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Coprinopsis laanii fruiting on mammal carcasses in an underground mine

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ABSTRACT

Fungi are important decomposers of organic material, including animal waste. Ammonia and postputrefaction fungi grow in soil enriched in ammonium and nitrogen from carcasses. In 2014, we observed mushrooms fruiting on the flesh of a dead muskrat (*Ondatra zibethicus*) in an abandoned underground copper mine in southeastern New Brunswick, Canada. We placed an adult beaver (*Castor canadensis*) carcass near the muskrat to facilitate fungal colonization and fruiting. The beaver carcass was colonized by a variety of molds, especially *Acaulium caviariforme*. We observed mushrooms of an unidentified copriniid on the flesh 6 years and 9 months after carcass placement. Using morphological and molecular (nuclear internal transcribed spacer [nrITS]) data, we identified the mushrooms as *Coprinopsis laanii*, a rarely encountered species generally considered lignicolous. We discuss the role of *C. laanii*, and other postputrefaction fungi, in cave environments.

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KEYWORDS
Ammonia fungi; cave fungi; corpse mushrooms; postputrefaction fungi; psathyrellaceae

INTRODUCTION

Fungi are important decomposers of organic material, including animal waste. "Ammonia fungi" are a chemoecological group of fungi that fruit after sudden additions of ammonia or other nitrogenous materials to soil (Sagara 1992). Some fungi grow in soil enriched in ammonium and nitrogen from animal carcasses, such as corpse finder mushrooms in the genus *Hebeloma* (Sagara et al. 2008). These fungi do not grow on buried cadavers per se, but rather on soil enriched with the subsequent release of nitrogen during the cadaver's decomposition (termed the cadaver decomposition island) (Carter and Tibbett 2003; Carter et al. 2007). Generally, carcasses decompose quickly, which may preclude the formation of fungal reproductive structures directly on the waste (Sagara 1995). However, various species of mold grow on carcasses (Hawksworth and Wiltshire 2011; Ishii et al. 2006; Nováková et al. 2018). Ammonia and postputrefaction fungi undergo a succession of fruiting where one set of fungi is later replaced by another (Sagara 1992, 1995). Early-stage fungi are generally saprotrophic and fruit from 1 to 10 months after enrichment of soil with urea or other nitrogenous materials, whereas late-stage fungi comprise ectomycorrhizal basidiomycetes that fruit from 1 to 4 years after soil enrichment (Fukiharu and Hongo 1995; Sagara 1992; Sagara et al. 2008).

Animal carcasses are sometimes found in caves where decomposition is slow and carcasses are colonized by molds (Nováková et al. 2018). During routine surveys of hibernating bats (Vanderwolf and McAlpine 2021), we found small mushrooms growing on the flesh of a muskrat carcass (*Ondatra zibethicus*) on the ground in an abandoned mine. Placing a carcass nearby produced additional mushrooms, and we collected sufficient material for identification via genetic sequencing.

MATERIALS AND METHODS

We conducted this study in Dorchester Mine, an ungated, abandoned, copper mine in southeastern New Brunswick, Canada, that operated intermittently from 1881 to 1917. The copper mineralization consists mostly of chalcocirte, and malachite. Chalcocite is found within small veinlets and nodules, whereas malachite is mostly associated with fossilized plant fragments within gray sandstones (Wright 1950). The gray sandstone is reported to contain 4–5% chalcocite (S. Hinds, New Brunswick Geological Surveys Branch, pers. comm.). A straight adit, with water of 10–50 cm depth flowing toward the mine mouth, branches ~450 m from the mine entrance into two tunnels. The total length of the mine is around 930 m (Wright 1950). We collected temperature and

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relative humidity data as part of another study (Vanderwolf and McAlpine 2021). Briefly, we placed an iButton (model DS1921G; ±1 C; Maxim Integrated Products, Sunnyvale, California, USA) set to record air temperature twice daily (02:30 and 14:30 h) about 1 m from the mine floor on a wall within 3 m of the carcasses from December 2011 to August 2017. The overall mean temperature over the recording period was 6.6 ± 0.24 C (max = 7.1 C, min = 6.1 C; n = 5659 measurements). We measured relative humidity with a Kestrel 3000 Handheld weather meter (MPN 0830; ±1% RH; Boothwyn, Pennsylvania, USA) during site visits. Kestrel measurements took some time to stabilize, so the device was temporarily positioned on the mine floor for measurements. The relative humidity was 79.6% on 31 March 2015 and 89% on 4 December 2014.

