Investigations in the boletes (Boletaceae) of southeastern USA: four novel species and three novel combinations

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Abstract

The Boletaceae is the largest family of fleshy fungi in the Boletales. Despite the extensive history of work in the Boletaceae in North America, novel species and genera are continually being described. Multigene molecular phylogenetic analyses of five loci were combined with thorough morphological studies to investigate the taxonomy of several boletes from the southeastern USA. Based on our results, we describe four new species: *Aureoboletus pseudoauriporus*, *Cyanoboletus bessettei*, *Hemileccinum floridanum*, and *Xerocomellus bolinii*. We also propose three combinations to reflect the results of our molecular analyses: *Cyanoboletus cyanetinctus* comb. nov., a bolete that is widespread across the eastern USA, *C. cyanetinctus* f. *reticulatus*, and *Lannmaoa sublurida*, a rarely-documented bolete that is so far known only from Florida.

Keywords – Boletales – ectomycorrhizal – phylogeny

Introduction

The boletes of the southeastern USA are diverse but poorly studied. Perhaps the first taxonomic work on the southeastern boletes began with Thomas Walter’s (Walter 1788) *Boletus dimidiatus*, nom. illeg. Several more southeastern boletes were described by von Schweinitz (1822) and Berkeley & Curtis (1853). Peck and Frost were both prolific with bolete studies in the late 1800s (Halling 1983, Both & Ortiz-Santana 2010). Murrill (1909) published the first monograph of the boletes of North America, although after its publication, he described many more species of boletes (Halling 1986), especially from Florida (Weber 1961, Halling 1986). Coker & Beers (1943) published a monograph of the boletes of North Carolina. Rolf Singer published a monograph on the boletes of Florida (Singer 1945a, b, 1947), which treated species common to the southeastern USA, endemic to Florida, and extralimital species from around the globe. Later, Murrill (1948) published a summation of Florida boletes, one of the last broad treatments of the boletes of the region. Other works broadly focused on boletes in the southeastern USA included Thiers (1963) and Grand (1970a, b, c). Both (1993) published a compendium of all boletes described in North America, providing diagnostic features as well as taxonomic notes on each species. Despite the extensive history and monographic treatments, novel species of boletes from the southeastern USA are continually being described...
Molecular phylogenetic analyses have redefined our understanding of the boletes. Once considered to consist of only a few genera, the Boletaceae has now increased to over 70 genera (Nuhn et al. 2013, Wu et al. 2014, 2016). In part, this expansion is due to the recognition of sequestrate (Yang et al. 2006, Smith et al. 2015, Castellano et al. 2016, Vadthanarat et al. 2018, Wu et al. 2018) and new lamellate (Farid et al. 2018, Zhang & Li 2018) genera. This increase of genera is also due to molecular phylogenetic analyses allowing taxonomists to better recognize synapomorphies, as many of the traditional characters used to classify the boletes were homoplastic. The broad relationships between genera are also better understood with analyses of molecular data. An analysis of 290 operational taxonomic units (OTUs) across 59 genus-level clades by Wu et al. (2014) also revealed six subfamily-level recognitions (Xerocomoideae, Leccinoideae, Boletoideae, Austroboletoideae, Zangioidae, and Chalcioporoideae), although some genera did not resolve to any of the known subfamilies (Solioccasus Trappe, Osmundson, Manfr. Binder, Castellano & Halling, Bothia Halling, T.J. Baroni & Manfr. Binder, Gymnogaster J.W. Cribb, Baorangia G. Wu & Zhu L. Yang, and Pseudoboletus Šutara), including one large grouping of genera (the Pulveroboletus group).

