cantharellales	RAS444
Multiclavula mucida	Hydnaceae	Cantharellales	RAS525
Hydnum cuspidatum	Hydnaceae	Cantharellales	RAS098
Hydnum umbilicatum	Hydnaceae	Cantharellales	RAS099
Hydnum canadense	Hydnaceae	Cantharellales	RAS100
Hydnum cuspidatum	Hydnaceae	Cantharellales	RAS205
Hydnum umbilicatum	Hydnaceae	Cantharellales	RAS356
Clavulina castaneipes	Hydnaceae	Cantharellales	RAS452
Sistotrema coronilla	Hydnaceae	Cantharellales	RLG5411
Sistotrema	Hydnaceae	Cantharellales	AhoTM38
Clavulina coralloides	Hydnaceae	Cantharellales	RAS490
Cantharellus corallinus	Hydnaceae	Cantharellales	RAS466
Hydnum mulsicolor	Hydnaceae	Cantharellales	MES3229
Hydnum oregonense	Hydnaceae	Cantharellales	RPL12318D
Hydnum vagabundum	Hydnaceae	Cantharellales	MO298808
Hydnum melitosarx	Hydnaceae	Cantharellales	RLP12318C
Sistotrema adnatum	Hydnaceae	Cantharellales	GB700
Sistotrema oblongisporum	Hydnaceae	Cantharellales	HHB707
Sistotrema brinkmanii	Hydnaceae	Cantharellales	LI3431
Sistotrema porulosum	Hydnaceae	Cantharellales	HHB7822
Sistotrema porulosum	Hydnaceae	Cantharellales	GB566
Sistotrema oblongisporum	Hydnaceae	Cantharellales	FP101802
Sistotrema brinkmanii	Hydnaceae	Cantharellales	71177
Sistotrema	Hydnaceae	Cantharellales	HHB11889
Sistotrema porulosum	Hydnaceae	Cantharellales	GB720
Sistotrema eximum	Hydnaceae	Cantharellales	T429
Sistotrema biggsiae	Hydnaceae	Cantharellales	RLG5457
Sistotrema raduloides	Hydnaceae	Cantharellales	FD550
Craterellus tubaeformis	Hydnaceae	Cantharellales	RAS485
Cantharellus cinnabarinus	Hydnaceae	Cantharellales	RAS511
Cantharellus vicinus	Hydnaceae	Cantharellales	RAS426
Cantharellus velutinus	Hydnaceae	Cantharellales	RAS456
Cantharellus flavolateritius	Hydnaceae	Cantharellales	RAS458
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	PBM4292
Cantharellus persicinus	Hydnaceae	Cantharellales	RAS468
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	RAS473
Cantharellus altipes	Hydnaceae	Cantharellales	RAS084
Cantharellus appalachiensis	Hydnaceae	Cantharellales	RAS146
Craterellus dubius	Hydnaceae	Cantharellales	MH17001
Craterellus fallax	Hydnaceae	Cantharellales	EEApvecm2
Craterellus fallax	Hydnaceae	Cantharellales	PBM3290
Craterellus	Hydnaceae	Cantharellales	MBP06012010
Craterellus ignicolor	Hydnaceae	Cantharellales	AJF19
Cantharellus velutinus	Hydnaceae	Cantharellales	AJF32
Craterellus tubaeformis	Hydnaceae	Cantharellales	AJF66
Craterellus	Hydnaceae	Cantharellales	RAS393
Craterellus fallax	Hydnaceae	Cantharellales	TENN072344
Hydnum subolympicum	Hydnaceae	Cantharellales	MH16009
Hydnum umbilicatum	Hydnaceae	Cantharellales	TFB13482
Hydnum	Hydnaceae	Cantharellales	MES3213

Table 4. Continued.

Burgellopsis nivea	Hydnaceae	Cantharellales	ATCCMYA4209
Minimedusa pubescens	Hydnaceae	Cantharellales	ATCCMYA2993
Sistotrema	Hydnaceae	Cantharellales	MES2607
Sistotrema	Hydnaceae	Cantharellales	MES2725
Sistotrema	Hydnaceae	Cantharellales	MES2816
Sistotrema	Hydnaceae	Cantharellales	L13431
Sistotrema oblongisporum	Hydnaceae	Cantharellales	HHB11889
Sistotrema adnatum	Hydnaceae	Cantharellales	GB700
Sistotrema eximum	Hydnaceae	Cantharellales	T429
Sistotrema	Hydnaceae	Cantharellales	15C2D12
Sistotrema	Hydnaceae	Cantharellales	CLS16
Sistotrema oblongisporum	Hydnaceae	Cantharellales	GB511
Sistotrema brinkmanii	Hydnaceae	Cantharellales	RLG6932
Sistotrema	Hydnaceae	Cantharellales	FP133188A
Burgoa verzuoliana	Hydnaceae	Cantharellales	CBS32075
Sistotrema coronilla	Hydnaceae	Cantharellales	HHB10070
Sistotrema raduloides	Hydnaceae	Cantharellales	FP135005
Sistotrema raduloides	Hydnaceae	Cantharellales	HHB14321
Sistotrema raduloides	Hydnaceae	Cantharellales	Whitney8
Sistotrema brinkmanii	Hydnaceae	Cantharellales	Colo51175
Sistotrema coronilla	Hydnaceae	Cantharellales	RAS538
Multiclavula mucida	Hydnaceae	Cantharellales	RAS525
Clavulina	Hydnaceae	Cantharellales	RAS497
Clavulina cinerea	Hydnaceae	Cantharellales	RAS494
Clavulina	Hydnaceae	Cantharellales	RAS569
Clavulina	Hydnaceae	Cantharellales	RAS634
Multiclavula mucida	Hydnaceae	Cantharellales	RAS392
Clavulina	Hydnaceae	Cantharellales	RAS642
Clavulina	Hydnaceae	Cantharellales	RAS643
Clavulina	Hydnaceae	Cantharellales	MES2892
Clavulina cinerea	Hydnaceae	Cantharellales	AM-AR17-014
Sistotrema	Hydnaceae	Cantharellales	FP102031
Hydnum	Hydnaceae	Cantharellales	MK08141509
Hydnum albidum	Hydnaceae	Cantharellales	RAS210
Hydnum washingtonianum	Hydnaceae	Cantharellales	isotype
Sistotrema brinkmanii	Hydnaceae	Cantharellales	Whit7
Sistotrema brinkmanii	Hydnaceae	Cantharellales	76-46/1
Hydnum vagabundum	Hydnaceae	Cantharellales	MO298808
Hydnum	Hydnaceae	Cantharellales	44LL
Craterellus	Hydnaceae	Cantharellales	NJJ15_053
Craterellus ignicolor	Hydnaceae	Cantharellales	RAS589
Craterellus dubius	Hydnaceae	Cantharellales	MH20007
Craterellus caeruleofuscus	Hydnaceae	Cantharellales	RAS674
Craterellus carolinensis	Hydnaceae	Cantharellales	PBM4446
Craterellus odoratus	Hydnaceae	Cantharellales	RAS331
Craterellus foetidus	Hydnaceae	Cantharellales	RAS277B
Craterellus calyculus	Hydnaceae	Cantharellales	MGW969
Craterellus fallax	Hydnaceae	Cantharellales	RAS576
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	SAT1519702
Craterellus	Hydnaceae	Cantharellales	MES3362
Craterellus caeruleofuscus	Hydnaceae	Cantharellales	LJ2020-1
Craterellus foetidus	Hydnaceae	Cantharellales	LJ2020-3
Cantharellus minor	Hydnaceae	Cantharellales	RAS644
Cantharellus altipes	Hydnaceae	Cantharellales	RAS639
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	MH20005
Cantharellus persicinus	Hydnaceae	Cantharellales	PBM3933
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	RAS056
Cantharellus flavolateritius	Hydnaceae	Cantharellales	PBM4264
Cantharellus	Hydnaceae	Cantharellales	PBM4265

Table 4. Continued.

Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	PBM4267
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	RAS447
Cantharellus velutinus	Hydnaceae	Cantharellales	PBM4290
Cantharellus tenuithrix complex	Hydnaceae	Cantharellales	RAS464
Cantharellus flavolateritius	Hydnaceae	Cantharellales	RAS474
Cantharellus corallinus	Hydnaceae	Cantharellales	RAS445
Cantharellus camphoratus	Hydnaceae	Cantharellales	RAS614
Cantharellus flavolateritius	Hydnaceae	Cantharellales	RAS590
Cantharellus corallinus	Hydnaceae	Cantharellales	RAS629
Cantharellus betularum	Hydnaceae	Cantharellales	RAS631
Cantharellus camphoratus	Hydnaceae	Cantharellales	RAS659
Cantharellus vicinus	Hydnaceae	Cantharellales	RAS669
Cantharellus minor	Hydnaceae	Cantharellales	RAS718
Cantharellus corallinus	Hydnaceae	Cantharellales	RAS757
Cantharellus quercophilus	Hydnaceae	Cantharellales	RAS758
Cantharellus	Hydnaceae	Cantharellales	RAS785
Burgella flavoparmeliae	Hydnaceae	Cantharellales	Buck38682
Neoburgoa freyi	Hydnaceae	Cantharellales	EZLF1256
Neoburgoa freyi	Hydnaceae	Cantharellales	EZ4455
Bulbilla applanata	Hydnaceae	Cantharellales	Flakus16422
Bulbilla applanata	Hydnaceae	Cantharellales	Flakus16424
Hyphodontia	Hyphodontiaceae	Hymenochaetales	HHB10247
Hyphodontia	Hyphodontiaceae	Hymenochaetales	HHB17983
Inocybe soluta	Inocybaceae	Agaricales	JH1356_20
Inocybe picrosma	Inocybaceae	Agaricales	PBM1758
Inocybe lilacina	Inocybaceae	Agaricales	PBM3940
Inocybe aff. *fibrosa*	Inocybaceae	Agaricales	RAS424
Inocybe	Inocybaceae	Agaricales	PBM4219
Peniophora	Peniophoraceae	Russulales	HHB19494
Peniophora	Peniophoraceae	Russulales	FP135449
Bjerkandera	Phanerochaetaceae	Polyporales	FP133581
Sebacina schweinitzii	Sebacinaceae	Sebacinales	SAT1518911
Sebacina schweinitzii	Sebacinaceae	Sebacinales	SAT1519202
Sebacina epigaea	Sebacinaceae	Sebacinales	RAS721
Sistotremastrum	Sistotremastraceae	Sistotremastrales	HHB432
Stereum hirsutum	Stereaceae	Russulales	IMI44225
Tulasnella violea	Tulasnellaceae	Cantharellales	FP134258
Tulasnella cf. *asymmetrica*	Tulasnellaceae	Cantharellales	RAS537 culture
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB10152
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB15716
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB7330
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB11831
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB13471
Tulasnella	Tulasnellaceae	Cantharellales	HHB17767
Tulasnella allantospora	Tulasnellaceae	Cantharellales	HHB14054
Tulasnella araneosa	Tulasnellaceae	Cantharellales	HHB10156
Tulasnella pruinosa	Tulasnellaceae	Cantharellales	HHB10208
Tulasnella violea	Tulasnellaceae	Cantharellales	HHB3860
Tulasnella	Tulasnellaceae	Cantharellales	LR-31
Tulasnella	Tulasnellaceae	Cantharellales	MES2153
Protomerulius minor		Auriculariales	RAS536
Auriculariales		Auriculariales	PR2284

Table 5. Locus-specific primers and PCR conditions.

Locus	Primer	Sequence (5'–3')	Reference	Thermocycler conditions
18S	NS1	GTAGTCATATGCTTGTCTC	White et al. 1990	1.5 min @ 95 C 35 cycles of: 1 min @ 95 C 1 min @ 54 C 1 min @ 72 C 10 min @ 72 C
	SR2	CGGCCATGCACCACC	Vilgalys lab; DePriest 1993	
ITS	ITS1F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns 1993	
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990	
28S	LR0R	ACCCGCTGAACTTAAGC	Cubeta et al. 1991	
	LR7	TACTACCACCAAGATCT	Vilgalys & Hester 1990	
rpb 1	RPB1-Af2	GAKTGTCCKGGWCATTTTGG	Matheny et al. 2002	Matheny et al. 2002
	RPB1-Crev	CCNGCDATNTCRTTRTCCATRTA		
rpb 2	bRPB2-6F	TGGGGYATGGTNTGYCCYGC	Matheny 2005	
	bRPB2-7.1R	CCCATRGCYTGYTTMCCCATDGC		
	Canth6F-1	TGGGGNATGGTNTGYCC	This study	
	Canth7.1R-1	CCCATNGCYTGYTTVCCCATNGC		
tef 1	tef1F	TACAARTGYGGTGGTATYGACA	Morehouse et al. 2003	Morehouse et al. 2003
	tef1R	ACNGACTTGACYTCAGTRGT		
	983F	GCYCCYGGHCAYCGTGAYTTYAT	Rehner & Buckley 2005	Rehner & Buckley 2005
	2218R	ATGACACCRACRGCRACRGTYTG		

Table 6. Total number of attempted and successfully sequenced samples with mean number of sequence variants produced for each locus.

Locus	Total # attempted samples	Total # of successful samples	Mean # of sequence variants per sample
18S	273	247	1.23
ITS	273	213	1.85
28S	273	178	1.25
rpb 1	153	15	1.93
rpb 2	253	164	2.63
tef 1	166	69	3.64

Table 7. Total number of attempted and successfully sequenced samples with mean number of sequence variants produced for each locus and maximum within-sample sequence variant divergence by taxonomic family.

Family	Total # attempted samples	Total # of successful samples (1 or more loci)	Mean # of sequence variants per sample						Max within-sample sequence variant divergence
			18S	ITS	28S	*rpb* 1	*rpb* 2	*tef* 1	
Hydnaceae	151	151	1.21	1.76	1.35	1.92	2.41	2.76	3.2%
Botryobasidiaceae	50	37	1.24	1.72	1.06	n/a	3.53	3.33	8.8%
Ceratobasidiaceae	38	27	1.46	2.08	1.18	n/a	4	5.5	5.6%
Tulasnellaceae	12	8	1.2	2.87	n/a	n/a	1.6	3.67	1.7%
Inocybaceae	5	5	1	2.4	1	1	1.8	n/a	2.8%
Other families	16	15	1.11	1.89	1.06	4	1.89	3.75	5.1%
Total	272	243	1.23	1.85	1.25	1.93	2.63	3.64	

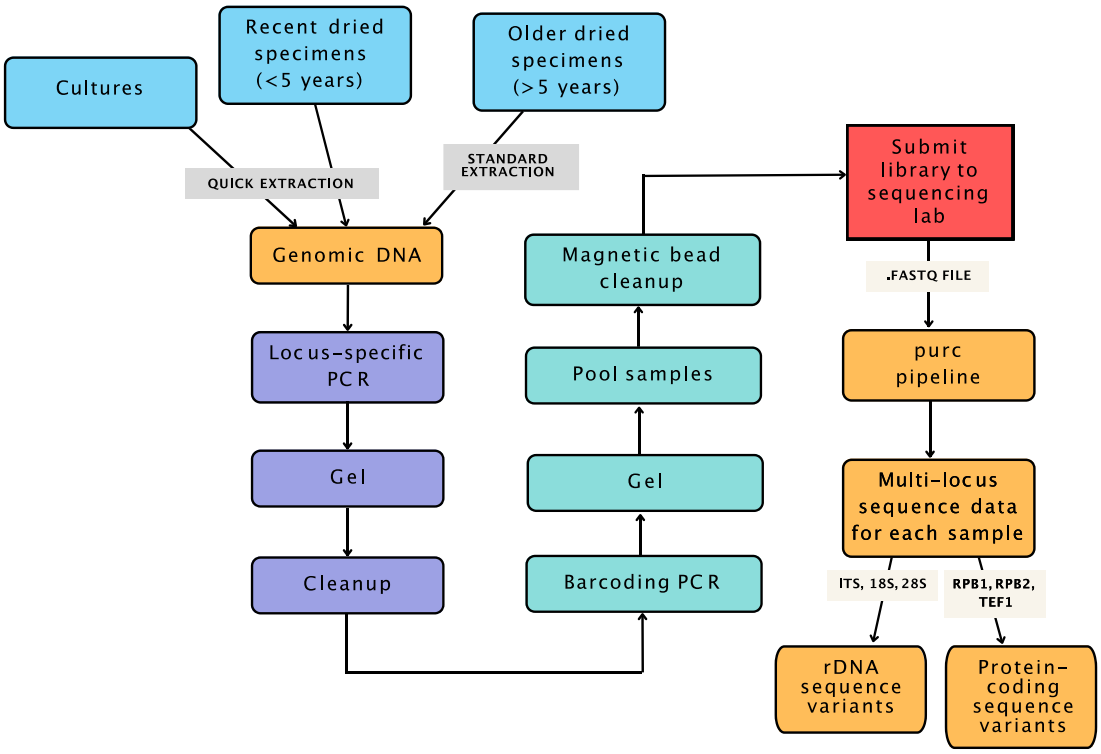

Figure 17. The PacBio multi-locus long-read high throughput sequencing (HTS) workflow.