We first observed the muskrat (*Ondatra zibethicus*) carcass in the mine on 12 March 2014 in the dark zone (area where no light penetrates) approximately 500 m from the mine entrance and adjacent to a pool of water >3 m depth leading to the flooded lower level of the mine. The carcass was in an advanced state of decay, although flesh was still present (FIG. 1). Mold growth was present, but no mushrooms were visible. We collected mushrooms growing on the flesh of the muskrat, especially the tail, on 4 December 2014, 31 March 2015, 14 July 2015, and 24 August 2017 (deposited in the New Brunswick Museum herbarium: NBM-FF-005101, NBM-FF-005142, NBM-FF-005750). However, the mushrooms were small, fragile, and easily autolyzed, so we did not obtain enough material for sequencing. To obtain more mushrooms, we placed two adult beaver (*Castor canadensis*) carcasses in the mine on 14 July 2015, one adjacent to the muskrat and the second about 3 m away in an alcove (FIG. 2). Beaver carcasses were frozen at the time of placement, and we made no attempt to inoculate the carcasses with spores from the muskrat carcass. The beaver carcass adjacent to the muskrat was not present on the next visit on 29 August 2016. The beaver carcass in the alcove remained and was heavily colonized with mold during our visits on 29 August 2016 and 24 August 2017, but no mushrooms were visible (FIG. 3). Many mushrooms were growing on the beaver carcass 8 April 2022 (FIG. 4), and we collected sufficient samples for genetic sequencing.

We used small fragments of dried basidiomes for DNA extraction to obtain one sequence of the unknown mushrooms. ALVALAB (http://www.alvalab.es/) conducted the molecular work using standard methods for the study of basidiomycete fungi (e.g., Justo and Hibbett 2011). We used the primer combination ITS1F and ITS4