Boletes serve vital ecological roles as ectomycorrhizae of the primary forest trees (Quercus and Pinus) of the southeastern USA, yet the extent of their diversity in this region is largely unknown. The aim of this paper is to update our understanding of boletes in southeastern North America, through multigene phylogenetic analyses. The name Boletus cyanetinctus is resurrected for a species closely related to Cyanoboletus pulverulentus (Opat.) Gelardi, Vizzini & Simonini. This paper provides the first phylogenetic analyses of a rarely documented bolete, Suillellus sublurid Murrill, which is transferred to Lanmaoa. We also describe four novel species, including one of Xerocomellus, an uncommon species of Cyanoboletus, a species that resembles Hemileccinum subglabripes (Peck) Halling, and one that resembles Aureoboletus auriporus (Peck) Pouzar. We also generated protein-coding sequences from the epitype of Pulchroboletus rubricitrinus, as well as from specimens of western Xerocomellus. Finally, we generated sequences from an herbarium specimen of Exsudoporus floridanus from Florida and discuss the generic concepts of Exsudoporus and Butyriboletus.

Materials & Methods

Sampling and morphological studies
Specimens were collected in situ between 2015–2020 and deposited at the University of South Florida Herbarium (USF). Additional collections were obtained on loan from Florida Museum of Natural History (FLAS) for study. Macroscopic descriptions were made using fresh basidiomes. Micromorphological features were observed with a phase contrast microscope (AmScope, Irvine, CA, USA). Distilled H₂O, lactoglycerol, KOH, and Phloxine B were used to rehydrate and stain sections (Singer 1986). Measurements were made at 1000 × with a calibrated ocular micrometer in Piximètre 5.9 R 1532 (http://piximetre.fr). Basidiospore dimensions are reported as length by width, with each measurement reported as the minimum, the average minus the standard deviation, the average plus the standard deviation, and the maximum. Spore dimensions are followed by the number of spores counted, N, and the average quotient mean, Q, where Q is the average length divided by the average width. Scanning Electron Microscopy (SEM) was performed at the Electron Microscopy Core Facility at the University of South Florida on an Aquila Hybrid Scanning Electron Microscope (Topcon, Tokyo, Japan).

DNA Extraction, PCR amplification, and sequencing
Genomic DNA was isolated as described in Farid et al. (2017). A subset of the samples was
extracted using the NucleoSpin Plant II Kit (Macherey-Nagel Inc. Bethlehem, Pennsylvania, USA). Portions of five gene regions were targeted for phylogenetic analysis: nuc rDNA internal transcribed spacer ITS1-5.8S-ITS (ITS), nuc 28S rDNA (28S), RNA polymerase II subunit 1 (RPB1), RNA polymerase II subunit 2 (RPB2), and translation elongation factor 1-alpha (TEF1) were amplified according to Farid et al. (2019). The primer pair ITS1-F/ITS4 (White et al. 1990). Gardes & Bruns (1993) were used to amplify ITS, LR0R/LR7 (Vilgalys & Hester 1990) for 28S. The bolete-specific primer pairs EF1-BF1/EF1-B-R, RPB1-B-F/RPB1-B-R, and RPB2-B-F1/RPB2-B-R (Wu et al. 2014) were used to amplify TEF1, RPB1, and RPB2, respectively. Crude PCR product was purified and sequenced at the DNA laboratory at Arizona State University with a 3730 DNA Analyzer (applied Biosystems, Carlsbad, CA, USA) using the same PCR primers for amplification, and additionally the internal 28S primers LR5 and LR3R were used (Vilgalys & Hester 1990).

A subset of samples (JAB 95 and JAB 80) was obtained using a nested PCR method. First, the primer pair gRPB1-Af/rRPB1-Cr (Matheny et al. 2002) were used to amplify a portion of the RPB1 gene; PCR products were then diluted in nanoPure H2O in a 1:100 ratio used in a second hemi-nested PCR using one of the original primers gRPB1-Af or rRPB1-Cr paired with an internal primer chosen from either RPB1-B-F or RPB1-B-R or one of two novel Boletales specific primers (Table 1).