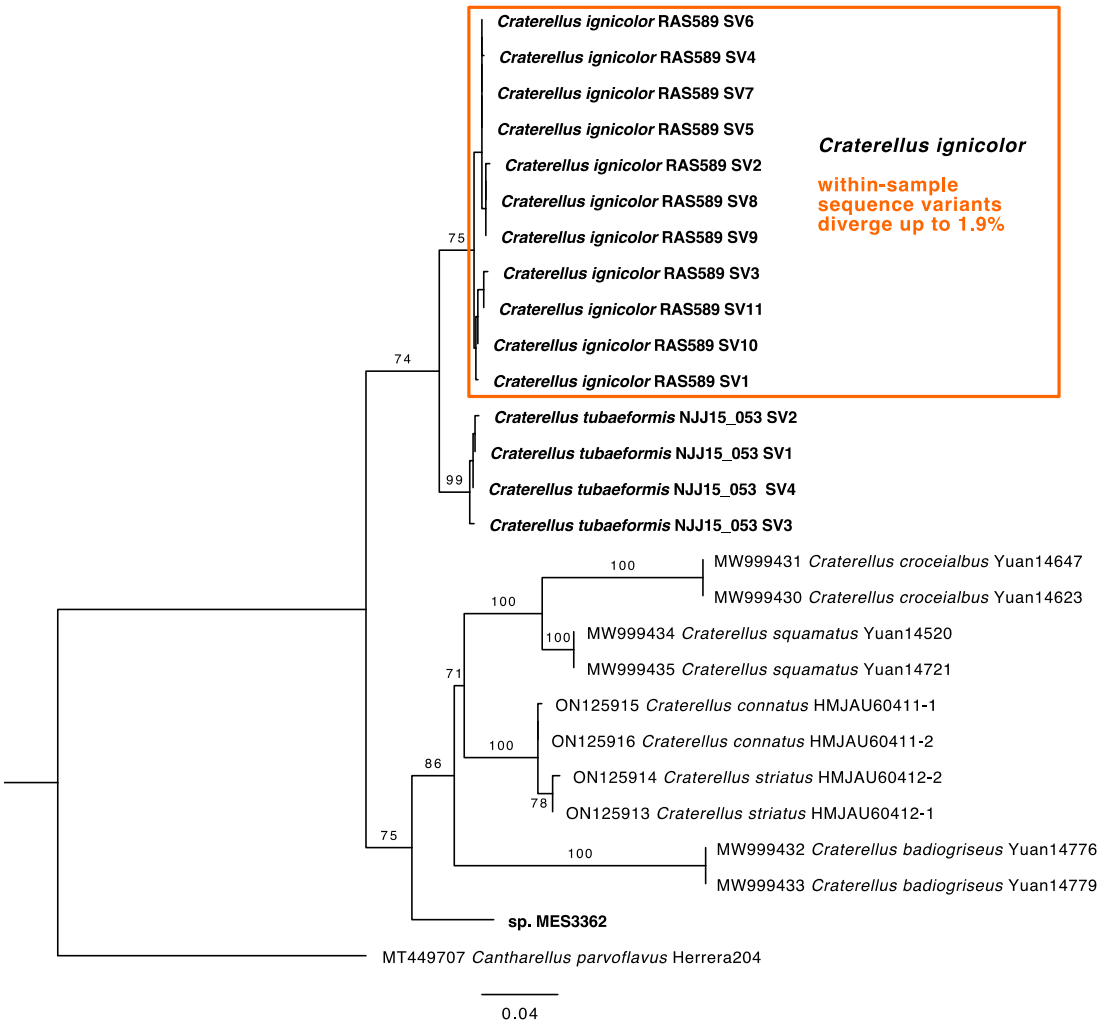

Figure 18. Intra-individual diversity of the *tef*1 locus across *Craterellus.* Sequences produced for this study are in bold text. Bootstrap support values are shown above branches. Within-sample sequence variant divergence up to 1.9% was detected among 11 sequence variants of *tef*1 in *Craterellus ignicolor* RAS589 (fruiting body isolate).

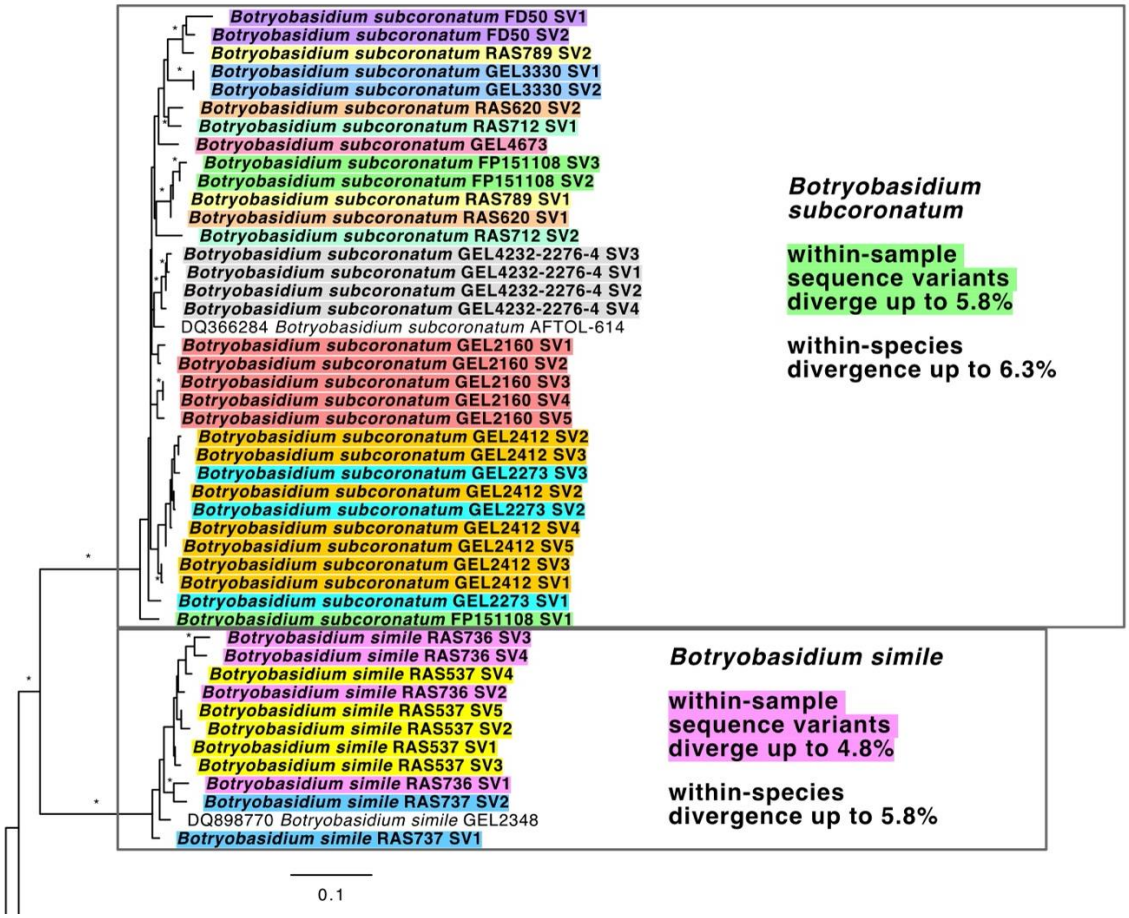

Figure 19. Intra-individual and intraspecific diversity of *rpb*2 across samples of *Botryobasidium subcoronatum* and *B. simile* (culture and fruiting body isolates). Sequences produced for this study are in bold text. Bootstrap support values ≥ 70 are denoted by an asterisk (*). Intraspecific sequence divergence in both species was greater than 5.5%.

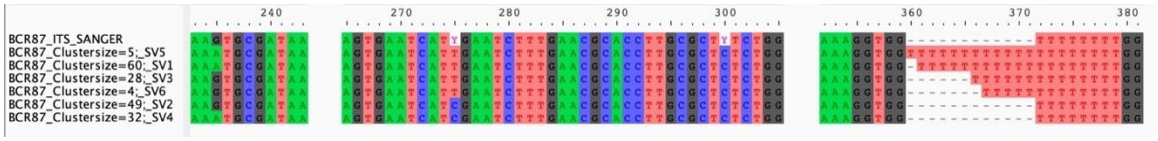

Figure 20. Comparison of intra-individual genetic variation captured by Sanger sequencing vs. PacBio HTS for a single *Hydnum* sample (fruiting body isolate). A Sanger sequence (top row) is shown in comparison to six PacBio long-read HTS sequence variants (SV1–6) from the ITS locus. Nucleotide positions are presented above sequences. Sequence polymorphisms captured in the Sanger sequence are reflected in the long-read HTS sequence variants at positions 275 and 300, but the polymorphic site at position 235 and length heterogeneity at sites 360–71 are not captured by the Sanger sequence.

	RAS538	RAS594
Method	**Taxa Recovered**	
Microscopy	*Sistotrema* , *Tulasnella*	*Botryobasidium*
ITS (Sanger)	*Sistotrema*	*Botryobasidium*
ITS (PacBio HTS)	*Sistotrema*	*Botryobasidium* , Ascomycete (Helotiales)
18S (PacBio HTS)	*Sistotrema*	*Botryobasidium* , *Tulasnella*
28S (PacBio HTS)	*Sistotrema*	*Botryobasidium*
rpb 2 (PacBio HTS)	*Sistotrema* , *Tulasnella*	*Botryobasidium* , *Tulasnella*
tef 1 (PacBio HTS)	*Sistotrema*	*Botryobasidium* , Ascomycete (Helotiales), *Rhizoctonia*

Figure 21. Two corticioid Cantharellales specimens containing non-target taxa. Fungal taxa detected varied by sequencing method and locus.

CHAPTER IV
A PHYLOGENETIC OVERVIEW OF THE CANTHARELLALES (CHANTERELLES AND ALLIES)

Abstract

The agaricomycete order Cantharellales contains approximately 1,000 species of fungi characterized by diverse morphological forms and nutritional strategies. Examples of these morphologies and ecological guilds include coralloid lichens that form symbioses with unicellular green algae, bulbil-forming lichenicolous species, corticioid free-living fungi that degrade dead organic carbon, pathogens that cause disease in agricultural crops, orchid root symbionts, and ectomycorrhizal mushroom-forming fungi. The order also includes popular edible mushrooms that are sold in markets worldwide. Despite recent advances to characterize relationships across the fungal tree of life, evolutionary relationships in the Cantharellales remain poorly understood. Here, I construct a multi-gene phylogeny of the Cantharellales using data generated from 323 specimens across the order to evaluate family-level relationships in the order. Four monophyletic families were recovered with strong measures of statistical support. These include Hydnaceae *sensu lato*, Tulasnellaceae, Botryobasidiaceae, and Ceratobasidiaceae. Across the order, three clades contain ectomycorrhizal fungi and seven clades contain lichenicolous forms. Seven different clades contained samples of the polyphyletic genus *Sistotrema*, six of which will require taxonomic reassignment to other genera. This study provides a framework for examining the evolution of form and function across this ecologically and morphologically diverse order of fungi.

Introduction

The Agaricomycetes, a class of predominantly mushroom-forming fungi, contains more than 20,000 described species that function in a variety of ecological roles as mutualists, pathogens, and decomposers in ecosystems throughout the globe (Hibbett et al. 2014). The Cantharellales is an order of mushroom-forming fungi that is sister to the rest of the Agaricomycetes (Varga et al. 2019; Sánchez-García et al. 2020). Members of the Cantharellales display a diverse array of morphologies and nutritional modes (Figure 22; all Tables and Figures are in Appendix), including popular ectomycorrhizal edible mushrooms such as the chanterelles and hedgehogs, corticioid (resupinate) saprobes that degrade dead wood, and destructive plant pathogens that cause blight on important crop plants such as peanuts, rice, and cucumbers (Anderson 1982; Moncalvo et al. 2006; Olariaga et al. 2017). Other species are lichenized, lichenicolous, or orchid symbionts (Lawrey et al. 2016; Oberwinkler et al. 2017).

The Cantharellales is an order of nearly 1,000 species (per indexfungorum.org). Seven families have been described within the order: Cantharellaceae, Clavulinaceae, Hydnaceae, Sistotremataceae, Botryobasidiaceae, Ceratobasidiaceae, and Tulasnellaceae. Early research into members of the order relied on basidiome, spore, and basidial morphology for classification. The diversity of basidiome and hymenophore morphologies across the Cantharellales meant stipitate members such as *Cantharellus* were categorized separately from corticoid Cantharellales like *Sistotrema*. Due to differing hymenophore types among stipitate Cantharellales, genera with gill-like (*Cantharellus*, *Craterellus*), coralloid (*Clavulina*, *Multiclavula*), and toothed (*Hydnum*)

morphologies were thought to be distantly related. However, characteristics of the basidia were used to infer a potential relationship among diverse basidiome types. For example, Penancier (1961) noted members of the Cantharellaceae, Clavulinaceae, Hydnaceae, and Corticiaceae (including *Sistotrema*) shared the presence of stichic basidia, meiosporangia characterized by a longitudinally oriented meiotic spindle. Donk (1964) suggested the presence of stichic basidia was a potentially shared derived trait indicating a close relationship among those taxa. Despite this, Jülich (1981) segregated the genus *Sistotrema* into its own family, Sistotremaceae, within the order Sistotremales based on basidium shape and number of spores.

The remaining corticioid Cantharellales were initially considered "tulasnelloid fungi". Later, the families Tulasnellaceae, Ceratobasidiaceae, and Botryobasidiaceae were recognized based on differences in basidium morphology and the presence or absence of repetitively germinating basidiospores (Juel 1897, Martin 1948, Donk 1956). However, taxonomists disagreed over how and whether these three families were closely related. The morphological classification developed by Rea (1922) segregated *Tulasnella* in its own order, Tulasnellales, based on its basidia with basally septate sterigmata. Oberwinkler (1982) retained Ceratobasidiaceae and Tulasnellaceae under Tulasnellales, while Botryobasidiaceae was considered distantly related. Jülich (1981) placed Ceratobasidiaceae and Botryobasidiaceae into the new orders Ceratobasidiales and Botryobasidiales, while noting a close relationship between Ceratobasidiales, Botryobasidiales, and Tulasnellales.

The earliest molecular phylogenetic studies referred to these various taxonomic groups as members of the "cantharelloid clade" (Pine et al. 1999; Binder et al. 2005; Moncalvo et al. 2006; Matheny et al. 2007). The families Cantharellaceae, Clavulinaceae, Hydnaceae, Botryobasidiaceae, Ceratobasidiaceae, and Tulasnellaceae have most commonly been recognized within the Cantharellales based on phylogenetic evidence (Moncalvo et al. 2006; Veldre et al. 2013; Diederich et al. 2014; Hibbett et al. 2014; Cao et al. 2021). A summary of classification schemes for Cantharellales is summarized in Table 8.

Today, as many as seven families have been recognized within the Cantharellales: Cantharellaceae, Clavulinaceae, Hydnaceae, Sistotremataceae, Botryobasidiaceae, Ceratobasidiaceae, and Tulasnellaceae. However, broader relationships among these families are unclear as studies have produced conflicting topologies, particularly regarding the positions of Botryobasidiaceae, Ceratobasidiaceae, and Tulasnellacae (Moncalvo et al. 2006; Veldre et al. 2013; Cao et al. 2021). The relatively few Cantharellales genomes that have been sequenced have done little to resolve family-level relationships, perhaps due to limited taxon sampling (Miyauchi et al. 2020; see also https://mycocosm.jgi.doe.gov/). One genus, *Sistotrema*, comprises five or more separate clades, three of which cannot be placed within existing families (Moncalvo et al. 2006). Several lichenicolous lineages in the Cantharellales have been recognized and described as unique genera but occupy unresolved positions in the order (Diederich et al. 2014; Lawrey et al. 2016; Diederich et al. 2022). To date, there is no densely sampled phylogeny of the Cantharellales that resolves these higher-level relationships and provides a robust internal framework to understand diversification and evolution of features within the group.

A phylogenetic framework can enable future studies of historical biogeography, species diversification, and evolutionary ecology. In particular, the Cantharellales is proportionally rich in ectomycorrhizal (ECM) species (Tedersoo et al. 2010). ECM fungi represent a major ecological guild that forms root symbioses with approximately 6,000 species of land plants and are critical to ecosystem health. The Cantharellales also contains a high number of lichenicolous lineages relative to other groups of mushroom-forming fungi (Diederich et al. 2022). Sánchez-García et al. (2020) suggested that the most recent common ancestor of the Cantharellales was a saprotroph, and the many corticioid Cantharellales lineages retained wood-decay capabilities. However, the order diverged before the origin of white rot capabilities in Agaricomycetes, and the mechanism of carbon degradation in these fungi has not yet been elucidated (Martin et al. 2016). In light of this, I constructed a multi-gene phylogeny of the Cantharellales to test the hypothesis that there are seven well-supported families in the order, and to enable future studies using phylogenetic comparative methods.

Materials and Methods

Taxon sampling

A literature search was conducted to compile a list of accepted genera within Cantharellales (Table 9). Samples were collected in the field, preserved, and accessioned at the University of Tennessee Herbarium (TENN). Cultures were attempted for samples of *Botryobasidium*, *Sistotrema*, and *Tulasnella* on malt extract or potato dextrose agar plates. Plates were incubated at 20–22° C. Additional samples comprised specimens from the University of Tennessee Herbarium, cultures from the Center for Forest Mycology Research, G. Langer (Northwest German Forest Research Institute), and M. Cubeta (North Carolina State University); and archival DNA isolates. A total of 223 Cantharellales specimens and one outgroup were selected for PacBio sequencing representing all seven families within the order. Sanger sequences from 24 collections were included to expand gene coverage and improve taxon sampling relative to estimated species diversity.

DNA extraction, gene sampling, and sequencing

Tissue samples were collected from specimens for DNA extraction using either an Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, Missouri) for fresh or recently dried specimens < 5 years old and cultures. Older dried specimens > 5 years old were extracted using an HP Fungal DNA Extraction Kit (Omega Bio-Tek, Norcross, Georgia, USA). For specimens > 25 years, DNA was extracted using the method outlined for type specimen extraction in Swenie et al. (2018).