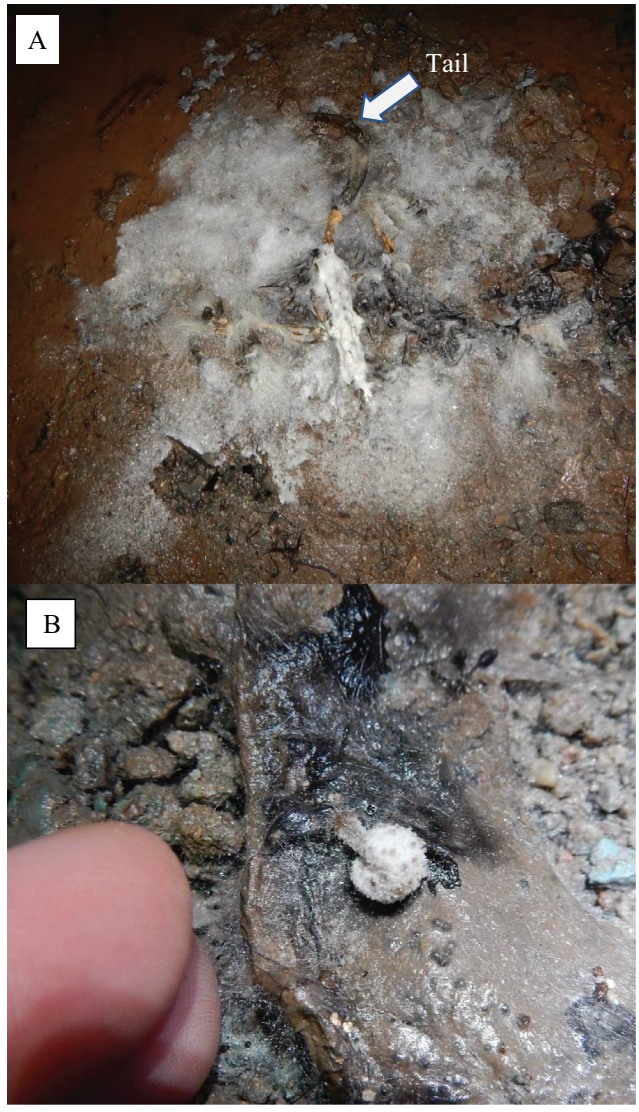

Figure 1. Dead muskrat (*Ondatra zibethicus*) in Dorchester Mine, New Brunswick, Canada, 12 March 2014, with white mold growing out from the carcass (A), and 31 March 2015, depicting one of the mushrooms growing out of the flesh (B).

(Gardes and Bruns 1993; White et al. 1990) for amplification and sequencing of the fungal barcode (nuc rDNA internal transcribed spacer ITS1-5.8S-ITS2). We edited and assembled raw data in ChromasPro (Technelysium, South Brisbane, Australia). We assembled an ITS data set of all publicly available sequences in GenBank with a sequence similarity to our collection ≥89% over the entire length of the sequences (as calculated in a standard BLAST search in the National Center for Biotechnology [NCBI] database). All sequences included in the analysis are of comparable length, with only a few base pair difference at either end of the ITS region. The only exception is the sequence of *Coprinopsis martinii* (GU23412), which is missing a good portion of ITS2 (about 150 bp). We aligned a total of 20 ITS sequences using MAFFT 7 (Katoh et al.

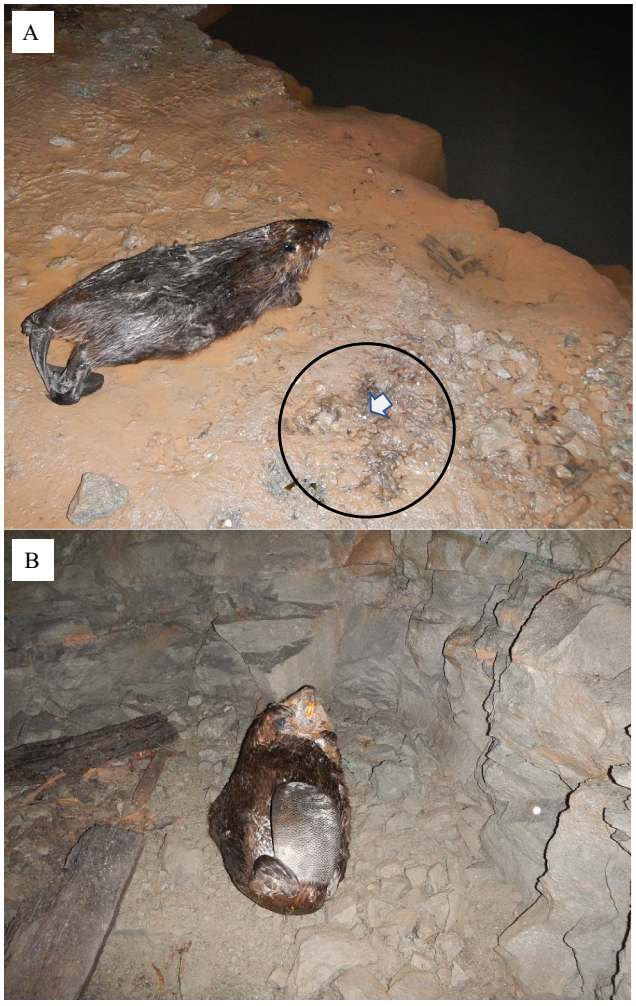

Figure 2. A. Beaver (*Castor canadensis*) carcass placed adjacent to muskrat (*Ondatra zibethicus*) carcass and a deep pool of water in Dorchester Mine, 14 July 2015. The remains of the muskrat are circled. The arrow indicates two mushrooms on the muskrat carcass. B. Beaver carcass placed in the alcove, 14 July 2015.