**Table 1** Primer design Boletales-specific **RPB1** primers

| Primer name       | Sequence (5’ → 3’)                |
|-------------------|-----------------------------------|
| RPB1mexF1bol      | CGRCATGTYGCGATCC                  |
| RPB1mexR2bol      | GGWTCTRCAGYTTCGCA                 |

**Alignments, model selection, and phylogenetic analyses**

A multi-locus phylogeny consisting of ITS, 28S, RPB1, RPB2, and TEF1. Alignments of each locus were made in R (R Core Team 2017) using MAFFT v. 7.471; alignments of rDNA used the predicted secondary structure to improve the alignment. Gblocks v. 0.91b (Katoh & Standley 2013) was used to remove ambiguous regions of the resultant alignments to improve phylogenetic inference. Models were selected for each locus using jModelTest 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012). Bayesian information criterion models were selected for each partition, though we report all the models selected (Table 2). The resultant alignments were combined in Sequence Matrix (http://www.ggvaidya.com/taxondna/), with taxa missing target loci encoded as missing data (Felsenstein 2004). Seventeen genera from the Boletaceae were included in the phylogenetic analyses (Fig. 1): *Aureoboletus* Pouzar, *Heimieccinum* Šutara, *Pulchroboletus* Gelardi, Vizzini & Simonini, *Heimioporus* E. Horak, *Alessioporus* Gelardi, Vizzini & Simonini, *Xerocomellus* Šutara, *Nigroboletus* Gelardi, Vizzini, E. Horak, T.H. Li & Ming Zhang, *Hortiboletus* Simonini, Vizzini & Gelardi, *Boletus* L., *Baorangia* G. Wu & Zhu L. Yang, *Cyanooboletus* Gelardi, Vizzini & Simonini, *Lammaoa* G. Wu & Zhu L. Yang, *Butyrboletus* D. Arora & J.L. Frank, *Suillellus* Murrill, *Gymnogaster* J.W. Cribb, *Chalciopus* Bataille, and *Buchwaldoboletus* Pilát.

Phylogenetic analyses were conducted using the CIPRES Gateway server V3.3 (Miller et al. 2010). Maximum likelihood (ML) was conducted with RAXML-HPC 8.2.10 (Stamatakis 2014) using 1000 non-parametric bootstrap replicates (BS) and a partitioned model. Bayesian inference (BI) was conducted with MrBayes 3.2.6 on XSEDE platform of the CIPRES Science Gateway server (Ronquist et al. 2012). Four Markov chain Monte Carlo simulations were run for ten million generations, sampling trees every thousand generations. Chain convergence was determined using Tracer V1.6 (Rambaut et al. 2018). The first 25% were discarded as burn-in, and a majority rule consensus tree was computed to obtain estimates for Bayesian posterior probabilities (BPP). BI trees were visualized in Figtree (Rambaut 2007) and exported into Inkscape, where bootstrap values were added to node labels. BPP above 0.90 and bootstrap values above 70% were reported. Alignment and phylogenetic trees were uploaded to http://www.treebase.org/ (submission ID 27951).
Table 2 Models selected for each locus using different model strategies in jModelTest 2.1.10. Abbreviations: AICc = Akaike information criterion. BIC = Bayesian information criterion. DT = Decision theory. GTR = Generalized time reversible model. HKY = Hasegawa, Kishino and Yano 1985 model. K80 = Kimura’s two parameter model. SYM = Symmetrical model. I = Invariant. G = Gamma

| Model Strategy | ITS         | 28S         | RPB1        | RPB2        | TEF         |
|----------------|-------------|-------------|-------------|-------------|-------------|
| AICc           | GTR+I+G     | GTR+I+G     | HKY+I+G     | SYM+I+G     | HKY+I+G     |
| BIC            | GTR+I+G     | GTR+I+G     | K80+I+G     | K80+I+G     | HKY+I+G     |
| DT             | HKY+I+G     | GTR+I+G     | K80+I+G     | K80+I+G     | HKY+I+G     |

Figure 1 – Phylogram generated from MrBayes based on ITS, 28S, RPB1, RPB2, and TEF1 sequence data. Nodes labeled with PP (≥ 0.90) followed by bootstrap replicate support (≥ 70). Colors represent
distinct genera. Specimens with molecular data generated in this study are bolded. Inset phylogeny depicts portion of phylogeny shown in figure.