A gene sampling strategy was established that targeted five nuclear loci: 18S rDNA (18S), ITS1+5.8S+ITS2 (ITS), the 5’ end of 28S rDNA (28S), and portions of two nuclear protein-coding genes, *rpb2* and *tef1*. These loci have been the most frequently used in previous phylogenetic studies of Cantharellales (Moncalvo et al. 2006; Lawrey et al. 2016; Olariaga et al. 2017; Cao et al. 2021). Select Cantharellales sequences were downloaded from GenBank if three or more loci were available from the same source

sample. Additional Cantharellales sequences were obtained from and JGI MycoCosm (https://mycocosm.jgi.doe.gov/) targeting protein-coding sequence data from genomes. Additional sequences were included from holotypes and additional species within the lichenized, lichenicolous, corticioid, and aquatic genera *Bergerella, Bulbilla*, *Burgoa*, *Bryoclavula, Membranomyces, Multiclavula, Minimedusa, Neoburgoa, Parmeliicida*, and *Rogersiomyces*, regardless of number of loci available per sample, due to lack of representation of these lineages. *Burgoa moriformis* was excluded due to high 28S sequence divergence. All specimens and GenBank accession numbers are listed in Table 9.

For Sanger sequencing, primers were used in the following combinations to amplify and sequence the ITS, 28S, *tef1,* and *rpb2* regions, respectively: ITS1F/ITS4, ITS1F/ITS2, or 5.8SR/ITS4 (White et al. 1990); LR0R/LR5 or LR0R/LR7 using LR5 as an internal sequence primer (Cubeta et al. 1991; Vilgalys and Hester 1990), tef1F/tef1R, 983F/2218R, or HEF1F/HEF1R (Morehouse et al. 2003; Rehner and Buckley 2005; Feng et al. 2016); and b6F/b7.1R (Matheny 2005). Sequencing was performed on an Applied Biosystems 3730 Genetic Analyzer at the University of Tennessee Genomics Core, and sequence reads were assembled in Sequencher 5.0.1 (Gene Codes, Ann Arbor, Michigan).

For PacBio high throughput multi-locus sequencing (MLST), methods are outlined in Chapter 3 of this dissertation. Briefly, multi-locus sequence data were generated for the 18S, ITS, 28S, *tef1,* and *rpb2* regions using a multiplexed two-step PCR amplification and library preparation for sequencing on the PacBio Sequel platform using one SMRT cell. Samples were spread across two sequencing runs and the resulting sequence data were demultiplexed, error-corrected, sorted by locus, and clustered into sequence variants with a read depth of four or greater.

DNA alignment and phylogenetic analyses

Sequences were aligned separately for each of the five loci using MUSCLE in AliView 1.27 (Larsson 2014). Minor adjustments were made manually to each alignment, and primer sequences were trimmed from the beginning and ends of sequences where necessary. Maximum likelihood (ML) phylogenetic analysis was performed separately for each of the five individual loci using RAxML 8.2.9 (Stamatakis 2014) with 1000 bootstrap replicates under the GTR+GAMMA substitution model as recommended by the user manual. *Dacrymyces* sp. FPL8953 and *Dacrymyces chrysospermus* AMH013 (Dacrymycetes) served as outgroups due to the sister relationships between Dacrymycetes and Agaricomycetes. *Holtermanniella wattica* CBS 9496 (Tremellomycetes) was added as an additional outgroup as Tremellomycetes are sister to the clade containing Aagaricomycetes and Dacrymycetes. After inspecting the individual gene phylogenies for strongly supported intergene conflict and potential rogue taxa, the phylogenies were concatenated in SeaView, excluding the 18S and ITS rDNA loci for *Cantharellus*, *Craterellus,* and *Tulasnella*. Data were partitioned by locus and coding region and an additional ML analysis was performed on the five-locus concatenated alignment. IQ-TREE 2.2 was used to test model fit for the partitioned five-locus dataset, and an additional ultrafast (UF) 1000 bootstrap analysis was run along with the SH-aLRT (SH) and aBayes branch support tests (Nguyen et al. 2015; Hoang et al. 2018; Guindon et

al. 2010; Anisimova et al. 2011). ML bootstrap proportions ≥70%, UF bootstraps ≥95%, SH-aLRT values ≥ 80%, and aBayes values ≥ 90% were considered evidence of strong support. FigTree 1.4.4 was used to visualize the phylogenies. Edits to the tree figures were made in Affinity Designer 1.10.6 (Serif Europe Ltd, United Kingdom).

Molecular dating analysis

The five-locus ML phylogeny was time calibrated by penalized likelihood in R 4.3.1 using the chronos function in ape (R Core Team 2023; Paradis & Schliep 2019). The node representing the most recent common ancestor of Cantharellales (Agaricomycetes) and Dacrymycetes was constrained to the Carboniferous period (299–359 Mya), an estimate supported by numerous previous studies (Floudas et al. 2012; Garnica et al. 2016; Varga et al. 2019; Sánchez-García et al. 2020; Miyachi et al. 2020).

Results

22 of the 25 accepted Cantharellales genera (Table 9) were represented by one or more sequences in the phylogenies. Sequences were not obtained from samples of *Ingoldiella*, and *Sistotremella* and *Afrocantharellus* were excluded due to high sequence divergence and limited gene sampling. Multiple studies have recovered *Afrocantharellus* as a sister clade to *Cantharellus* (Tibuhwa et al. 2012; Buyck et al. 2014; Shao et al. 2014). Cao et al. (2021) found *Sistotremella* occupied an uncertain phylogenetic position within the Hydnaceae.

The various single gene phylogenies contained 976 sequences from 323 samples (Table 9; sequence data are available in the NCBI Sequence Read Archive, BioProject PRJNA945373). Splicesomal introns of *tef1* and *rpb2* were retained if alignable across all taxa. One 410 bp insertion was excluded from 28S. The 18S alignment contained 223 sequences and 1228 sites; the ITS alignment 227 sequences and 1081 sites; the 28S alignment 209 sequences and 1570 sites; the *tef1* alignment 112 sequences and 1370 sites; and the *rpb2* alignment 211 sequences and 1177 sites. The five-locus concatenated alignment contained sequences from 303 samples and included 6426 sites.

The Cantharellales was recovered as a monophyletic group with strong statistical support across the multi-gene (Figure 23) and single-gene (Figures 24–28) phylogenies. The five-locus phylogeny supported four monophyletic families in the Cantharellales with high ML bootstrap values: Hydnaceae *sensu lato* (including the formerly recognized families Cantharellaceae, Clavulinaceae, and Hydnaceae *sensu stricto*), Tulasnellaceae, Botryobasidiaceae, and Ceratobasidiaceae. These results support the previous classifications of Hibbett et al. (2014) and Cao et al. (2021) (Table 8). Ceratobasidiaceae was strongly supported as sister to the rest of the order, and Botryobasidiaceae as sister to the clade containing Tulasnellaceae and Hydnaceae *sensu lato*. Tulasnellaceae and Hydnaceae were identified as sister groups in the five-locus phylogeny. Topologies varied across the single-gene phylogenies, but no strongly supported gene conflict was detected at nodes deeper than the genus level. Hydnaceae *sensu lato* received strong bootstrap support in the multi-gene analysis and the 18S, 28S, and *tef1* phylogenies. Tulasnellaceae was poorly supported across all single-gene phylogenies except 18S,

where it was sister to *Cantharellus* and *Craterellus* probably due to effects of long branch attraction. The 18S and ITS loci exhibited unusually high rates of change for *Tulasnella*, *Cantharellus*, and *Craterellus*, as noted previously (Feibelman et al. 1994; Moncalvo et al. 2006).

The family Botryobasidiaceae contained one genus, *Botryobasidium*, with several strongly supported subclades. Taxonomic revision would be required to determine whether the genus merits subdivision into additional genera. Similarly, the family Tulasnellaceae contained a single genus, *Tulasnella*, species of which were highly divergent including at low-copy protein-coding loci *tef1* and *rpb2*. The genera *Ceratobasidium*, *Rhizoctonia*, and *Thanatephorus* were each polyphyletic. Species-level clades received strong support in a few instances, such as *Ceratobasidium bulbillifaciens* and *C. oryzae-sativae*, but some anastomosis groups of *Rhizoctonia solani* (i.e., AG-1, AG-4) were not monophyletic.

The multi-gene phylogeny recovered seven disparate clades of *Sistotrema* in the Hydnaceae *sensu lato*. *Sistotrema sensu stricto*, containing the ECM pileate-stipitate type species *S. confluens* and additional corticoid ECM *Sistotrema* taxa, was monophyletic. *Sistotrema sensu stricto* plus additional corticioid *Sistotrema* lineages formed a grade (paraphyletic group) giving rise to the ECM pileate-stipitate mushroom genus *Hydnum*. The lichenicolous bulbil-forming genus *Neoburgoa* formed a strongly supported sister group to *Hydnum* and *Sistotrema sensu stricto*. Together, these taxa were closely related to the lichenicolous *Burgellopsis* and saprotrophic *Sistotrema raduloides*. *Burgellopsis*, another lichenicolous bulbil-forming lineage, was represented by a single specimen that was weakly supported as sister to the aforementioned groups. The lichenicolous genus *Minimedusa*, previously considered closely related to *Hydnum* and *Sistotrema sensu stricto* (Lawrey et al. 2007), was found to be polyphyletic. *Minimedusa polyspora* (type) and *M. obcoronata* formed a strongly supported clade together, whereas *Minimedusa pubescens* formed a separate well supported sister clade with *Sistotrema coronilla* and *S. adnatum*. The sole member of the lichenized coral genus *Bryoclavula*, *B. phycophila*, was recovered as sister to *Minimedusa*, *Sistotrema coronilla*, and *S. adnatum* but with weak statistical support. A clade containing the lignicolous members of the bulbil-forming genus *Burgoa* and lignicolous corticioid species *Sistotrema sernanderi*, *S. biggsiae*, and *S. eximum* formed a clade sister to the rest of the Hydnaceae *sensu lato* with weak support.

Taxa classified in the family Clavulinaceae included the stipitate and mostly coralloid ECM *Clavulina* mushrooms, the corticioid ECM genus *Membranomyces*, three independent clades of saprotrophic *Sistotrema*, the aquatic lignicolous corticioid *Rogersiomyces*, lichenicolous genera *Bulbilla*, *Burgella*, and *Parmeliicida*, and the lichenized coralloid *Multiclavula*. Broader relationships across this group mostly received strong statistical support, excepting *Clavulina*, which was recovered as paraphyletic when ITS sequences of *Membranomyces* were included. Samples of *Sistotrema brinkmanii* formed a genetically diverse but monophyletic group sister to *Clavulina*, *Membranomyces*, and *Sistotrema luteoviride*. *Burgella*, *Bulbilla*, *Rogersiomyces*, and three unidentified *Sistotrema* samples formed a weakly-supported clade. Sister to the remainder of the Clavulinaceae was a clade containing *Multiclavula* and *Parmeliicia*.

The family Cantharellaceae contained the edible ectomycorrhizal chanterelle and trumpet mushrooms (*Cantharellus* and *Craterellus*, respectively). The sister relationship between these two genera was strongly supported across all phylogenetic analyses, as reported from previous studies (Moncalvo et al. 2006; Matheny et al. 2007; Wilson et al. 2012; Cao et al. 2021). Lichenicolous Cantharellales were also confined to the Hydnaceae *sensu lato*, except *Ceratobasidium bulbillifaciens* in the Ceratobasidiaceae.

The time-calibrated phylogeny (Figure 29) estimated the age of the Cantharellales at 324 Mya. Within the Hydnaceae *sensu lato*, the phylogeny suggests three potential transitions to an ECM nutritional mode were observed during the Mesozoic Era (252–66 Mya): one in the common ancestor of *Clavulina* and *Membranomyces*, one in the common ancestor of *Hydnum* and *Sistotrema* sensu stricto, and one in the common ancestor of *Cantharellus* and *Craterellus* (Figure 29). Two additional transitions to ECM habit occurred in the Ceratobasidiaceae (Veldre et al. 2013). Seven clades in the Cantharellales phylogeny contained lichenicolous taxa (Figure 29).

Discussion

This is the first contribution towards a densely-sampled phylogeny of the Cantharellales based on multi-gene sampling. The five-locus phylogeny strongly supports the recognition of four families within the order, rather than a classification of seven families. A more inclusive Hydnaceae (Hydnaceae *sensu lato*) is monophyletic across the multi-gene and protein-coding phylogenies with strong statistical support in the ML multi-gene and three of the five individual gene trees. The previously recognized families Cantharellaceae, Clavulinaceae, and Hydnum *sensu stricto* receive strong statistical support in the multi-gene tree but show weak bootstrap support and are polyphyletic within the individual gene trees. An alternative classification recognizing these three families would require erecting three additional families to accommodate the clades labeled *incertae cedis* in Figure 23, but weakly supported relationships along the backbone of Hydnaceae *sensu lato* and a lack of clear shared characteristics make this a less parsimonious option.

The family Hydnaceae *sensu lato* encompasses the largest number of genera and the widest variety of morphological and functional groups in the Cantharellales. Stichic basidia, in which the meiotic spindle is vertically oriented, may be a synapomorphy for this clade (Donk 1933, Pine et al. 1999). Stichic basidia have been confirmed in samples of the stipitate ECM genera *Hydnum*, *Cantharellus*, and *Clavulina*, as well as the lichenized stipitate genus *Multiclavula*, but have not been investigated in the remaining genera in the family, including lichenicolous and bulbil-forming lineages and the corticioid *Sistotrema* groups. The families Tulasnellaceae, Botryobasidiaceae, and Ceratobasidiaceae each form strongly supported monophyletic groups and can be distinguished from one another on the basis of morphological and ecological traits (Anderson 1982; Langer 1994; Oberwinkler et al. 2017). However, taxon sampling is limited at the genus level for all three families and does not include sequences from all *Rhizoctonia* anastomosis groups, or synonyms of *Tulasnella* such as *Epulorhiza* and *Gloeotulasnella*, which lack multi-locus data and have highly divergent rDNA sequences.

The protein-coding loci *tef1* and *rpb2* provide greater phylogenetic resolution than the more widely used nuclear rDNA loci (18S, ITS, and 28S) across the Cantharellales phylogeny, particularly at deeper nodes. These two loci are also useful for species-level recognition in some Hydnaceae genera such as *Cantharellus* (see Chapter 2 of this dissertation), *Hydnum*, and *Clavulina*. Use of these and other protein-coding gene markers is recommended in addition to rDNA loci for future systematic studies of the Cantharellales. The inclusion of ITS sequences of the corticioid ectomycorrhizal genus *Membranomyces* renders *Clavulina* paraphyletic; however, no other loci besides ITS are available from *Membranomyces* samples, so additional gene sampling is needed to confirm this result.

Species recognition within each of the largely corticoid families Tulasnellaceae, Ceratobasidiaceae, and Botryobasidiaceae will require further studies that incorporate intra-individual genetic variation into species delimitation (see Chapter 3 of this dissertation). *Rhizoctonia* and *Thanatephorus* are two non-monophyletic genera in the Ceratobasidiaceae. Several anastomosis groups in *Rhizoctonia* are also non-monophyletic, reflecting the need for further taxonomic work in this family. The Tulasnellaceae traditionally contained the genera *Gloeotulasnella* and *Epulorhiza*, which are now considered synonyms of *Tulasnella* (Roche et al. 2010). Similarly, the Botryobasidiaceae now contains a single genus, *Botryobasidium*. *Botryohypochnus* is considered a synonym of *Botryobasidium* (Langer 1994), and *Botryobasidium* was recommended for use over the asexual synonym *Haplotrichum* (Stalpers et al. 2021).

This is the first systematic study of the Cantharellales to include protein-coding sequences of lichenicolous taxa. While all lichenicolous genera other than *Minimedusa* are strongly supported as monophyletic, in some cases their broader placement within Hydnaceae *sensu lato* remains poorly resolved probably owing to limited gene sampling (i.e, *Bergerella*, *Burgellopsis*, *Burgoa*). The lichenicolous species *Burgoa moriformis*, represented by a single 28S sequence, was omitted due to its uncertain position on a long branch. It remains unclear whether this species is more closely related to species in the Tulasnellaceae as previously suggested (Diederich et al. 2022), or other species of *Burgoa* in Hydnaceae. Greater taxon sampling and sequencing of protein-coding genes from lichenicolous type species are expected to increase confidence in the phylogenetic position of these lichenicolous lineages.

Broadly recognized, the genus *Sistotrema* contains 125 species (based on data from Indexfungorum.org). Traditionally, corticioid or pileate-stipitate species possessing urniform basidia with 6–8 sterigmata, smooth spores, and a monomitic hyphal system with oily inclusions in the subicular hyphae were placed in *Sistotrema* (Eriksson et al. 1984). Later phylogenetic studies of *Sistotrema* species revealed the genus as polyphyletic (Larsson et al. 2004; Moncalvo et al. 2006). The dozens of *Sistotrema* samples included in this study were distributed across seven distantly related groups. Based on the five-locus phylogeny (Figure 23), *Sistotrema sensu stricto* is monophyletyic. Additional *Sistotrema* samples, some of which are ECM, plus *Sistotrema sensu stricto* form a grade giving rise to *Hydnum*. The other six independent *Sistotrema* lineages require taxonomic reassignment into other genera. Several lichenicolous Cantharellales form monophyletic groups containing *Sistotrema* species. For example, the genus *Burgoa* contains both saprotrophic and lichenicolous bulbil-forming species

that have been suggested as anamorphic forms of the sexual species *Sistotrema eximum* and *S. sernanderi* (Lawrey et al. 2007). Sequences produced for this study of *S. biggsiae* match sequences of the type species *Burgoa verzuoliana*; like *B. verzuoliana, S. biggsiae* produces bulbils (Croan et al. 1999). Together, these samples form a strongly supported clade, including samples labeled S. *eximum* and *S. sernanderi*, suggesting several *Sistotrema* species outside of *Sistotrema sensu stricto* may be reassigned to the genus *Burgoa*. Similarly, three specimens sequenced for this study labeled *S. oblongisporum* and another labeled *Sistotrema* sp. form a strongly supported clade with two *Burgella* samples. The aquatic anamorphic genus *Ingoldiella* was later found to form a sexual state in culture which was described as *S. hamatum* (Nawawi & Webster 1982). However, the relationship of *S. hamatum* to other *Sistotrema* species remains unknown due to a lack of sequence data.