2019) and the strategy FFT-NS-i. We used a sequence of *Coprinus cortinatus* as an outgroup. We inspected and manually corrected the alignment in AliView (Larsson 2014). We ran a maximum likelihood (ML) analysis in RAxML 8.2.12 under a GTRGAMMAI model as recommended (Stamatakis 2014) with 1000 rapid bootstrap (BS) replicates, using resources at the CIPRES Science Gateway (Miller et al. 2010, see Supplemental data).

RESULTS AND DISCUSSION

Our sample (GenBank OR269905) appears in the phylogeny together with a GenBank sequence of *Coprinopsis laanii* (Q249276) (Kits van Wav.) Redhead, Vilgalys & Moncalvo (Basidiomycota: Psathyrellaceae; FIG. 5). Both sequences appear as the sister clade to a sequence of *C. martinii*, and part of a well-supported clade that includes other species of *Coprinopsis* subsection *Narcotici: C. trispora, C. narcotica, C. sclerotiger* (=? *C. tuberosus*), and *C. stercorea* (TreeBASE S30569). Species of subsection *Narcotici* are characterized by the mealy-powdery veil covering the pileus, made up of globose elements, and the spores usually with distinct myxosporium.

Coprinopsis is a large genus with close to 200 species found worldwide on soil, wood, plant debris, and dung (Lebel et al. 2022; Redhead et al. 2001). Although occurrences are not all confirmed with genetic data, *Coprinopsis laanii* has previously been documented on cut tree stumps, wood chips, and fallen and rotted wood in Finland, Slovakia, Germany, Poland, Belgium, the Netherlands, Bulgaria, Scotland, Spain, and Sri Lanka but is considered rare (Kits van Waveren 1968; Runge 1986; Ódor et al. 2001; Denchev et al. 2007; Fernando 2009; Oosterbaan et al. 2009; Arrillaga et al. 2011; Adamčík et al. 2016; Von Bonsdorff et al. 2016; Gierczyk et al. 2017; Vandekerkhove et al. 2018; Watling and Riddiford 2021). Unconfirmed online sources, such as iNaturalist, suggest an even wider global distribution in temperate environments in both the Northern and Southern hemispheres. Although *Coprinopsis laanii* has not been previously documented in caves or mines, *C. atramentaria* and *C. radiata* have been found on wood and walls in caves in Europe and North America (Vanderwolf et al. 2013). *Coprinopsis laanii* is considered an ammonia fungus, as treating cut tree stumps with urea increased colonization by *C. laanii* (Pratt and Redfern 2001).

In our study site, carcass decomposition is slow and primarily conducted by microbes. Necrophagous insect taxa and vertebrate scavengers are absent due to the distance from the mine entrance and because the floor of the entrance adit is covered by a stream for much of its length, which may discourage vertebrates. Although we did not attempt to identify the variety of molds present on the carcasses, masses of black ascocarps of *Acaulium caviariforme* were readily identifiable and occurred extensively on the flesh of the muskrat and beaver carcasses. This species is commonly found on carcasses in caves and mines (Nováková et al. 2018). The slow decomposition and fruiting process may also be due to constant low temperatures (6.6 C) in the mine. The temperature optima for spore germination of several other species