Figure 1 – Continued.
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Results

Phylogenetic analyses

The final dataset consisted of 305 specimens comprising 141 ITS, 234 28S, 140 RPBI, 165 RPB2, 216 TEF1 sequences (Supplementary Table 1). A total of 143 sequences were generated for this study. The six species from this study were distributed across five genera. One species of *Aureoboletus* forms a strongly supported clade (0.96 BPP, 96 bootstrap replicate support), with somewhat strong support (0.91 BPP, 94 bootstrap replicate support) as a sister clade with *Aureoboletus auriporus* (Peck) Pouzar. A strongly supported clade in *Hemileccinum* with somewhat strong support (0.96 BPP, but <70 bootstrap replicate support) was sister to a clade of *Hemileccinum subglabripes* (Peck) Halling. In *Xerocomellus*, a strongly supported clade was sister to an unnamed *Xerocomellus* sp. (HKAS 56311) from China. Three specimens of *Nigroboletus roseonigrescens* Gelardi, Vizzini, E. Horak, T.H. Li & Ming were strongly supported as basal to all *Xerocomellus* sequences included in the analyses. Two species in *Cyanoboletus* were recovered in the analyses. This first *Cyanoboletus* species is in a strongly supported clade (1 BPP, 98 bootstrap replicate support) containing *Cyanoboletus pulverulentus* s.str., and *Cyanoboletus sinopulverulentus* (Gelardi & Vizzini) Gelardi, Vizzini & Simonini, although *C. sinopulverulentus* did not receive strong support as sister to either of these species. The second *Cyanoboletus* species formed a strongly supported sister clade to an unnamed *Cyanoboletus* sp. (HKAS 76850) from China, and a clade containing *Cyanoboletus instabilis* (W.F. Chiu) G. Wu & Zhu L. Yang. A species of *Lannmaoa* formed a strongly supported sister clade to *Lannmaoa roseocrispans* A.E. Bessette, A.R. Bessette, Nuhn & Halling. *Pulchroboletus rubricitrinus* (Murrill) Farid & A.R. Franck, which was strongly supported as a sister clade to *Pulchroboletus roseoilbidus* (Alessio & Littini) Gelardi, Vizzini & Simonini, was consistent with the results from the nucDNA analysis in Farid et al. (2017). Our collection of *Exsudoporus floridanus* formed a strongly supported clade with *Exsudoporus floridanus* from Belize (1.0 BPP, 100 bootstrap replicate support), while the *Exsudoporus* clade was strongly supported as sister to *Butyriboletus* (1.0 BPP, 0.96 bootstrap replicate support).

*Aureoboletus pseudoauriporus* J.A. Bolin, A.R. Bessette, A.E. Bessette, L.V. Kudzma, A. Farid & J.L. Frank sp. nov.

Mycobank number: MB840856; Facesoffungi number: FoF 10467

Etymology – The epithet *pseudoauriporus* is from the Latin “pseudo” = false in reference to this bolete so closely resembling, but differing from, *Aureoboletus auriporus*.

Typification – USA, Florida, Palm Beach County, Jupiter, Abacoa Natural Area, 1 Mar 2019, J.A. Bolin 320 (holotype USF 301510).

Diagnosis – Medium-sized basidiocarps with a glabrous, non-viscid pinkish tan unchanging pileus that becomes tan with age, or sometimes retains pinkish tones. The hymenophore is bright yellow when young, becomes darker yellow and then dingy yellow with age, and does not stain when bruised or cut. The stipe is typically longitudinally striate for one-third or more of its length. Basidiospores measure (14-)15–17(-18) × 5–6.5 μm.