The dense species sampling presented here reveals a more complex picture of evolution of form and function in the Cantharellales than previous studies. The Cantharellales is shown to be an ancient group of fungi that diverged during the Carboniferous (Figure 29). The time-calibrated phylogeny suggests that early Cantharellales may have associated with progymnosperms (Lutzoni et al. 2018; Strullu-Derrien et al. 2018), and that most extant lineages in the order diverged after the Permian-Triassic extinction event. The evolution of ECM lineages may have coincided with the diversification of ECM conifers during the Mesozoic.

The evolution of lichenicolous forms, however, presents a more complex picture. While broader relationships across lichenicolous clades are not entirely clear, the topology suggests a hypothesis of multiple transitions to a lichenicolous habit in Cantharellales with subsequent transitions to other nutritional modes. While the clades containing lichenicolous Cantharellales vary in age between 50–250 Mya, this is considerably younger than the origin of ascolichens (Lutzoni et al. 2018).

Previous studies of agaricomycete diversification have considered lichenicolous fungi as parasitic corticioid forms, and other non-parasitic corticioid Cantharellales as saprotrophs. Yet, little is known about the functional ecology of lichenicolous and saprotrophic Cantharellales. Saprotrophic taxa in the order have a limited repertoire of lignocellulolytic enzymes and are thought to rely on decomposing low-lignin non-woody substrates (Martin et al. 2016). Additional research utilizing metabolomics and functional genomics is needed to understand whether all the presumed saprotrophic lineages in the Cantharellales are functionally similar.

The role of ECM symbiosis in the diversification of the Cantharellales remains unclear. While one recent study concluded that fruiting body form and not nutritional mode drove diversification in Agaricomycetes (Sánchez-García et al. 2020), multiple traits may underlie shifts in diversification rates (Caetano et al. 2018). Such dynamics have not been studied on a finer scale within the Cantharellales. Given the overwhelming species richness in the genera that produce stipitate basidiomes and function as ECM symbionts (*Hydnum*, *Clavulina*, *Cantharellus*, and *Craterellus*), I predict that the combination of stipitate basidiomes and ECM symbiosis promoted increased diversification in the Cantharellales. Future studies are planned to test this hypothesis using phylogenetic comparative methods.

REFERENCES

Anderson, N. A. (1982). The Genetics and Pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology*, *20*(1), 329–347. https://doi.org/10.1146/annurev.py.20.090182.001553

Anisimova, M., Gil, M., Dufayard, J. F., Dessimoz, C., & Gascuel, O. (2011). Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology*, *60*(5), 685–699.

Binder, M., Hibbett, D. S., Larsson, K. H., Larsson, E., Langer, E., & Langer, G. (2005). The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). *Systematics and Biodiversity*, *3*(2), 113–157. https://doi.org/10.1017/S1477200005001623

Buyck, B., Kauff, F., Eyssartier, G., Couloux, A., & Hofstetter, V. (2013). A multilocus phylogeny for worldwide Cantharellus (Cantharellales, Agaricomycetidae). *Fungal Diversity*, *64*(1), 101–121. https://doi.org/10.1007/s13225-013-0272-3

Caetano, D. S., O'Meara, B. C., & Beaulieu, J. M. (2018). Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution*, *72*(11), 2308–2324. https://doi.org/10.1111/evo.13602

Cao, T., Hu, Y. P., Yu, J. R., Wei, T. Z., & Yuan, H. S. (2021). A phylogenetic overview of the Hydnaceae (Cantharellales, Basidiomycota) with new taxa from China. *Studies in Mycology*, *99*, 100–121. https://doi.org/10.1016/j.simyco.2021.100121

Corner, E. J. H. (1950). A monograph of *Clavaria* and allied genera. *A monograph of* Clavaria *and allied genera.*

Croan, S. C., Burdsall Jr, H. H., & Rentmeester, R. M. (1999). Preservation of tropical wood-inhabiting basidiomycetes. *Mycologia*, *91*(5), 908–916.

Diederich, P., & Lawrey, J. D. (2007). New lichenicolous, muscicolous, corticolous and lignicolous taxa of *Burgoa* s. l. and *Marchandiomyces* s. l. (anamorphic Basidiomycota), a new genus for *Omphalina foliacea*, and a catalogue and a key to the non-lichenized, bulbilliferous basidiomycetes. *Mycological Progress*, *6*(2), 61–80. https://doi.org/10.1007/s11557-007-0523-3

Diederich, P., Lawrey, J. D., Capdet, M., Pereira, S., Romero, A. I., Etayo, J., Flakus, A., Sikaroodi, M., & Ertz, D. (2014). New lichen-associated bulbil-forming species of Cantharellales (Basidiomycetes). *Lichenologist*, *46*(3), 333–347. https://doi.org/10.1017/S0024282913000583

Diederich, P., Millanes, A. M., Wedin, M., & Lawrey, J. D. (2022). Flora of Lichenicolous Fungi, Volume 1.

Donk, M. A. (1933). Revision der niederländischen Homobasidiomycetae-Aphyllophoraceae. *Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht*, *9*(1), 1–278.
Donk, M. A. (1956). NOTES ON RESUPINATE HYMENOMYCETES-II* The tulasnelloid fungi. *Reinwardtia*, *3*(3), 363–379.

Eriksson, J., Hjortstam, K., & Ryvarden, L. (1984). The Corticiaceae of North Europe, Vol. 7: *Schizopora–Suillosporium*. *Fungiflora, Oslo*.

Feibelman, T., Bayman, P., & Cibula, W. G. (1994). Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research*, *98*(6), 614–618. https://doi.org/10.1016/S0953-7562(09)80407-3

Feng, B., Wang, X.-H., Ratkowsky, D., Gates, G., Lee, S. S., Grebenc, T., & Yang, Z. L. (2016). Multilocus phylogenetic analyses reveal unexpected abundant diversity and significant disjunct distribution pattern of the Hedgehog Mushrooms (*Hydnum* L.). *Scientific Reports*, *6*(April), 25586. https://doi.org/10.1038/srep25586

Floudas, D., Binder, M., Henrissat, B., Martínez, A. T., Otillar, R., Spatafora, J. W., Yadav, J. S., Coutinho, P. M., Vries, R. P. De, Ferreira, P., Findley, K., & Foster, B. (2012). The Paleozoic Origin of Enzymatic Lignin Decomposition Reconstructed from 31 Fungal Genomes. *Science*, *336*(June), 1715–1719. https://doi.org/10.1126/science.1221748

Garnica, S., Riess, K., Schön, M. E., Oberwinkler, F., & Setaro, S. D. (2016). Divergence times and phylogenetic patterns of Sebacinales, a highly diverse and widespread fungal lineage. *PLoS ONE*, *11*(3), 1–16. https://doi.org/10.1371/journal.pone.0149531

Gónzalez, D., Rodriguez-Carres, M., Boekhout, T., Stalpers, J., Kuramae, E. E., Nakatani, A. K., Vilgalys, R., & Cubeta, M. A. (2016). Phylogenetic relationships of Rhizoctonia fungi within the Cantharellales. *Fungal Biology*, *120*(4), 603–619. https://doi.org/10.1016/j.funbio.2016.01.012

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321

Hawksworth, D. L., & Lücking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol Spectrum*, *5*(4). https://doi.org/10.1128/microbiolspec.FUNK-0052-2016.

Hibbett, D. S., Bauer, R., Binder, M., Giachini, A. J., Hosaka, K., Justo, A., ... & Thorn, R. G. (2014). 14 Agaricomycetes. *Systematics and Evolution: Part A*, 373–429.

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*(2), 518–522.

Juel, H.O. (1897). *Muciporus* und die Familie der Tulasnellaceen. *Bihang till Kongliga Svenska Vetenskaps-Akadernim Handlingar 23*(III: 12). 1–29.

Jülich, W. (1981). Higher taxa of Basidiomycetes. *Biblio. Mycol.*, *85*.

Langer, G. (1994). *Die gattung* Botryobasidium *Donk (Corticiaceae, Basidiomycetes)*. Gebrüder Borntraeger.

Larsson, K.-H., Larsson, E., & Kõljalg, U. (2004). High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research*, *108*(9), 983–1002. https://doi.org/10.1017/S0953756204000851

Larsson, K.-H. (2007). Re-thinking the classification of corticioid fungi. *Mycological Research*, *111*(9), 1040–1063. https://doi.org/10.1016/j.mycres.2007.08.001

Lawrey, J. D., Binder, M., Diederich, P., Molina, M. C., Sikaroodi, M., & Ertz, D. (2007). Phylogenetic diversity of lichen-associated homobasidiomycetes. *Molecular Phylogenetics and Evolution*, *44*(2), 778–789. https://doi.org/10.1016/j.ympev.2006.12.023

Lawrey, J. D., Zimmermann, E., Sikaroodi, M., & Diederich, P. (2016). Phylogenetic diversity of bulbil-forming lichenicolous fungi in Cantharellales including a new genus and species. *The Bryologist*, *119*(4), 341–349. https://doi.org/10.1639/0007-2745-119.4.341

Lutzoni, F., Nowak, M. D., Alfaro, M. E., Reeb, V., Miadlikowska, J., Krug, M., Arnold, A. E., Lewis, L. A., Swofford, D. L., Hibbett, D., Hilu, K., James, T. Y., Quandt, D., & Magallón, S. (2018). Contemporaneous radiations of fungi and plants linked to symbiosis. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-07849-9

Matheny, P. B. (2005). Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molecular Phylogenetics and Evolution*, *35*(1), 1–20. https://doi.org/10.1016/j.ympev.2004.11.014

Brandon Matheny, P., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Henrik Nilsson, R., Hughes, K. W., Hofstetter, V., Ammirati, J. F., & Schoch, C. L. (2007). Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi).

Molecular Phylogenetics and Evolution, *43*(2), 430–451. https://doi.org/10.1016/j.ympev.2006.08.024

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, *14*(12), 760–773. https://doi.org/10.1038/nrmicro.2016.149

Martin, G. W. (1945). The classification of the Tremellales. *Mycologia 37*, 527–542.

Miyauchi, S., Kiss, E., Kuo, A., Drula, E., Kohler, A., Sánchez-García, M., ... & Martin, F. M. (2020). Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nature Communications*, *11*(1), 5125.

Moncalvo, J.-M., Nilsson, R. H., Koster, B., Dunham, S. M., Bernauer, T., Matheny, P. B., Porter, T. M., Margaritescu, S., Weiss, M., Garnica, S., Danell, E., Langer, G., Langer, E., Larsson, E., Larsson, K.-H., & Vilgalys, R. (2006). The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia*, *98*(6), 937–948. https://doi.org/10.3852/mycologia.98.6.937

Morehouse, E. A., James, T. Y., Ganley, A. R., Vilgalys, R., Berger, L., Murphy, P. J., & Longcore, J. E. (2003). Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology*, *12*(2), 395–403. https://doi.org/10.1046/j.1365-294x.2003.01732.x

Nakasone, K.K. (2020). *Boidinella* gen. nov. (Cantharellales, Basidiomycota). *Cryptogamie, Mycologie*, *32*(2), 191–197.

Nawawi, A., & Webster, J. (1982). *Sistotrema hamatum* sp. nov., the teleomorph of *Ingoldiella hamata*. *Transactions of the British Mycological Society*, *78*(2), 287–291.

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274.

Oberwinkler, F. (1982). The significance of the morphology of the basidium in the phylogeny of basidiomycetes. In *Basidium and basidiocarp: Evolution, cytology, function, and development* (pp. 9–35). New York, NY: Springer New York.

Oberwinkler, F., Cruz, D., & Suárez, J. P. (2017). Biogeography and Ecology of Tulasnellaceae. *Ecol. Stud.* 230: 237–271. https://doi.org/10.1007/978-3-319-56363-3

Olariaga, I., Moreno, G., Manjón, J. L., Salcedo, I., Hofstetter, V., Rodríguez, D., & Buyck, B. (2017). *Cantharellus* (Cantharellales, Basidiomycota) revisited in Europe through a multigene phylogeny. *Fungal Diversity*, *83*(1), 263–292. https://doi.org/10.1007/s13225-016-0376-7

Olariaga, I., Huhtinen, S., Læssøe, T., Petersen, J. H., & Hansen, K. (2020). Phylogenetic origins and family classification of typhuloid fungi, with emphasis on *Ceratellopsis*, *Macrotyphula* and *Typhula* (Basidiomycota). *Studies in Mycology*, *96*, 155–184. https://doi.org/10.1016/j.simyco.2020.05.003

Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528.

Penancier, N. (1961). Recherches sur l'orientation des fuseaaux mitotiques dans la baside des Aphyllophorales. *Trav. Lab. (La Jaysinia)*, *2*, 57–71.

Pilz, D., Norvell, L., Danell, E., & Molina, R. (2003). Ecology and management of commercially harvested chanterelle mushrooms. Portland (OR): US Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Pine, E. M., Hibbett, D. S., & Donoghue, M. J. (1999). Phylogenetic relationships of cantharelloid and clavaroid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia*, *91*(6), 944–963.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rea, C. (1922) British Basidiomycetae: a handbook of the larger British Fungi. Cambridge University Press.

Rehner, S. A., & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, *97*(1), 84–98. https://doi.org/10.3852/mycologia.97.1.84

Roberts, P. (1998). *Oliveonia* and the origin of the holobasidiomycetes. *Folia Cryptogamica Estonica*, *33*, 27–132.

Roche, S. A., Carter, R. J., Peakall, R., Smith, L. M., Whitehead, M. R., & Linde, C. C. (2010). A narrow group of monophyletic *Tulasnella* (Tulasnellaceae) symbiont lineages are associated with multiple species of *Chiloglottis* (Orchidaceae): Implications for orchid diversity. *American Journal of Botany*, *97*(8), 1313–1327. https://doi.org/10.3732/ajb.1000049

Sánchez-García, M., & Matheny, P. B. (2016). Is the switch to an ectomycorrhizal state an evolutionary key innovation in mushroom-forming fungi? A case study in the Tricholomatineae (Agaricales). *Evolution*, 1–15. https://doi.org/10.1111/evo.13099

Sánchez-García, M., Ryberg, M., Khan, F. K., Varga, T., Nagy, L. G., & Hibbett, D. S. (2020). Fruiting body form, not nutritional mode, is the major driver of diversification in

mushroom-forming fungi. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(51), 32528–32534. https://doi.org/10.1073/pnas.1922539117

Shao, S.-C., Buyck, B., Hofstetter, V., Tian, X.-F., Geng, Y.-H., Fu-Qiang, Y., & Liu, P.-G. (2014). *Cantharellus hygrophorus*, a New Species in Subgenus Afrocantharellus from Tropical Southwestern China. *Cryptogamie, Mycologie*, *35*(3), 283–291. https://doi.org/10.7872/crym.v35.iss3.2014.283

Spirin, V., Volobuev, S., Viner, I., Miettinen, O., Vlasák, J., Schoutteten, N., ... & Larsson, K. H. (2021). On *Sistotremastrum* and similar-looking taxa (Trechisporales, Basidiomycota). *Mycological Progress*, *20*, 453–476.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313.

Strullu-Derrien, C., Selosse, M. A., Kenrick, P., & Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses: From palaeomycology to phylogenomics. *New Phytologist*. https://doi.org/10.1111/nph.15076

Tedersoo, L., May, T. W., & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: Global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza 20*(4), 217–263. https://doi.org/10.1007/s00572-009-0274-x

Tedersoo, L., & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, *27*(3–4), 83-99.

Tibuhwa, D. D., Savić, S., Tibell, L., & Kivaisi, A. K. (2012). Afrocantharellus gen. stat. nov. is part of a rich diversity of African Cantharellaceae. *IMA Fungus*, *3*(1), 25–38.

Varga, T., Krizsán, K., Földi, C., Dima, B., Sánchez-García, M., Sánchez-Ramírez, S., Szöllősi, G. J., Szarkándi, J. G., Papp, V., Albert, L., Andreopoulos, W., Angelini, C., Antonín, V., Barry, K. W., Bougher, N. L., Buchanan, P., Buyck, B., Bense, V., Catcheside, P., … Nagy, L. G. (2019). Megaphylogeny resolves global patterns of mushroom evolution. *Nature Ecology and Evolution*, *3*(4), 668–678. https://doi.org/10.1038/s41559-019-0834-1

Veldre, V., Abarenkov, K., Bahram, M., Martos, F., Selosse, M. A., Tamm, H., Kõljalg, U., & Tedersoo, L. (2013). Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecology*, *6*(4), 256–268. https://doi.org/10.1016/j.funeco.2013.03.004

Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, *172*(8), 4238–4246.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, *18*(1), 315–322.

Wilson, A. W., Aime, M. C., Dierks, J., Mueller, G. M., & Henkel, T. W. (2012). Cantharellaceae of Guyana I: New species, combinations and distribution records of *Craterellus* and a synopsis of known taxa. *Mycologia*, *104*(6), 1466–1477. https://doi.org/10.3852/11-412

Zamora, J. C., Pérez-Ortega, S., & Rico, V. J. (2014). *Heteroacanthella ellipsospora* sp. nov., the first lichenicolous basidiomycete with acanthoid basidia. *Lichenologist*, *46*(1), 17–23. https://doi.org/10.1017/S0024282913000765

APPENDIX

Table 8. Previous classification schemes for Cantharellales.