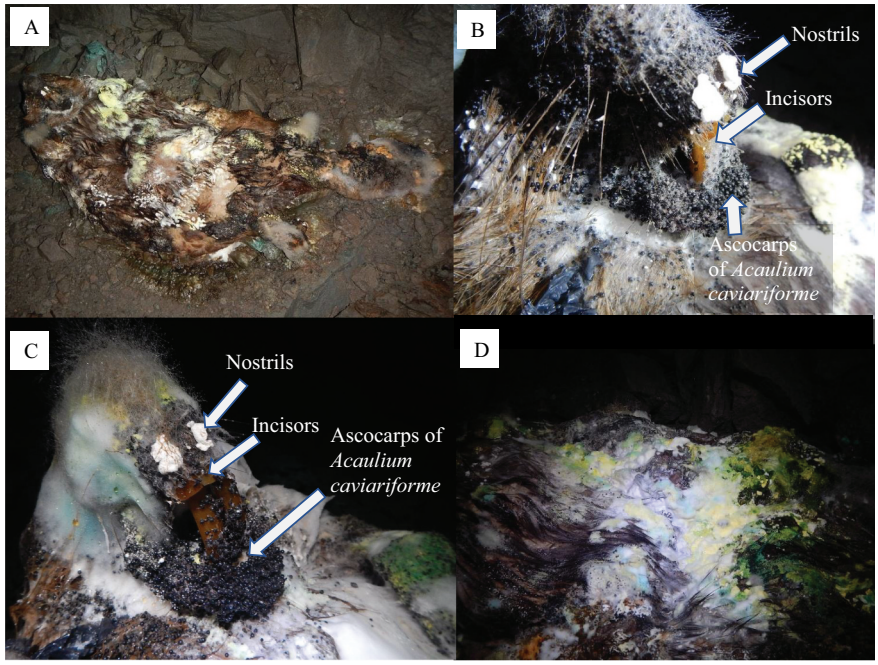

Figure 3. Beaver (*Castor canadensis*) carcass in the alcove in Dorchester Mine showing heavy mold growth, 29 August 2016 (A, B) and 24 August 2017 (C, D).

Figure 4. Body and face (orange incisors visible) of beaver (*Castor canadensis*) carcass in the alcove in Dorchester Mine with *Coprinopsis laanii* mushrooms visible, 8 April 2022.

of *Coprinopsis* belonging to the ammonia fungi group is 15–30 C (Raut et al. 2011).

Coprinopsis spp. and other agarics are considered early-stage (1–10 months) postputrefaction fungi (Fukiharu and Hongo 1995; Sagara 1992; Sagara et al. 2008). We did not observe *C. laanii* fruiting until 6 years and 9 months after we placed the beaver carcass in the mine. Although our surveys were irregular (29 August 2016, 24 August 2017, and 8 April 2022) and fruiting likely started earlier than our first detection, fruiting had not started 2 years 2 months after carcass placement. The *C. laanii* mushrooms were fruiting on the muskrat 14 July 2015 and 24 August 2017, and this may have been the source of the fungus on the beaver, or potentially the fungus originated from plant material in the gut of both animals. Late-stage postputrefaction fungi are ectomycorrhizal, and these fungi do not appear in plots where roots are excluded (Sagara 1995). Although ectomycorrhizal fungi associated with tree roots, including *Hebeloma* spp., are present in caves and mines in our study region (Poelman et al. 2021), roots do not extend deep enough at our study site to reach the carcass' location. Likely this explains why we did not observe late-stage postputrefaction fungi on carcasses in the mine.

Coincident with the appearance of *C. laanii* on the beaver carcass, the flesh was mushy and the surface black (FIG. 4). The location of buried and decomposing vertebrate cadavers may be stained with a black color at the soil surface when ammonia fungi are present (Sagara et al. 2008). The black staining is caused by the output of excess ammonia by ammonia fungi, which increases soil pH, solubilizing black humic and fulvic acids (Sagara et al. 2008). A similar process may have occurred with the carcass at our study site.