Description – *Pileus* 5–8.5 cm broad, convex at first, remaining so well into maturity; surface glabrous, color variable, pinkish to pinkish red or pinkish tan, usually losing pinkish tones when mature, unchanging when bruised, tastes acidic; margin incurved, even or narrowly sterile; staining pale yellow-orange then fading to light brown with the application of KOH, pale blue-green fading quickly or slowly with NH$_4$OH, slowly staining light greenish gray or negative with FeSO$_4$. Context white, unchanging or faintly and slowly turning pink or light yellow near the hymenium; staining yellow-orange with KOH, slowly light greenish gray or negative with NH$_4$OH, and light blue-green or negative with FeSO$_4$. Odor and taste not distinctive. *Hymenophore* tubulose, bright yellow when young, becoming darker yellow and then dingy yellow with age, not staining when bruised or cut; pores rounded, 1–2 per mm; tubes 4–12 mm deep. *Stipe* 4–6 cm long, 8–12 mm at the apex, 1–1.4 cm thick at the base, typically equal or slightly enlarged downward, sometimes with a pinched base; surface typically dry but viscid when wet, typically longitudinally striate for one-third or more of its...
length, whitish, sometimes with pale pink tones, not staining when bruised; context white, firm and woody toward the base, often staining faintly pinkish; with white basal mycelium.

**Basidiospores** light to medium brown in fresh deposit, (14–)15–17(–18) × 5–6.5 µm, n = 30, Q = 2.79, elliptical in face view, inequilateral in profile, thick-walled, smooth, lacking an apical pore, yellow-brown in KOH or Melzer’s. **Basidia** 25–38 × 8–13 µm, clavate, 2-sterigmate, hyaline in KOH or Melzer’s. **Basidioles** 12–23 × 6.5–8 µm, clavate, thin-walled, hyaline in KOH or Melzer’s. **Hymenial cystidia** 30–50 × 10–15 µm, cylindrical, sometimes with a capitate to capitulate apex. **Hymenophoral trama** boletoid, with lateral elements, 4–12 µm wide, moderately divergent, hyaline in KOH or Melzer’s. **Pileipellis** an ixotrichoderm, terminal elements 7–22 µm wide, highly variable, thin-walled, smooth, hyaline in KOH, with golden yellow contents in Melzer’s. **Pileus trama** hyphae loosely interwoven, highly variable, 6–32 µm wide, smooth, thin-walled, hyaline in KOH or Melzer’s. **Stipitipellis** mostly parallel, slightly interwoven, 4–12 µm wide, hyaline in KOH or Melzer’s, with fascicles of clavate or fusiform caulocystidia. **Caulocystidia** of two types; clavate, 24–42 × 12–22 µm, with yellowish contents in KOH, thin-walled, smooth; fusiform 32–39 × 8–12 µm, hyaline in KOH, thin-walled, smooth. **Stipe trama** interwoven, 6–13 µm wide, hyaline in KOH or Melzer’s, thin-walled, smooth. Clamp connections absent.

**Figure 2** – Field photograph of *Aureoboletus pseudoauriporus*. A J.A. Bolin 488. B J.A. Bolin 157. C J.A. Bolin 124. D J.A. Bolin 130. Photo credit: J.A. Bolin.
Habit, Habitat & Distribution – solitary or scattered in sandy soil with oak in a scrubby flatwood community; known from central Florida, distribution limits yet to be determined.

Material examined – USA, Florida, Hillsborough County, Brandon, S of Camden Visconti entrance pond, adjacent to canal, 27°55’27.9"N 82°20’22.9"W, 5 Oct 2016, A. Farid 501 (USF 288287); Tampa, Violet Cury Nature Preserve, 4 Jun 2017, A. Farid 592 (USF 301502); Tampa, Trout Creek Nature Preserve, Xeric hammock beneath Quercus geminata, 3 May 2019, A. Farid 919 (USF 301507); Lake County, Lake Louisa State Park, Clermont, 24 Oct 2019, J.A. Bolin 448 (USF 301492); Miami-Dade County, Everglades National Park, 5 Jul 2019, A. Farid 959 with A.R. Franck and R.E. O’Donovan (EVER 144770); Palm Beach County, Frenchman’s Forest natural Area, 28 May 2018, J.A. Bolin 167 (USF 301497); Hypoluxo Scrub Natural Area, Lantana, 21 Nov 2017, J.A. Bolin 80 (USF 301487); ibid., 70 Sep 2019, 7 Sep 2019, J.A. Bolin 106 (USF 301489); ibid., 6 Nov 2017, J.A. Bolin 130, (USF 301493); Jupiter, Abacoa Natural Area, 1 Mar 2019, J.A. Bolin 320 (holotype USF 301510); ibid., 13 Aug 2019, J.A. Bolin 418 (USF 301483).