Author	Orders recognized	Genera recognized	Notes
Julich (1981)	Botryobasidiales	Botryobasidiaceae	
		Botryohypochnaceae	
		Hypochnellaceae	Atheliales
	Cantharellales	Clavulinaceae	
		Cantharellaceae	
		Craterellaceae	
		Hydnaceae	
	Ceratobasidiales	Ceratobasidiaceae	
		Cejpomycetaceae	
	Tulasnellales	Tulasnellaceae	
Veldre et al. (2013)	Cantharellales	Botryobasidiaceae	
		Ceratobasidiaceae	
		"core group"	contains Cantharellaceae, Clavulinaceae, Hydnaceae
		Tulasnellaceae	
Hibbett et al. (2014)	Cantharellales	Botryobasidiaceae	
		Ceratobasidiaceae	
		Hydnaceae	synonyms: Cantharellaceae, Clavulinaceae, Sistotremataceae
		Tulasnellaceae	
Diederich et al. (2014)	Cantharellales	Botryobasidiaceae	
		Ceratobasidiaceae	
		Clavulinaceae	
		Hydnaceae	
		Tulasnellaceae	
Cao et al. (2021)	Cantharellales	Botryobasidiaceae	
		Ceratobasidiaceae	
		Hydnaceae	
		Tulasnellaceae	

Table 9. Accepted and former Cantharellales genera based on present and previous studies.

Accepted Cantharellales genera

Genus	Family	Type species	Synonymy	Ecology	Basidiome morphology	Notes
Afrocantharellus	Hydnaceae	*A. symoensii*	*Cantharellus* subgen. *Afrocantharellus*	ectomycorrhizal	pileate-stipitate	
Cantharellus	Hydnaceae	*C. cibarius*	*Goossensia*	ectomycorrhizal	pileate-stipitate	
Craterellus	Hydnaceae	*C. cornucopioides*	*Pseudocraterellus*	ectomycorrhizal	pileate-stipitate	
Clavulina	Hydnaceae	*C. cristata*		ectomycorrhizal	coralloid-stipitate, pileate-stipitate, effuso-coralloid, resupinate	
Membranomyces	Hydnaceae	*M. spurius*		ectomycorrhizal	corticioid	
Hydnum	Hydnaceae	*H. repandum*	*Dentinum*	ectomycorrhizal	pileate-stipitate	
Sistotrema s. str.	Hydnaceae	*S. confluens*		ectomycorrhizal	pileate-stipitate, corticioid	Sister to *Hydnum*
Sistotrema pro parte	Hydnaceae	n/a	*Uthatobasidium* pro parte (anamorph), *Pellicularia* pro parte	saprotrophic	corticioid	Polyphyletic array of taxa not sister to to *Hydnum*
Multiclavula	Hydnaceae	*M. corynoides*		lichenized	coralloid-stipitate	
Bryoclavula	Hydnaceae	*B. phycophila*		lichenized	coralloid-stipitate	
Ingoldiella	Hydnaceae	*I. hamata*		saprotrophic (aquatic)	n/a; conidia on aquatic litter	anamorph of 8-spored species *Sistotrema hamatum* (Nawawai & Webster 1982)
Sistotremella	Hydnaceae	*S. perpusilla*		saprotrophic	corticioid	
Burgella	Hydnaceae	*B. flavoparmeliae*		lichenicolous	bulbilliferous	*B. flavoparmeliae* may be anamorph of *Sistotrema oblongisporum* (Diedrich et al. 2014)
Bergerella	Hydnaceae	*B. atrofusca*		lichenicolous	bulbilliferous	
Burgellopsis	Hydnaceae	*B. nivea*		lichenicolous	bulbilliferous	
Neoburgoa	Hydnaceae	*N. freyi*		lichenicolous	bulbilliferous	
Minimedusa	Hydnaceae	*M. polyspora*		lichenicolous	bulbilliferous	
Parmeliicida	Hydnaceae	*P. pandemica*		lichenicolous	bulbilliferous	
Burgoa	Hydnaceae, Tulasnellaceae	*B. verzuoliana*	= anamorph of *Sistotrema biggsiae*	saprotrophic	bulbilliferous	Polyphyletic; *B. moriformis* is in Tulasnellaceae (Lawrey et al. 2016)
Bulbilla	Hydnaceae	*B. applanata*	*Adamflakia applanata* (nom. inval.)	lichenicolous	bulbilliferous	
Rogersiomyces	Hydnaceae	*R. okefenokeensis*	*Hyphobasidiofera*	saprotrophic (aquatic)	gymnocarpous on aquatic litter	
Botryobasidium	Botryobasidiaceae	*B. subcoronatum*	*Botryohypochnus, Haplotrichum* (anamorph), *Pellicularia* pro parte	saprotrophic	corticioid	
Ceratobasidium	Ceratobasidiaceae	*C. calosporum*	*Pellicularia* pro parte	parasitic, saprotrophic, orchid mycorrhizal, endophytic, ectomycorrhizal, lichenicolous	corticioid	Polyphyletic (Velre et al. 2013, Gonzalez et al. 2016)
Rhizoctonia	Ceratobasidiaceae	*R. solani*	*Thanatephorus* (teleomorph), *Uthatobasidium* pro parte (anamorph), *Aquathanatephorus* , *Cejpomyces* , *Ceratorhiza* , *Oncobasidium* , *Tofispora, Ypsilondium* , *Pellicularia* pro parte	parasitic, saprotrophic, orchid mycorrhizal, endophytic	corticioid	Polyphyletic (Velre et al. 2013, Gonzalez et al. 2016)
Tulasnella	Tulasnellaceae	*T. lilacina*	*Gloeotulasnella* (Roche et al. 2010), *Epulorhiza* (anamorph), *Pseudotulasnella* , *Stilbotulasnella*	orchid mycorrhizal, saprotrophic	corticioid	

Genera formerly classified in Cantharellales

Genus	Family	Type species	Synonymy	Ecology	Basidiome morphology	Notes
Aphelaria	incertae cedis	*A. dendroides*		unknown	coralloid-stiptate	Corner (1950) described fruitbodies as leathery and suggested connections to *Pterula* and *Tremellodendron*
Boidinella	incertae cedis	*B. globulispora*		unknown	corticioid	No sequence data
Gloeomucro	Agaricales	*G. nodosum*		saprotrophic	gelatinous-pendulant	Unpublished ITS sequence data shows affinity to *Mucronella* (Agaricales)
Oliveonia	Auriculariales	*O. fibrillosa*	*Oliveorhiza (anamorph), Heteromyces, Monosporonella, Sebacinella*	unknown	corticioid	*Oliveorhiza* placed in Exidiales (Auriculariales) (Roberts 1998)
Heteroacanthella	Sebacinales or Auriculariales	*H. variabilis*	*Acanthellorhiza*	saprotrophic, lichenicolous	corticioid	Closest to *Oliveonia* (Auriculariales) (Oberwinkler et al. 1990, Zamora et al. 2014)
Osteomorpha	nom. inval.	*O. fragilis*	anamorph of *Trechispora*	unknown	n/a	
Paullicorticium	incertae cedis	*P. pearsonii*		saprotrophic	corticioid	Placed in Cantharellales due to 4+ sterigmata. Several species transferred to Trechisporales and Russulales based on molecular sequence data, but type species has not been sequenced.
Pellicularia	Hydnaceae, Botryobasidiaceae, Ceratobasidiaceae	*P. koleroga*		unknown	corticioid	Most species synonymized with *Botryobasidium, Rhizoctonia* , *Sistotrema*
Repetobasidium	incertae cedis	*R. vile*	*Peniophora vilis*	saprotrophic	corticioid	Two species confirmed in Hymenochaetales based on sequence data (Olariaga et al. 2020); type species has not been sequenced.
Scotomyces	incertae cedis	*S. fallax*	*Corticium fallax*	unknown	corticioid	Molecular evidence places *Scotomyces* outside of Ceratobasidiaceae (Larsson et al. 2007)
Suillosporium	Hydnodontaceae	*S. cystidiatum*		saprotrophic	corticioid	Sequences of *S. cystidiatum* show placement in Trechisporales (Spirin et al. 2021)
Waitea	incertae cedis	*W. circinata*			corticioid	Molecular evidence places *Waitea* outside of Ceratobasidiaceae (Larsson et al. 2007)

Table 10. Specimen data for sequences used in phylogenies. Sequences labeled "MLST" (PacBio) or "Sanger" are new to this study and were generated by that method.

Species	Type status	Locality	Field/voucher number	ITS	18S	28S	tef1	RPB2
Botryobasidium		USA: Tennessee	RAS537	MLST	MLST	MLST		MLST
Botryobasidium		USA: Illinois	FP102090	MLST	MLST	MLST		MLST
Botryobasidium			GEL2597-10	MLST	MLST	MLST	MLST	MLST
Botryobasidium			GEL3304-1	MLST	MLST	MLST		
Botryobasidium			GEL3416	MLST	MLST	MLST	MLST	
Botryobasidium			GEL3335-1	MLST	MLST	MLST	MLST	MLST
Botryobasidium		USA: North Carolina	RAS563	MLST	MLST	MLST		MLST
Botryobasidium		USA: North Carolina	RAS565	MLST	MLST	MLST		MLST
Botryobasidium		USA: North Carolina	RAS594	MLST	MLST	MLST	MLST	MLST
Botryobasidium		USA: North Carolina	RAS600	MLST	MLST	MLST	MLST	MLST
Botryobasidium		USA: Tennessee	RAS736	MLST	MLST	MLST		MLST
Botryobasidium		USA: Tennessee	RAS737	MLST	MLST	MLST		MLST
Botryobasidium		USA: North Carolina	RAS712	MLST	MLST	MLST	MLST	MLST
Botryobasidium		Chile: Los Lagos, Puyehue National Park	MES-2797	MLST	MLST			MLST
Botryobasidium		Chile: Los Lagos, Vicente Perez Rosales National Park	MES-2884	MLST	MLST	MLST		
Botryobasidium		Chile: Los Lagos, Puyehue National Park	MES-1763	MLST	MLST			
Botryobasidium asperulum		USA: Illinois	FP102150	MLST	MLST	MLST		MLST
Botryobasidium asperulum		USA: Tennessee	RAS552	MLST	MLST	MLST		MLST
Botryobasidium asperulum		USA: Tennessee	RAS578	MLST	MLST	MLST		MLST
Botryobasidium botryosum			none				Genome	Genome
Botryobasidium cf. *candicans*		USA: Tennessee	RAS583	MLST	MLST			
Botryobasidium conspersum		Chile: Osorno	MES-1018	MLST	MLST	MLST		
Botryobasidium isabellinum		USA: Tennessee	RAS579	MLST	MLST	MLST		MLST
Botryobasidium isabellinum		USA: Florida	RAS769	MLST				
Botryobasidium isabellinum			GEL2109			AF393047		AY218475
Botryobasidium laeve		USA: Florida	RAS762	MLST				
Botryobasidium obtusisporum		Canada	GEL3030		DQ898739	DQ898729		DQ898769
Botryobasidium simile		Canada	GEL2348	KP171641	DQ898740	DQ898730		DQ898770
Botryobasidium subcoronatum		USA: Massachussets	FD50	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		USA: Minnesota	FP101015	MLST	MLST	MLST		
Botryobasidium subcoronatum		USA: Michigan	FP151108	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		Germany: Bavaria, Bavarian Forest	GEL4232+2276-4	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		Germany: Bavaria, Oberallgäu	GEL2273	MLST	MLST	MLST		MLST
Botryobasidium subcoronatum		Réunion	GEL4673	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		Norway: Åkershus	GEL2160	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		USA: North Carolina	RAS620	MLST	MLST	MLST		MLST
Botryobasidium subcoronatum		Germany: Bavaria, Oberallgäu	GEL2412	MLST	MLST	MLST	MLST	MLST
Botryobasidium subcoronatum		USA: Florida	RAS770	MLST	MLST			
Botryobasidium subcoronatum		USA: Florida	RAS789	MLST	MLST	MLST		MLST
Botryobasidium subcoronatum		USA	AFTOL-614	DQ200924				DQ366284
Botryobasidium vagum		Taiwan	GEL3330	MLST	MLST	MLST	MLST	MLST
Botryobasidium vagum		USA: North Carolina	RAS559	MLST	MLST			
Botryobasidium vagum			GEL2136	MLST	MLST	MLST		
Bryoclavula phycophila	HOLOTYPE	Japan	HM293	LC508117		LC508118		
Bulbilla applanata	TYPE	Bolivia	Flakus16422	KC336078	MLST	MLST		
Bulbilla applanata		Bolivia	Flakus16424	MLST	MLST			
Burgella flavoparmeliae	TYPE	USA	Buck38682	MLST	NG_061056			
Burgella lutea	TYPE	Bolivia	Etayo27623	KC336076		KC336075		
Burgellopsis nivea	TYPE	UK	ATCCMYA4209	MLST	MLST	MLST		MLST
Bergerella atrofusca	TYPE	Austria	Berger34240	MN902070		MN902070		
Burgoa anomala		Japan	JCM30265	LC228688		LC228747		

Table 10. Continued.

Species	Type status	Locality	Field/voucher number	ITS	18S	28S	tef1	RPB2
Burgoa verzuoliana		Czech Republic: South Bohemia	CBS32075	MLST	MLST	MLST	MLST	MLST
Burgoa verzuoliana	TYPE	Italy	CBS131.38	NR145334		NG058614		
Cantharellus		USA: Tennessee	PBM4265		MLST	MLST	MLST	MLST
Cantharellus altipes		USA: Virginia	RAS084		MLST	MLST		MLST
Cantharellus altipes		USA: Tennessee	RAS639		MLST	MLST	MLST	MLST
Cantharellus ambohitantelyensis	HOLOTYPE	Madagascar	BB08.336	KF981366		KF294656	JX192989	KF294733
Cantharellus anzutake		Japan	C23				Genome	Genome
Cantharellus appalachiensis		USA: Tennessee	RAS146		MLST	MLST		
Cantharellus betularum		USA: North Carolina	RAS631		MLST	MLST	MLST	MLST
Cantharellus camphoratus		USA: North Carolina	RAS614		MLST	MLST	MLST	MLST
Cantharellus camphoratus		USA: North Carolina	RAS659		MLST	MLST	MLST	MLST
Cantharellus cinnabarinus		USA: North Carolina	RAS511		MLST	MLST		MLST
Cantharellus corallinus		USA: North Carolina	RAS466					MLST
Cantharellus corallinus		USA: North Carolina	RAS445		MLST	MLST		MLST
Cantharellus corallinus		USA: North Carolina	RAS629		MLST	MLST	MLST	MLST
Cantharellus corallinus		USA: Florida	RAS757		MLST	MLST	MLST	MLST
Cantharellus deceptivus		USA: Tennessee	RAS056		MLST	MLST	MLST	MLST
Cantharellus deceptivus		USA: North Carolina	RAS447		MLST	MLST	MLST	MLST
Cantharellus eyssartieri	HOLOTYPE	Madagascar	BB99.528			MK422936	MH643965	MT004803
Cantharellus flavolateritius		USA: Tennessee	RAS474		MLST	MLST		MLST
Cantharellus flavolateritius		USA: Tennessee	RAS458		MLST	MLST		MLST
Cantharellus flavolateritius		USA: Tennessee	PBM4264		MLST	MLST	MLST	MLST
Cantharellus flavolateritius		USA: Tennessee	RAS474		MLST	MLST	MLST	MLST
Cantharellus flavolateritius		USA: Tennessee	RAS590		MLST	MLST	MLST	MLST
Cantharellus flavus		USA: Tennessee	RAS473		MLST	MLST		MLST
Cantharellus flavus		USA: Tennessee	RAS464		MLST	MLST	MLST	MLST
Cantharellus ibityensis	HOLOTYPE	Madagascar	BB08.196	KF981368		KF294650	GQ914980	KF294727
Cantharellus lateritius		USA: Florida	RAS785		MLST	MLST		MLST
Cantharellus minor		USA: Tennessee	RAS644		MLST			
Cantharellus minor		USA: North Carolina	RAS718		MLST			
Cantharellus persicinus		USA: Tennessee	RAS468		MLST	MLST		MLST
Cantharellus phasmatis		USA: Tennessee	PBM4267		MLST	MLST		
Cantharellus quercophilus		USA: Florida	RAS758		MLST	MLST	MLST	MLST
Cantharellus sebosus	HOLOTYPE	Madagascar	BB08.234	NR154789		KF294652	JX192986	KF294729
Cantharellus spectaculus		USA: North Carolina	SAT1519702			MLST		MLST
Cantharellus spectaculus		USA: Tennessee	PBM3933		MLST	MLST	MLST	MLST
Cantharellus subfloridulus	HOLOTYPE	Central African Republic	BB16.016			MK422947	MG450686	MT004812
Cantharellus tanzanicus	HOLOTYPE	Tanzania	BB98.040			KF294622	JX192977	KF294696
Cantharellus tenuithrix		USA: Tennessee	PBM4292		MLST	MLST		MLST
Cantharellus tenuithrix		USA: North Carolina	MH20005		MLST		MLST	MLST
Cantharellus tomentosoides	HOLOTYPE	Central African Republic	BB16.007			MK422937	MG450685	MT004804
Cantharellus velutinus		USA: Tennessee	RAS456		MLST	MLST		MLST
Cantharellus velutinus		USA: Tennessee	PBM4290		MLST	MLST		MLST
Cantharellus velutinus		USA: Tennessee	AJF32		MLST	MLST		MLST
Cantharellus vicinus	HOLOTYPE	USA: Tennessee	RAS426		MLST	MLST		MLST
Cantharellus vicinus		USA: Tennessee	RAS669		MLST	MLST	MLST	MLST
Ceratobasidium		Japan	C517	MLST	MLST	MLST		
Ceratobasidium		Japan	C662	MLST	MLST	MLST	MLST	MLST
Ceratobasidium		Japan	A001C	MLST	MLST	MLST		
Ceratobasidium		Japan	70B	MLST	MLST	MLST		
Ceratobasidium		Japan	SIR1	MLST	MLST	MLST		
Ceratobasidium			AH6	MLST	MLST	MLST		
Ceratobasidium		Japan	C653	MLST	MLST	MLST		
Ceratobasidium		Japan	C620	MLST	MLST	MLST		
Ceratobasidium		USA: Florida	RH7	MLST	MLST	MLST		MLST

Table 10. Continued.