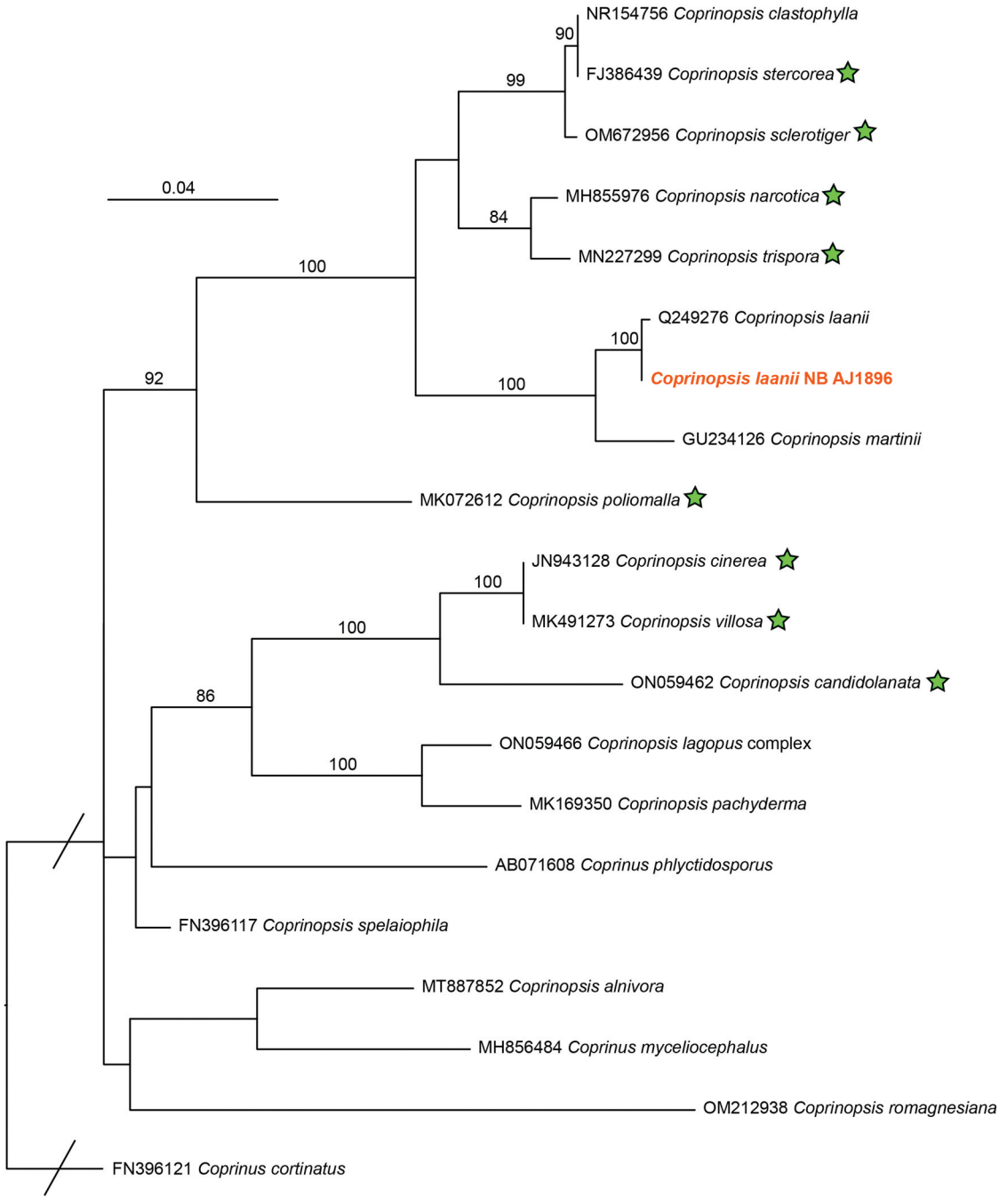

Figure 5. Maximum likelihood analysis of an ITS data set of all publicly available sequences in GenBank with a sequence similarity ≥89% to our mushroom sample (in orange, GenBank OR269905). *Coprinus cortinatus* is the outgroup. TreeBASE S30569. Species marked with a star have been reported to grow on dung (or other nitrogen-enriched substrates).