Notes – This species is a part of a cryptic species complex. It greatly resembles Aureoboletus auriporus (Peck) Pouzar, and its distribution limits are yet to be established. Aureoboletus auriporus differs from A. pseudoauriporus by the lack of longitudinal striations on the stipe. The pileus of A. auriporus is reported to turn red with the application of NH₂OH (Baroni 2017). The spore size of A. auriporus was not originally reported in the protologue, though Peck (1889) later provided an expanded description and reported the spores as 7.5–10 × 4–5 µm. Both (1998) studied the type specimen, obtaining a spore size of 9.8–15.5 × 3.96–5.75 µm, with a mean dimension of 13.15 × 4.73 µm, Q = 2.12–3.39, Qm = 2.75. Both (1998) also provided a description based on collections primarily from New York and Rhode Island, but also included a specimen from Tennessee, and did not include the type specimen. The spores reported were slightly larger than the type, at 11.0–16.05 × 4.4–6.38 µm, mean dimension 14.36 × 5.19 µm, and the spore quotient was similar, at Q = 2.2–3.19, Qm = 2.78. The spores of A. pseudoauriporus are somewhat larger, at (14–)15–17(–18) × 5–6.5 µm, x = 16.45 × 5.92 µm, and the spore quotient is nearly identical, at Q = 2.79.

Aureoboletus viridiflavus Coker & Beers ex Klofac is a similar species, and has been treated as a synonym of A. auriporus in the past (Singer 1947, Both 1998), which differs primarily by the pileus colors, which was described as “olivaceous gold with reddish areas”, the pileus when young tomentose-felted, less viscid, a lack of distinctly projecting margin, the hymenophore longer, to 17.5 mm (4–12 mm in A. pseudoauriporus), and the stipe, which bruises “brick red” and is not viscid (white, sometimes with pale pink tones, and not bruising in A. pseudoauriporus). The spore size is similar to A. auriporus, reported as 11.5–15(–16.6) × 4–5 µm in the protologue. Aureoboletus pseudoauriporus has somewhat longer and wider spores, measuring (14–)15–17(–18) × 5–6.5 µm. Aureoboletus subacidus (Murrill ex Singer) Pouzar is a somewhat similar species that shares reddish tones in the pileus, citrine yellow tubes, a whitish stipe, occurs in Florida, and is associated with Quercus spp. It can readily be distinguished from A. pseudoauriporus by the presence of the floccose yellow velar remnants left on the upper portion of the stipe, the scrobiculate pileus, and the yellow pileal context (Singer 1947).

So far, A. pseudoauriporus is the only species in the complex known from Florida. Although A. pseudoauriporus has been observed in southeastern Georgia (USA) by the authors, no collections were made. Aureoboletus innixus (Frost) Halling, A. R. Bessette & A. E. Bessette is similar but it has a dry, somewhat velutinous, dull reddish-brown pileus, and lacks longitudinal striations on its stipe. Aureoboletus roxanae (Frost) Klofac has whitish pores when young which eventually become pale yellow, and a yellow to pale orange-yellow stipe with a distinct dull orange zone at the apex.

Cyanoboletus bessettei A.R. Bessette, L.V. Kudzma, & A. Farid sp. nov.  
Figs 3, 10G–I  
Mycobank number: MB 840857; Facesoffungi number: FoF 10466

Etymology – The epithet bessettei honors American mycologist, Alan E. Bessette.

Typification – USA, South Carolina, Berkeley County, Francis Marion National Forest, State Route 402, approximately 1.25 mi. north of Huger, under oak and pine, 17 Sep 2016, A.R. Bessette ARB1393 (Holotype USF 301500).
Diagnosis – Medium-sized basidiocarps with a dry, reddish brown to buffy brown pileus and a reddish-brown stipe with a pale-yellow apex and white basal mycelium. The hymenophore surface is pale yellow and stains blue-green then olive when bruised. It has pale yellow context that stains blue-green then slowly turns peach to dull pinkish orange when exposed. The basidiospores measure (8–)9–11(–12) × 3.5–5 µm and are narrowly ovate to subelliptic. It fruits on the ground with oak and pine during fall.