Species	Type status	Locality	Field/voucher number	ITS	SSU	LSU	tef1	RPB2
Ceratobasidium		Argentina: Bariloche, Nahuel Huapi National Park	MES-2155	MLST	MLST	MLST		
Ceratobasidium		Argentina: Bariloche, Nahuel Huapi National Park	MES-2154	MLST	MLST	MLST		
Ceratobasidium		USA: Florida	379				Genome	Genome
Ceratobasidium		USA: Florida	394				Genome	Genome
Ceratobasidium		USA: Florida	395				Genome	Genome
Ceratobasidium		USA: Illinois	423				Genome	Genome
Ceratobasidium AG-1			DN8442				Genome	Genome
Ceratobasidium bulbillifaciens		Germany	CBS129339	MLST	MLST	Genbank		
Ceratobasidium bulbillifaciens		Germany: Bayern	CBS132236	MLST	MLST		MLST	MLST
Ceratobasidium cereale		Japan	BRGWP2	MLST	MLST			
Ceratobasidium cereale		Japan	KOUO41FW		MLST			
Ceratobasidium cornigerum		France	CBS148.54	DQ278937		KP171656		DQ301714
Ceratobasidium globisporum		Australia	CBS569.83	DQ278942		MH873365		DQ301723
Ceratobasidium oryzae-sativae		Japan	C511	MLST	MLST	MLST		
Ceratobasidium oryzae-sativae		USA: California	PG1	MLST	MLST	MLST		
Ceratorhiza hydrophila		Ecuador	E14504F			MT381951	MT381955	MT381954
Clavulina		Bhutan: Chukha, Bjachho	RMS22290					Sanger
Clavulina		USA: Tennessee	PBM4135	MLST	MLST	MLST		
Clavulina		USA: North Carolina	RAS487	MLST	MLST	MLST		MLST
Clavulina		USA: North Carolina	RAS497	MLST	MLST	MLST	MLST	MLST
Clavulina		USA: Tennessee	RAS569	MLST	MLST	MLST	MLST	MLST
Clavulina		USA: North Carolina	RAS634	MLST	MLST	MLST	MLST	MLST
Clavulina		USA: Tennessee	RAS642	MLST	MLST			MLST
Clavulina		USA: Tennessee	RAS643	MLST	MLST			MLST
Clavulina		Chile: Los Lagos, Vicente Perez Rosales National Park	MES-2892	MLST	MLST	MLST		MLST
Clavulina		USA: Massachusetts	MB03-034	DQ202266		AY745694	DQ028589	DQ366286
Clavulina caespitosa	HOLOTYPE	Guyana	TH8709	NR_119560		DQ056370		JN228234
Clavulina castaneipes		USA: North Carolina	RAS452	MLST	MLST			MLST
Clavulina cerebriformis	HOLOTYPE	Guyana	MCA4022	NR121504		JN228222		JN228233
Clavulina cinerea		USA: North Carolina	RAS494	MLST	MLST	MLST	MLST	MLST
Clavulina cinerea		Argentina: Río Negro	AM-AR17-014	MLST	MLST	MLST	MLST	MLST
Clavulina cinereoglebosa	PARATYPE	Guyana	TH8561	NR119975		JN228232		JN228246
Clavulina coralloides		USA: North Carolina	RAS490	MLST	MLST			MLST
Clavulina cristata		USA: North Carolina	RAS323	MLST	MLST	MLST		MLST
Clavulina gracilis		India: West Bengal, Darjeeling	RMS22170					Sanger
Clavulina humicola	HOLOTYPE	Guyana	TH8737	DQ056368		DQ056367		JN228244
Clavulina mahiscolorata	HOLOTYPE	Mexico	FCME27662	MH542554		MN049496		MN053719
Clavulina monodiminutiva	HOLOTYPE	Guyana	TH8738	NR_119559		DQ056372		JN228237
Clavulina rosiramea	HOLOTYPE	Guyana	TH8954	NR_120086		JQ677044		JQ677048
Clavulina rugosa		USA: North Carolina	RAS327	MLST	MLST			MLST
Clavulina sprucei		Guyana	TH9122	HQ680355		JN228223		JN228236
Clavulina tuxtlasana	HOLOTYPE	Mexico	FCME27279	MK547196		MT903238		MK519633
Craterellus		Australia: Tasmania	MBP06012010	MLST	MLST	MLST		
Craterellus		USA: Tennessee	RAS393	MLST	MLST			
Craterellus		USA: North Carolina	NJJ15_053	MLST	MLST	MLST		MLST
Craterellus		Chile: Villarica National Park	MES-3362	MLST	MLST	MLST	MLST	MLST
Craterellus caeruleofuscus		USA: Virginia	RAS674	MLST	MLST			
Craterellus caeruleofuscus		USA: North Carolina	LJ2020-1	MLST	MLST			
Craterellus calyculus		USA: Tennessee	MGW969	MLST	MLST			
Craterellus carolinensis		USA: North Carolina	PBM4446	MLST	MLST	MLST		
Craterellus dubius		USA: North Carolina	MH17001	MLST	MLST	MLST		MLST
Craterellus dubius		USA: North Carolina	MH20007	MLST	MLST	MLST	MLST	

Table 10. Continued.

Species	Type status	Locality	Field/voucher number	ITS	18S	28S	tef1	RPB2
Craterellus fallax		USA	EEApvecm2	MLST	MLST	MLST		
Craterellus fallax		USA: North Carolina	PBM3290	MLST	MLST	MLST		
Craterellus fallax		USA: Tennessee	TENN072344	MLST	MLST	MLST		
Craterellus fallax		USA: Tennessee	RAS576	MLST	MLST	MLST		
Craterellus foetidus		USA: Tennessee	RAS277B	MLST	MLST	MLST		
Craterellus foetidus		USA: North Carolina	LJ2020-3	MLST	MLST		MLST	
Craterellus ignicolor		USA: North Carolina	RAS444	MLST	MLST	MLST		MLST
Craterellus ignicolor		USA: Tennessee	AJF19	MLST	MLST	MLST		
Craterellus ignicolor		USA: Tennessee	RAS589	MLST	MLST	MLST	MLST	MLST
Craterellus odoratus		USA: North Carolina	RAS331	MLST	MLST	MLST		
Craterellus tubaeformis		USA: Tennessee	RAS387	MLST	MLST	MLST		MLST
Craterellus tubaeformis		USA: North Carolina	RAS485	MLST	MLST			
Craterellus tubaeformis		USA: Tennessee	AJF66	MLST	MLST	MLST		MLST
Haplotrichum		USA: Florida	VDZ15	MLST	MLST	MLST	MLST	MLST
Haplotrichum aureum		USA: Tennessee	RAS571	MLST	MLST	MLST	MLST	MLST
Haplotrichum conspersum		USA: Tennessee	RAS259	MLST	MLST			
Haplotrichum conspersum		USA	AFTOL-1766	DQ911612		DQ521414	DQ521420	
Haplotrichum rubiginosum		USA: Florida	RAS776	MLST				
Haplotrichum simile		USA: Georgia	RAS794	MLST				
Haplotrichum simile		USA: Georgia	RAS793	MLST				
Hydnum		Belize: Guacamallo	MES-3213	MLST	MLST			
Hydnum			MK08141509	MLST	MLST	MLST	MLST	Sanger
Hydnum		Belize: Cayo District	44LL	Sanger	MLST		Sanger	
Hydnum aerostatisporum		USA: Tennessee	RAS157	MLST	MLST	MLST	Sanger	MLST
Hydnum albidum		USA: Tennessee	BPL932	MLST	MLST	MLST	Sanger	MLST
Hydnum albidum		USA: North Carolina	RAS225	MLST	MLST		Sanger	MLST
Hydnum albidum		USA: North Carolina	RAS210	MLST	MLST	MLST	Sanger	MLST
Hydnum alboaurantiacum		USA: North Carolina	RAS104	MLST	MLST	MLST	Sanger	MLST
Hydnum albomagnum		USA	AFTOL-471	DQ218305	AY665777	AY700199	DQ234568	DQ234553
Hydnum canadense		USA: North Carolina	RAS100	Sanger	MLST		MLST	MLST
Hydnum cuspidatum		USA: Tennessee	RAS162	MLST	MLST		Sanger	MLST
Hydnum cuspidatum		USA: North Carolina	RAS098	MLST	MLST		Sanger	MLST
Hydnum cuspidatum		USA: North Carolina	RAS205	MLST	MLST			MLST
Hydnum ferruginescens		USA: Tennessee	RAS229	MLST	MLST	MLST	MLST	MLST
Hydnum melitosarx		USA: California	RLP12318C	MLST	MLST	MLST	Sanger	
Hydnum mulsicolor		USA: North Carolina	RAS108	MLST	MLST	MLST	Sanger	MLST
Hydnum mulsicolor		Belize: Guacamallo	MES-3229	MLST	MLST			MLST
Hydnum oregonense		USA: California	RPL12318D	MLST	MLST		Sanger	MLST
Hydnum rufescens		Sweden: Uppsala	UP504				Genome	Genome
Hydnum subconnatum		USA: Georgia	RAS169	MLST	MLST	MLST	Sanger	MLST
Hydnum subolympicum		USA: Georgia	RAS168	MLST	MLST	MLST	Sanger	MLST
Hydnum subolympicum		USA: North Carolina	MH16009	MLST	MLST	MLST	Sanger	Sanger
Hydnum subtilior		USA: North Carolina	PBM3868	Sanger	MLST		Sanger	Sanger
Hydnum subtilior		USA: Florida	RAS180	MLST	MLST	MLST	Sanger	MLST
Hydnum umbilicatum		USA: New York	BD14	Sanger	MLST		Sanger	Sanger
Hydnum umbilicatum		USA: North Carolina	RAS099	MLST	MLST		Sanger	MLST
Hydnum umbilicatum		USA: North Carolina	RAS356	MLST	MLST		Sanger	MLST
Hydnum umbilicatum		USA: Tennessee	TFB13482	MLST	MLST		Sanger	MLST
Hydnum vagabundum		USA: Georgia	MO298808	MLST	MLST			
Hydnum vagabundum		USA: Georgia	MO298808	MLST	MLST			
Membranomyces delectabilis			GB0090523	JQ638714				
Membranomyces spurius			H6025454	JQ638713				
Membranomyces spurius		United Kingdom: England, Hertfordshire	K(M):131627	MZ159352				

Table 10. Continued.

Species	Type status	Locality	Field/voucher number	ITS	18S	28S	tef1	RPB2
Minimedusa obcoronata		Thailand	CBS120605	GQ303278		GQ303309		
Minimedusa polyspora	ISOTYPE	Cuba	CBS113.16	NR_145335		NG_057640		
Minimedusa pubescens		Belgium: Booischot	ATCCMYA2993	MLST	MLST	MLST		MLST
Multiclavula mucida		USA: Tennessee	RAS392	MLST	MLST	MLST	MLST	MLST
Multiclavula mucida		USA: Tennessee	RAS525	MLST	MLST	MLST	MLST	MLST
Multiclavula petricola	ex-type	Japan	HMasumoto356	LC516464		LC516465		
Neoburgoa freyi		Switzerland: Kanton Wallis, Oberwald, Grimselpass	EZLF1256	MLST	MLST	MLST	MLST	MLST
Neoburgoa freyi			EZ4455	MLST	MLST	MLST		
Parmeliicida pandemica	TYPE	France	Diederich18144	MZ509450		MZ509450		
Parmeliicida pandemica		Austria	Berger35564	MZ509446		MZ509446		
Rhizoctonia butinii	HOLOTYPE	Norway	11026	KF386035				
Rhizoctonia calle			RC1	MLST	MLST			
Rhizoctonia endophytica			RE1	MLST	MLST			
Rhizoctonia endophytica		Canada	DAOM138188	KP171640		KP171655		KP171658
Rhizoctonia solani		India	BRS17	MK481078		MN078809	MN078941	
Rhizoctonia solani AG-1			IB				Genome	Genome
Rhizoctonia solani AG-3			Rhs1AP				Genome	Genome
Rhizoctonia solani AG-8			WAC10335				Genome	Genome
Rogersiomyces malaysianus		Vietnam	LE-BIN3507	KT779284		KT779286		
Sistotrema		USA: Kentucky	FP102155	MLST	MLST	MLST		MLST
Sistotrema		USA: Oregon	AhoTM38	MLST	MLST	MLST		MLST
Sistotrema		USA: Wisconsin	HHB11889	MLST	MLST	MLST		
Sistotrema		Chile: Los Lagos, Puyehue National Park	MES-2607	MLST	MLST	MLST		
Sistotrema		USA: Wisconsin	FP102031	MLST	MLST	MLST		MLST
Sistotrema			MUCL035158				Genome	Genome
Sistotrema		Argentina: Bariloche, Nahuel Huapi National Park	MES-2153			MLST		
Sistotrema adnatum		Canada: British Columbia	GB700	MLST	MLST	MLST	MLST	MLST
Sistotrema biggsiae		USA: Maryland	HHB1663	MLST	MLST	MLST		MLST
Sistotrema biggsiae		USA: New York	RLG5457	MLST	MLST	MLST		MLST
Sistotrema biggsiae		Chile: Los Lagos, Puyehue National Park	MES-2816	MLST		MLST		
Sistotrema brinkmanii		USA: Alaska	WBC60142T	MLST	MLST	MLST		MLST
Sistotrema brinkmanii		Costa Rica: San Jose Province	LI3431	MLST	MLST	MLST	MLST	MLST
Sistotrema brinkmanii		Norway: Sonsterud	71177	MLST	MLST	MLST		
Sistotrema brinkmanii		USA: Colorado	Colo51175	MLST	MLST	MLST	MLST	MLST
Sistotrema brinkmanii		Canada: Ontario	Whit7	MLST	MLST	MLST		MLST
Sistotrema brinkmanii		Norway: Nordland	76-46/1	MLST	MLST	MLST		MLST
Sistotrema cf. *coronilla*		Chile: Los Lagos, Puyehue National Park	MES-2725	MLST	MLST	MLST		MLST
Sistotrema chloroporum	HOLOTYPE	Japan	TUMH64399	NR_178117		LC642057	LC717879	LC667369
Sistotrema confluens		USA: Tennessee	RAS950	Sanger			Sanger	Sanger
Sistotrema confluens		Sweden	AFTOL-613	DQ267125	AY757260	AY647214		DQ381837
Sistotrema coronilla		USA: Michigan	HHB10379	MLST	MLST	MLST		
Sistotrema coronilla		USA: Tennessee	RAS538	MLST	MLST	MLST	MLST	MLST
Sistotrema coronilla		USA: New York	RLG5411	MLST	MLST	MLST		MLST
Sistotrema coronilla		USA: Minnesota	HHB10070	MLST	MLST	MLST	MLST	MLST
Sistotrema eximum		Canada: Ontario	T429	MLST	MLST	MLST		
Sistotrema farinaceum		Canada: British Columbia	GB659	MLST	MLST	MLST		MLST
Sistotrema flavorhizomorphae	HOLOTYPE	Japan	TUMH64409	NR_178118		LC642067	LC717878	LC667371
Sistotrema hirschii		USA: Idaho	TCH8A	MLST	MLST	MLST		MLST
Sistotrema luteoviride	HOLOTYPE	Finland	H:HK23176	NR_158892				

Table 10. Continued.

Species	Type status	Locality	Field/voucher number	ITS	18S	28S	tef1	RPB2
Sistotrema muscicola			OMC1658				Genome	Genome
Sistotrema oblongisporum		USA: Washington DC	HHB707	MLST	MLST	MLST		MLST
Sistotrema oblongisporum		USA: Wisconsin	FP101802	MLST	MLST	MLST		
Sistotrema porulosum		USA: Michigan	HHB7822	MLST	MLST	MLST		
Sistotrema porulosum		Canada: Quebec	GB566	MLST	MLST	MLST		
Sistotrema porulosum		Canada: British Columbia	GB720	MLST	MLST	MLST		
Sistotrema raduloides		USA: North Carolina	HHB4356	MLST	MLST	MLST		MLST
Sistotrema raduloides		USA: Massachussets	FD550	MLST	MLST	MLST	MLST	MLST
Sistotrema raduloides		USA: New York	FP135005	MLST	MLST	MLST	MLST	MLST
Sistotrema raduloides		USA: Wisconsin	HHB14321	MLST	MLST	MLST		MLST
Sistotrema raduloides		Canada: Ontario	Whitney8	MLST	MLST	MLST	MLST	MLST
Sistotrema raduloides			OMC1660				Genome	Genome
Sistotrema resinicystidium		USA: Michigan	HHB10232	MLST	MLST	MLST		MLST
Sistotrema sernanderi			OMC1753				Genome	Genome
Thanatephorus cucumeris		USA: Alaska	56RS	MLST	MLST			
Thanatephorus cucumeris		USA: Maine	10RS	MLST	MLST	MLST		
Thanatephorus cucumeris			RSBWB	MLST	MLST	MLST		
Thanatephorus cucumeris			RSIOEEA	MLST	MLST	MLST		
Thanatephorus cucumeris		Panama	CBS700.82	DQ278946		MH873283	DQ301660	DQ301727
Thanatephorus cucumeris			MPI-SDFR-AT-0096				Genome	Genome
Thanatephorus practicola			B195	MLST	MLST			
Thanatephorus practicola			RS293	MLST	MLST			
Thanatephorus practicola		USA: Georgia	RS359		MLST	MLST		
Tulasnella			GEL5130			DQ898731		DQ898771
Tulasnella		USA: Illinois	419				Genome	Genome
Tulasnella		USA: Illinois	425				Genome	Genome
Tulasnella		USA: Florida	427				Genome	Genome
Tulasnella araneosa		USA: Wisconsin	HHB10156	MLST				MLST
Tulasnella asymmetrica		Japan	AFTOL-1678	DQ520101				DQ836004
Tulasnella calospora		USA: Florida	UAMH9824				Genome	Genome
Tulasnella calospora			AL13/4D				Genome	Genome
Tulasnella cf. *asymmetrica*		USA: Tennessee	RAS537 culture	MLST				MLST
Tulasnella inquilina		USA: South Carolina	UAMH7632				Genome	Genome
Tulasnella violea		USA: Minnesota	FP134258	MLST			MLST	MLST
Tulasnella violea		USA: Wisconsin	HHB10152	MLST	MLST		MLST	MLST
Tulasnella violea		USA: Alaska	HHB15716	MLST	MLST		MLST	MLST
Tulasnella violea		USA: Wisconsin	HHB7330	MLST	MLST		MLST	
Tulasnella violea		USA: California	HHB13471	MLST	MLST		MLST	
Tulasnella violea		USA: Tennessee	HHB3860	MLST	MLST		MLST	
Uthatobasidium fusisporum		USA	AFTOL-611	DQ398957				DQ381842
Outgroups								
Dacrymyces		USA	FPL8953	DQ205684			DQ028587	DQ381845
Dacrymyces chrysospermus		USA	AMH013	MLST	MLST	MLST		
Holtermanniella wattica	TYPE		CBS9496	NR_138371	NG_062964	NG_058307	KF037099	KF036828

Figure 22. Morphological and ecological diversity among Cantharellales fungi. Clockwise from top left: the ectomycorrhizal edible mushroom *Hydnum umbilicatum*; corticioid (crust-like) teleomorph of the crop pathogen *Rhizoctonia solani*; an ectomycorrhizal coralloid *Clavulina* mushroom; the lichenized fungus *Multiclavula mucida*; the corticioid saprobe *Botryobasidium rubiginosum*; and the bulbil-forming lichenicolous *Neoburgoa freyi*. Photo of *Rhizoctonia solani* by Maria A. Kunetsova; photo of *Neoburgoa freyi* by Erich Zimmerman; all other photos by Rachel A. Swenie.