Mushrooms growing on the flesh of animal carcasses have rarely been documented. Future surveys of animal carcasses in caves and mines may yield further insights into the ecology of these uncommon fungi. These humid environments with limited or no access by scavengers and necrophagous insects may provide useful sites for experimental placement of host carcasses.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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LITERATURE CITED

Adamčík S, Aude E, Bässler C, Christensen M, Van Dort K, Fritz Ö, Glejdura S, Heilmann-Clausen J, Holec J, Jančovicová S, et al. 2016. Fungi and lichens recorded during the cryptogam symposium on natural beech forests, Slovakia 2011. Czech Mycol. 68:1–40. doi:10.33585/cmy.68101.

Arrillaga P, Albizu J, Lasa J, Martin J, Teres J. 2011. Especies raras o poco conocidas de hongos macromicetos. V Zizak. 8:35–49.

Carter DO, Tibbett M. 2003. Taphonomic mycota: fungi with forensic potential. J Forensic Sci. 48(1):2002169. doi:10.1520/JFS2002169.

Carter DO, Yellowlees D, Tibbett M. 2007. Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften. 94 (1):12–24. doi:10.1007/s00114-006-0159-1.

Denchev CM, Fakirova VI, Gyosheva MM, Petrova RD. 2007. Macromycetes in the Pirin Mts (SW Bulgaria). Acta Mycol. 42(1):21–34. doi:10.5586/am.2007.002.

Fernando KMEP. 2009. Species richness and ecological characterization of wood- inhabiting agaric fungi on home-garden logs in semi- urbanized areas in Colombo suburbs. Vidyod J Sci. 14:177–187.

Fukiharu T, Hongo T. 1995. Ammonia fungi of Iriomote Island in the southern Ryukyus, Japan and a new ammonia fungus, *Hebeloma luchuense*. Mycoscience. 36(4):425–430. doi:10.1007/BF02268627.

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

Gierczyk B, Szczepkowski A, Kujawa A, Ślusarczyk T, Zaniewski P. 2017. Contribution to the knowledge of fungi of the Kampinos National Park (Poland) with particular emphasis on the species occurring in burnt places. Acta Mycol. 52(1):1–18. doi:10.5586/am.1093.

Hawksworth DL, Wiltshire PEJ. 2011. Forensic mycology: the use of fungi in criminal investigations. Forensic Sci Int. 206 (1–3):1–11. doi:10.1016/j.forsciint.2010.06.012.

Ishii K, Hitosugi M, Kido M, Yaguchi T, Nishimura K, Hosoya T, Tokudome S. 2006. Analysis of fungi detected in human cadavers. Legal Med. 8(3):188–190. doi:10.1016/j.legalmed.2005.12.006.

Justo A, Hibbett D. 2011. Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five–marker dataset. Taxon. 60(6):1567–1583. doi:10.1002/tax.606003.

Katoh K, Rozewicki J, Yamada K. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings Bioinf 20 (4):1160–1166. doi:10.1093/bib/bbx108.

Kits van Waveren E. 1968. The "stercorarius group" of the genus *Coprinus*. Persoonia. 5:131–176.

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics. 300:3276–3278. doi:10.1093/bioinformatics/btu531.

Lebel T, Davoodian N, Bloomfield MC, Syme K, May TW, Hosaka K, Castellano MA. 2022. A mixed bag of sequestrate fungi from five different families: boletaceae, Russulaceae, Psathyrellaceae, Strophariaceae, and Hysterangiaceae. Swansona. 36:33–65.

Miller MA, Pfeiffer W, Schwartz T 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop. New Orleans, LA. p. 1–8.

Nováková A, Kubátová A, Sklenář F, Hubka V. 2018. Microscopic fungi on cadavers and skeletons from cave and mine environments. Czech Mycology. 70(2):101–121. doi:10.33585/cmy.70201.

Ódor P, Heilmann-Clausen J, Christensen M, Aude E, van DKW, Piltaver A, Siller I, Veerkamp MT, Walleyn R, Standovár T, et al. 2001. Diversity and composition of dead wood inhabiting fungal and bryophyte communities in semi-natural beech forests in Europe.