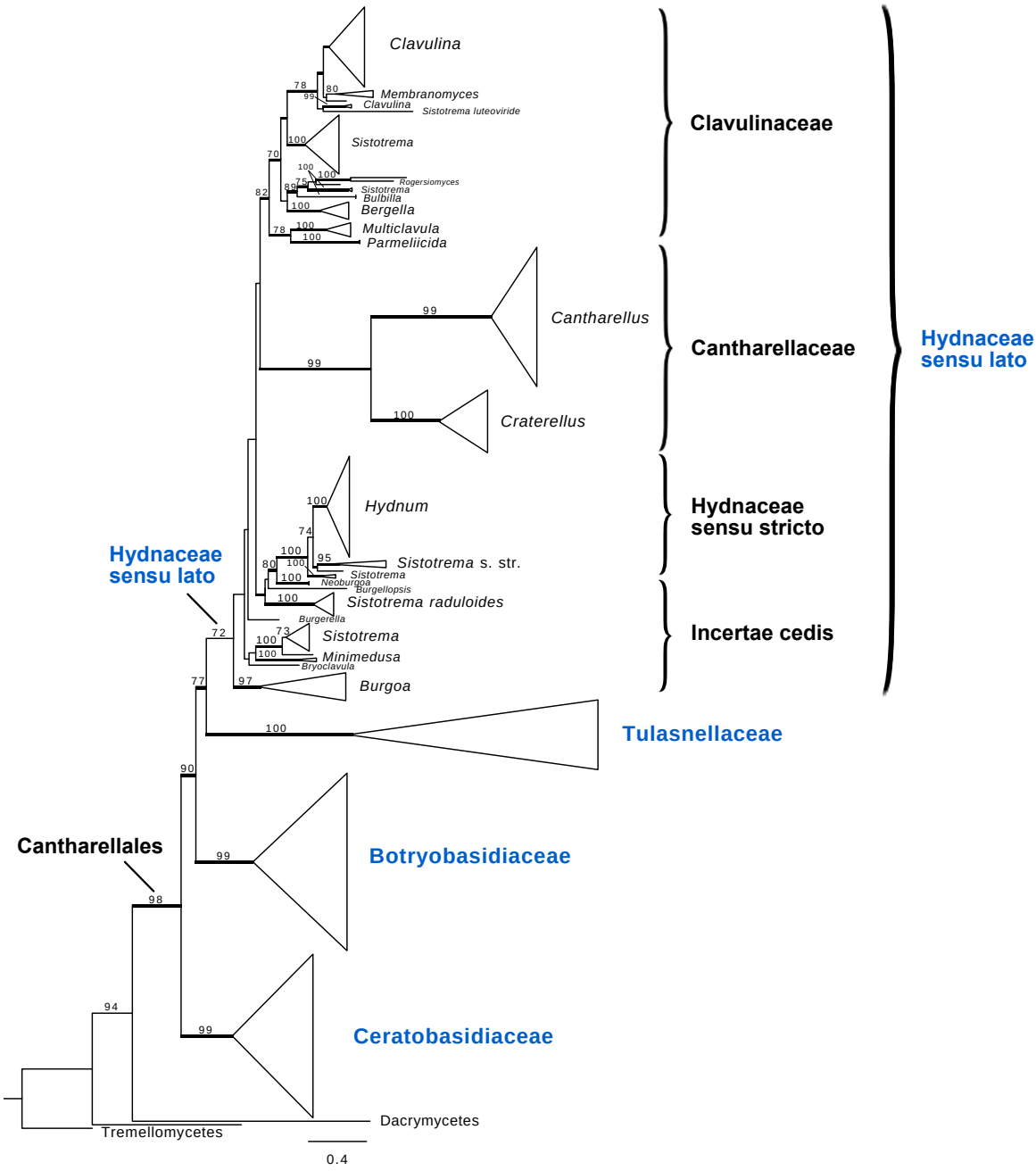

Figure 23. Multi-locus (18S, ITS, 28S, *tef1*, *rpb2*) phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. ML bootstrap values ≥ 70% are shown on branches. Clades strongly supported by UF bootstrap, SH, and aBayes metrics are indicated by bold branches.

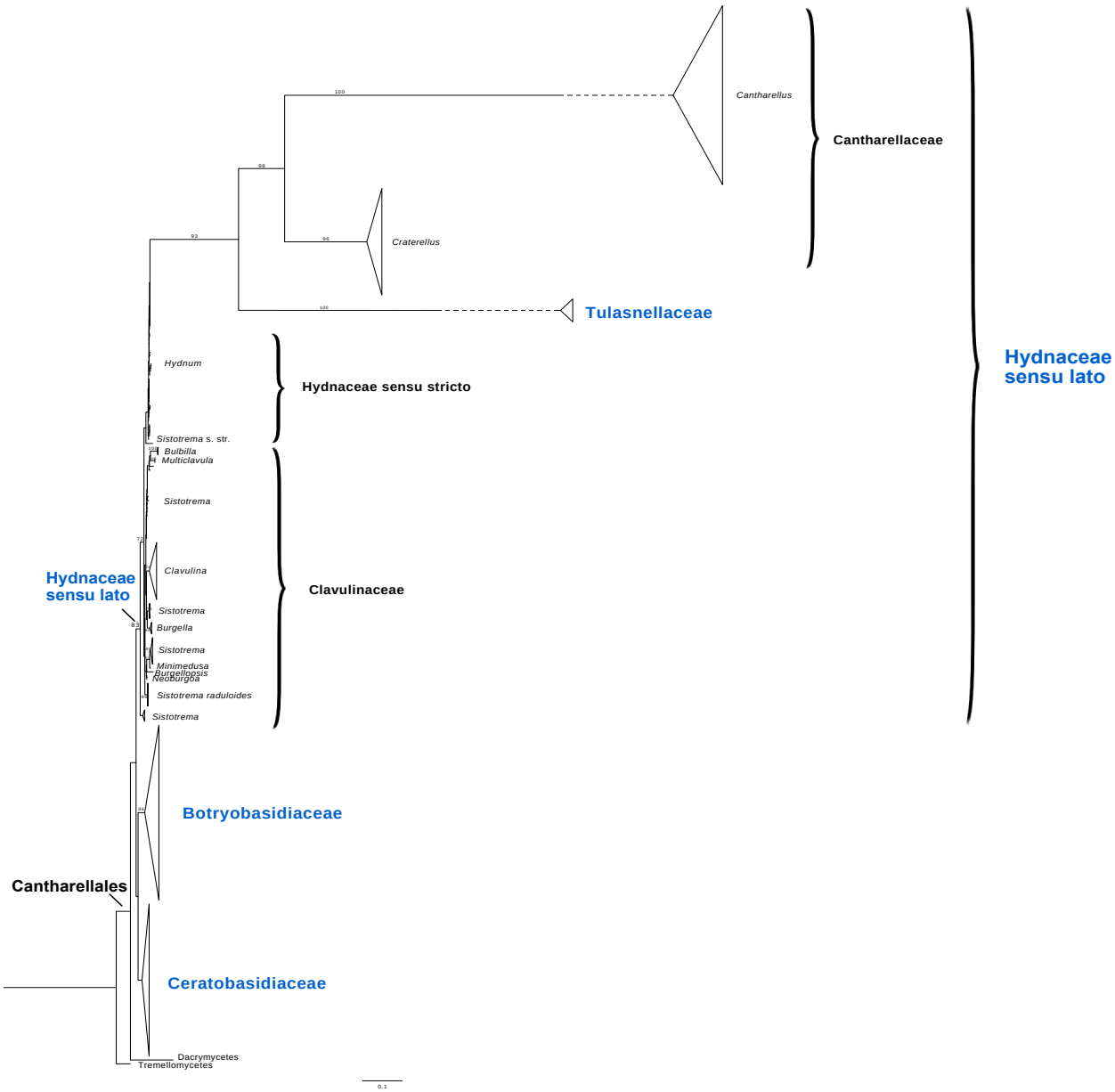

Figure 24. 18S phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. ML bootstrap values ≥ 70% are shown on branches. Dotted line indicates a branch is displayed as half of full length.

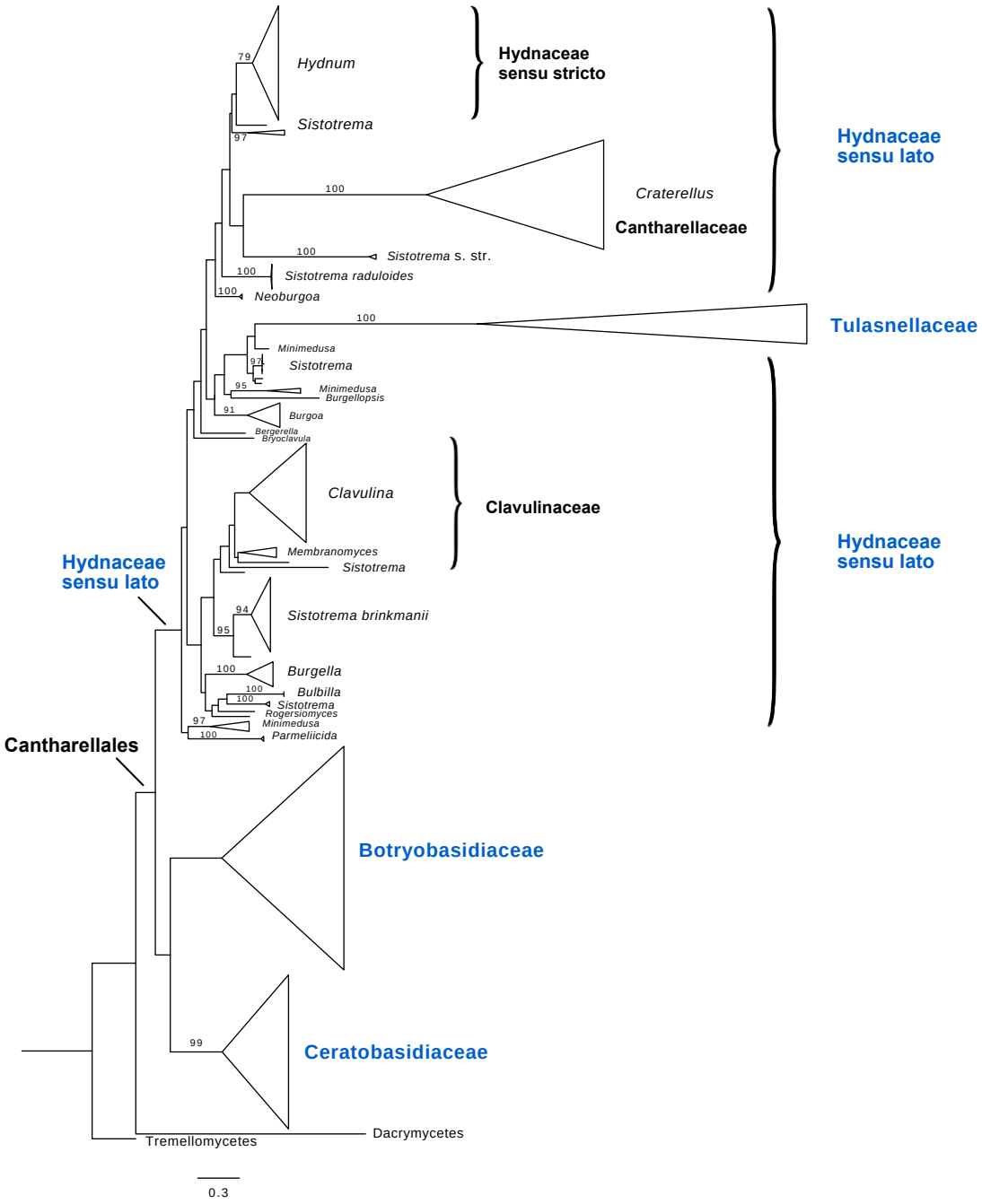

Figure 25. ITS phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. ML bootstrap values ≥ 70% are shown on branches.

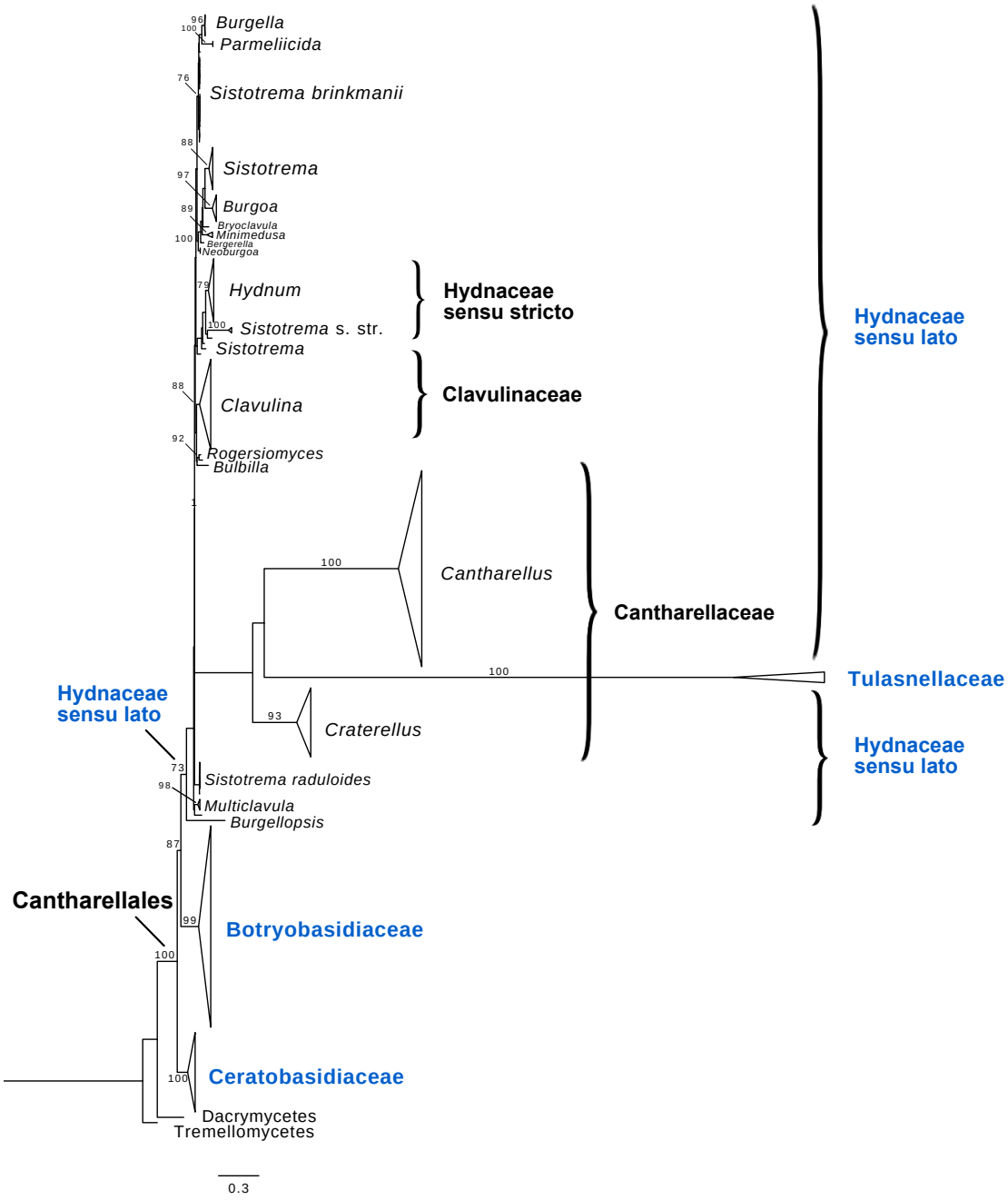

Figure 26. 28S phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. ML bootstrap values ≥ 70% are shown on branches.