Oosterbaan A, van den Berg CA, de Boer T, de Jong JJ, Moraal LG, Niemeijer CM, Veerkamp M, Verkaik E. 2009. Storm en bosbeheer; Afwegingen voor het laten liggen of ruimen van stormhout. Wageningen.

Poelman A, Weerasuriya N, Vanderwolf KJ, Malloch D, McAlpine DF, Thorn RG. 2021. Fungi associated with aeroponic roots in caves and mines of New Brunswick. Fungal Ecol. 52:101074. doi:10.1016/j.funeco.2021.101074.

Pratt JE, Redfern DB. 2001. Infection of Sitka spruce stumps by spores of *Heterobasidion annosum*: control by means of urea. Forestry. 74:73–78. doi:10.1093/forestry/74.1.73.

Raut JK, Suzuki A, Yoshihara M. 2011. Effects of environmental factors on basidiospore germination of ammonia fungi *Coprinopsis* spp. collected from different geographical areas. Mycoscience. 52(5):300–311. doi:10.1007/S10267-011-0107-6.

Redhead SA, Vilgalys R, Moncalvo J, Johnson J, Hopple J. 2001. Coprinus Pers. and the disposition of Coprinus species sensu lato. Taxon. 50(1):203–241. doi:10.2307/1224525.

Runge A. 1986. Pilzsukzession während der Finalphase auf Pappelstümpfen. Zeitschrift Für Mykologie. 52:217–224.

Sagara N. 1995. Association of ectomycorrhizal fungi with decomposed animal wastes in forest habitats: a cleaning symbiosis? Can J Bot. 73(S1):S1423–S1433. doi:10.1139/b95-406.

Sagara N. 1992. Experimental disturbances and epigeous fungi. In: Carroll G, Wicklow D, editors. The fungal community: its organization and role in the ecosystem. 2nd ed. New York (NY): Marcel Dekker; p. 427–454.

Sagara N, Yamanaka T, Tibbett M. 2008. Soil fungi associated with graves and latrines: toward a forensic mycology. In: Tibbett M, Carter D, editors. soil analysis in forensic taphonomy: chemical and biological effects of buried human remains. Boca Raton (FL): CRC Press, Taylor and Francis Group; p. 68–102.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313. doi:10.1093/bioinformatics/btu033.

Vandekerkhove K, Deforce K, Bastiaens J. 2018. Historic-ecological position of beech in the area of the Sonian Forest and an overview of beech-forest-related biodiversity present in the forest. *Rapporten van het Instituut voor Natuur- en Bosonderzoek*. 29:41. doi:10.21436/inbor.14173748.

Vanderwolf KJ, Malloch D, Mcalpine DF, Forbes GJ. 2013. A world review of fungi, yeasts, and slime molds in caves. Int J Speleol. 42(1):77–96. doi:10.5038/1827-806X.42.1.9.

Vanderwolf KJ, McAlpine DF. 2021. Hibernacula microclimate and declines in overwintering bats during an outbreak of white-nose syndrome near the northern range limit of infection in North America. Ecol Evol. 11(5):2273–2288. doi:10.1002/ece3.7195.

Von Bonsdorff T, Niskanen T, Liimatainen K, Kytövuori I, Huhtinen S, Vauras J, Höijer P, Kekki T, Lahti M, Puolasmaa A, et al. 2016. New national and regional biological records for Finland 8. Contributions to agaricoid, gastroid and ascomycetoid taxa of fungi 5. Memoranda Soc pro Fauna et Fl Fenn. 92:120–128.

Watling R, Riddiford NJ. 2021. Checklist of the non-lichenised fungi of Fair Isle, Scotland. Glasg Nat. 27 (3):28–41. doi:10.37208/tgn27322.

White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press; p. 315–322.

Wright W. 1950. Dorchester copper deposit. Westmorland County, New Brunswick: N.B. Fredericton.