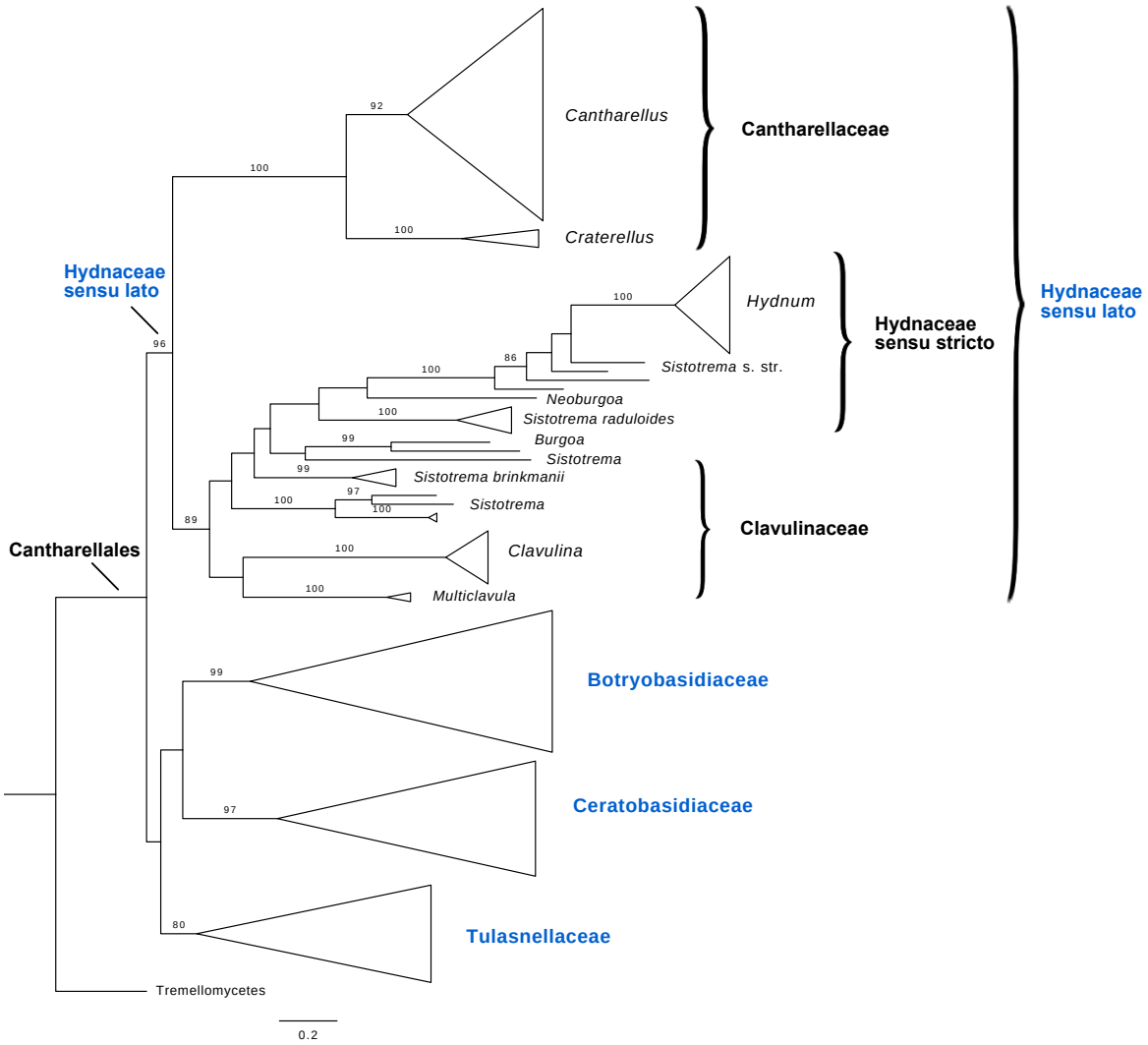

Figure 27. *tef1* phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. ML bootstrap values ≥ 70% are shown on branches.

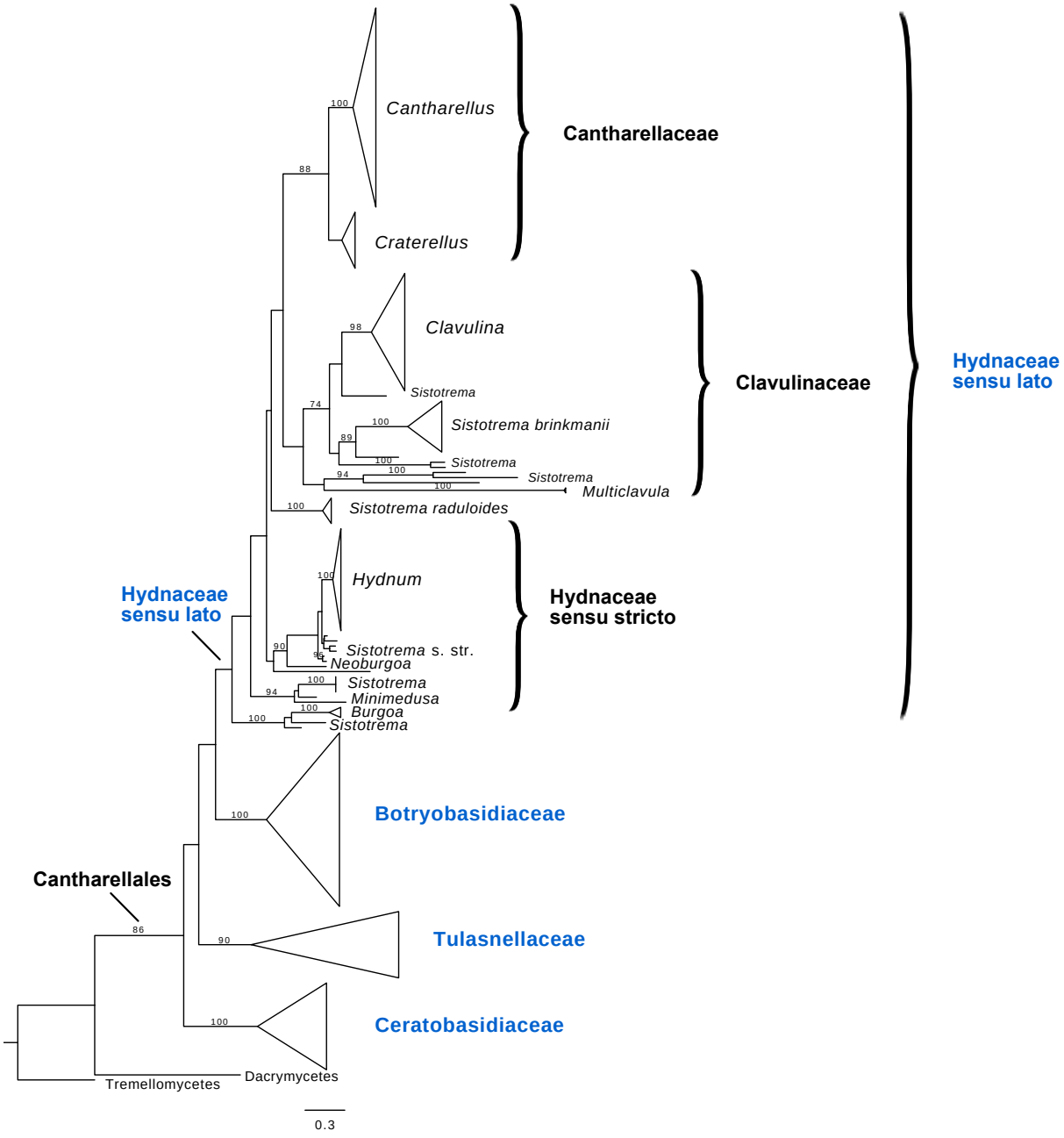

Figure 28. *rpb2* phylogeny of the Cantharellales. Family designations are denoted in bold text with accepted families in blue. Bootstrap values ≥ 70% are shown on branches.

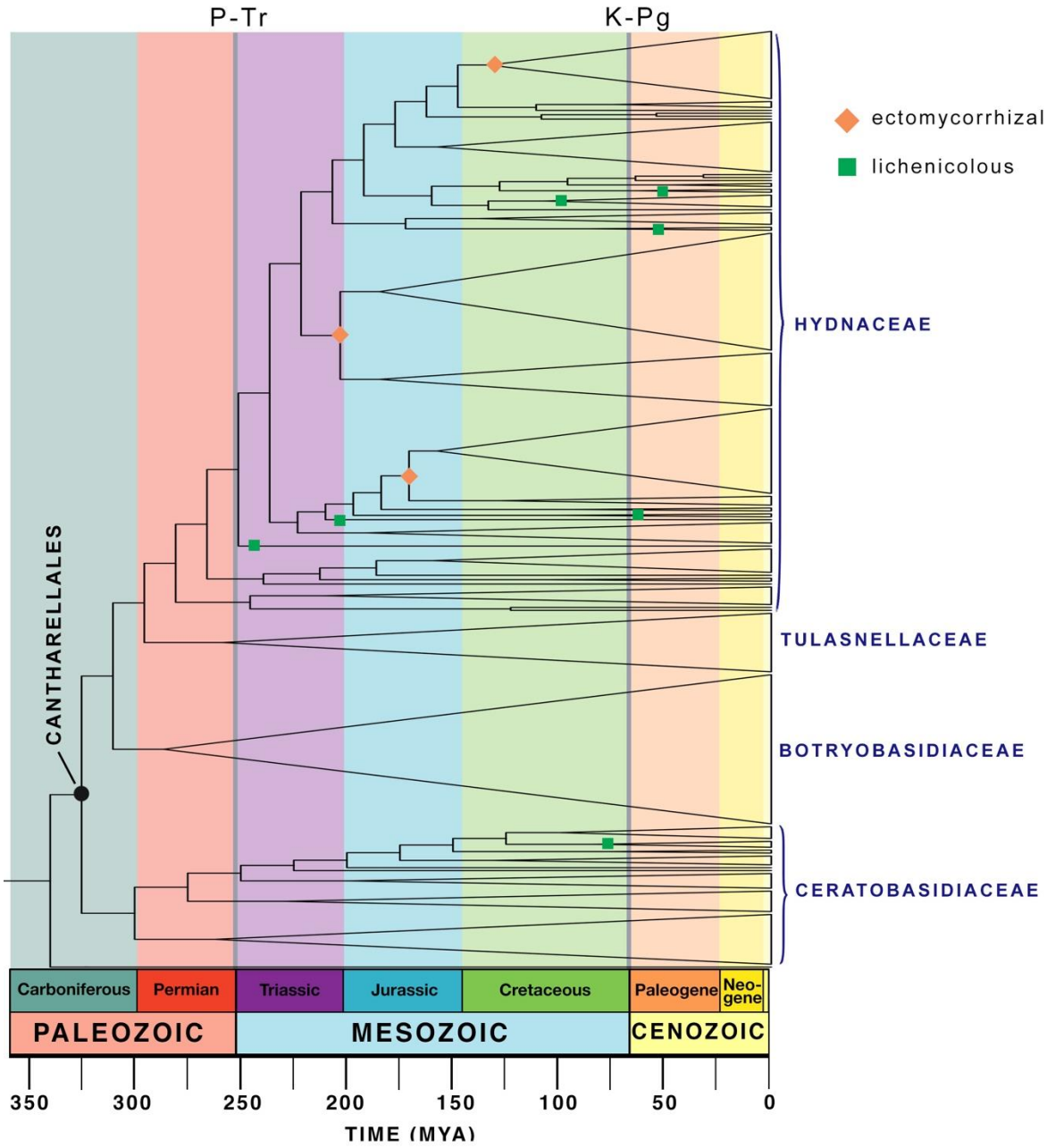

Figure 29. Time-calibrated phylogeny of the Cantharellales showing clades containing biotrophic (ectomycorrhizal and lichenicolous) species. Mass-extinction events are indicated with shaded bars.

CONCLUSION

This dissertation provides important contributions to understand fungal diversity, phylogenetic relationships, and evolution in the Cantharellales. Within the Cantharellales, I conducted systematic and taxonomic revision of two economically important genera of wild edible mushrooms in eastern North America: *Hydnum*, the hedgehog mushrooms, and *Cantharellus*, the chanterelle mushrooms. *Hydnum* was long thought to consist of very few globally-distributed species based on a morphological species concept, but species estimates based on molecular diversity have grossly exceeded that expectation. In eastern North America alone, I found evidence for sixteen species of *Hydnum* by integrating morphological, ecological, and molecular phylogenetic data from modern and historical type collections. In contrast to original concepts of *Hydnum* diversity, most *Hydnum* species are continental endemics (Feng et al. 2016). Indeed, only one eastern North American species, *Hydnum mulsicolor*, is shared between North America and Europe. Other more recent studies from China and Japan have revealed no *Hydnum* species that are shared between Asia and North America.

The chanterelle mushrooms (genus *Cantharellus*), in contrast, exhibit higher species diversity than *Hydnum* in eastern North America, with 27 described species. My research shows that while most chanterelle species in eastern North America appear to range widely across the region, a few exhibit intercontinental distribution patterns. Collections from the southern Appalachian Mountains revealed the first records of *Cantharellus camphoratus*, *C. betularum*, and *C. cibarius* from the United States. *Cantharellus camphoratus* is a fir- and hemlock-associated species with known populations in Atlantic Canada and the northeastern U.S., the highest elevations of the southern Appalachians, and montane Japan. I also show the iconic type species of the genus, *Cantharellus cibarius,* is not restricted to Europe as previously thought. Rather, *C. cibarius* appears to have a circumboreal distribution across Europe, Asia, and North America and may have been redescribed in North America as *C. enelensis* and *C. roseocanus*. Further multi-locus sampling of these and other eastern North American *Cantharellus* taxa is desirable to draw a clearer picture of species distributions.

Furthermore, I found multi-locus phylogenetic analysis is critical to species delimitation in *Cantharellus*. Gene conflict is abundant among *Cantharellus* species, and multi-locus analysis provides the most insight into species diversity among these morphologically variable fungi. Single-locus species identification has led to redescription of existing species; some cases, such as *C. tenuithrix*, are best referred to as complexes in the absence of additional multi-locus sampling from type materials. Nonetheless, novel unnamed *Cantharellus* species remain, even in a relatively well-studied region like North America. Based on micromorphology, ecology, and multi-locus phylogenetic analysis, I described one new species from oak habitat in Tennessee, *C. vicinus*, and elevated another species from the southern Appalachians to the species level, *C. intensissimus*. In total, I recognized 27 *Cantharellus* species in eastern North America, thirteen of which occur in the southern Appalachian Mountains.

Analyses of species diversity and distribution patterns provide insight into the historical biogeography and diversification processes that have shaped lineages. Knowledge of species diversity and their distributions is necessary to understand the

wider ecological impacts of human use of these mushrooms. Across the Cantharellales more broadly, many unanswered questions remain concerning patterns and timing of diversification events. However, no well-supported phylogeny of the order exists to provide a framework for future biogeographic and ecological studies. Fungal genomics is still in its infancy and there exist draft genomes but no reference genomes for Cantharellales species. Most modern phylogenies are built from Sanger sequencing data, which is costly and time-consuming to produce.

To expedite the construction of a large-scale Cantharellales phylogeny, I developed a new method for generating multi-locus sequence data from a fungal specimens, cultures, and archival DNA extracts using the PacBio Sequel sequencing platform. PacBio high-throughput sequencing technology produces high-quality long reads from hundreds of samples, including heterogeneous and contaminated samples. Multiplexing loci together saves additional time and cost compared to traditional Sanger sequencing methods. Using this new method yielded clean sequences and allelic variants for multiple loci from hundreds of specimens representing all major lineages and ecological groups of Cantharellales. PacBio sequencing succeeded for many difficult samples where Sanger sequencing had failed, often due to length polymorphisms, corticioid compound organisms, or presence of protist contaminants. I recovered particularly high levels of within-sample sequence divergence in corticioid Cantharellales– up to 8.8% even in single-copy protein-coding loci. Many of these species have binucleate and multinucleate hyphae, which can be genetically divergent within a single individual. Species delimitation based on phylogenetic and morphological criteria has proved problematic in these groups and species limits remain unresolved. However, this PacBio sequencing method provides a promising path forward to characterizing individual and species-level genetic variation in these fungi.

Lastly, I combined PacBio, Sanger, and JGI genome sequence data to generate the first densely sampled multi-locus phylogeny of the Cantharellales. Single-gene phylogenies revealed the small subunit (18S) and internal transcribed spacer (ITS) nuclear ribosomal DNA loci contained considerable length heterogeneity in the genera *Cantharellus*, *Craterellus*, and *Tulasnella*, and were thus excluded from the multi-locus phylogenetic analysis. Overall, the multi-gene phylogeny supports recognition of four monophyletic families within the Cantharellales: Hydnaceae *sensu lato*, Botryobasidiaceae, Ceratobasidiaceae, and Tulasnellaceae. Stichic basidia may be a synapomorphy for the Hydnaceae, but this has not yet been investigated in the lichenicolous or saprobic corticioid members of in the family. Three independent transitions to an ECM nutritional mode are predicted within the Hydnaceae. Overall, *rpb2* remains the most suitable locus for species recognition and phylogenetic inference across the Cantharellales, while acknowledging that multiple divergent copies of this gene may exist within a species.

There remain many outstanding questions about the evolution of nutritional modes and species diversification in the Cantharellales, such as: What is the nutritional mode of non-pathogenic and non-mycorrhizal Cantharellales species? Did acquisition of a stipitate morphology with an ECM nutritional mode drive species diversification? How did species diversity change across time and space? Further studies of diversification,

biogeography, and functional ecology of the Cantharellales are warranted to understand the mechanisms and outcomes of evolution in this important group of Agaricomycetes.

VITA

Rachel A. Swenie was born in Illinois. She earned a Bachelor of Fine Arts in Photography from Columbia College Chicago and went on to found and run a mycology laboratory and mushroom farm at The Plant in the Back of the Yards neighborhood of Chicago. Keen to learn more about fungal diversity, Rachel returned to school to study biology and mycology. She conducted doctoral studies on systematics and taxonomy of chanterelle mushrooms and allies under the mentorship of Brandon Matheny and received her Ph.D. in Evolutionary Biology from the University of Tennessee, Knoxville in 2023. Rachel is an avid field mycologist and co-author of a forthcoming field guide to mushrooms of the southern Appalachian Mountains.