Description – Pileus 2.7–8 cm broad, convex with an incurved margin that remains into maturity; surface subtomentose to nearly glabrous, dry, buffy brown overall when very young, becoming paler toward the margin and retaining darker brownish coloration on the disc at maturity, staining blue-green then dark olive-green and finally brown when bruised; margin with a narrow band of sterile tissue, sometimes undulating or lobed in age; context pale yellow, staining blue-green then slowly turning peach to dull pinkish orange when exposed; odor unpleasant, odd, chemical-like; taste slightly acidic or not distinctive. Cuticle stains dark amber with the application of KOH, pale olive with FeSO₄, and amber with an expanding blue-green outer ring with NH₄OH. Context stains yellow, then pale orange with the application of KOH or NH₄OH and is negative with FeSO₄. Hymenophore tubulose, pale yellow, staining blue-green, then olive when bruised; pores angular to irregular, 2–3 per mm; tubes 4–8 mm deep. Stipe 2.5–4 cm long, 1–2 cm thick, nearly equal or flaring at the apex, pinched at the base; surface longitudinally striate, dry, distinctly pale yellow at the apex, reddish brown below, with white basal mycelium, staining blue-green then reddish-brown; context pale yellow, slowly staining blue-green at the apex, then becoming bright chrome yellow.

Figure 3 – Field photograph of *Cyanoboletus bessettei* (ARB 1393). Photo credit: A.R. Bessette.

*Basidiospores* olive-brown in fresh deposit, (8–)9–11(–12) × 3.5–5 µm, n = 30, Q = 2.30, narrowly ovate to subelliptic in face view, obscurely inequilateral in profile, thin-walled, smooth, lacking an apical pore, yellowish in KOH or Melzer’s, inamyloid; spores sometimes collapsing when mounted in Melzer’s. *Basidia* 23–36 × 5.5–9 µm, mostly clavate, few cylindro-clavate, (2)4-sterigmate, hyaline in KOH, grayish yellow in Melzer’s. *Basidioles* 19–31 × 5–8.5 µm, clavate.
Cyanoboletus cyaneitinctus (Murrill) A. Farid, A.R. Franck & J.A. Bolin comb. nov.

Figs 4, 5A–G, 10A–C

MycoBank number: MB 840858; Facesoffungi number: FoF 10465

Basionym – Ceriomyces cyaneitinctus Murrill, Lloydia 6: 225 (1943).

Synonyms – Boletus cyaneitinctus (Murrill) Murrill, Lloydia 6: 228 (1943).

Typification – USA, Florida, Alachua County, Gainesville, Kelley’s Hammock, 3 Aug 1938, West and Murrill s.n. (holotype FLAS-F-17986); Hillsborough County, Tampa, Learning Gate Community grounds, 4 May 2019, A. Farid 920 (epitype here designated USF 301499).

= Boletus mutabilis Morgan, J. Cincinnati Soc. Nat. Hist. 7: 6 (1884), nom. illegit., Art. 53.1.

Diagnosis – Brownish or rarely reddish pulvinate pileus, bright yellow hymenophore, stipe, and context, all surfaces rapidly and brilliantly cyanescent.

Description – Pileus 3–8 cm wide, pulvinate or convex when young becoming broadly convex at maturity, bister, umber, mahogany, and dark brown overall, rarely entirely red in the pileus, glabrous to tomentose, tacky when wet, sometimes rimulose at maturity, blackening instantly where handled. Hymenophore tubulose, yellow, darkening to a gold color when mature, tubes 5–20 mm long, bluing instantly and strongly when handled; pore mouths subangular when mature, 0.5–1 mm in diameter. Stipe 3–6 × 0.5–2 cm, equal to ventricose, bright yellow, smooth or sometimes reticulate on the upper third, sometimes with flashes of reddish to brownish-red floccons, particularly towards the base of the stipe, bluing instantly and strongly when handled, basal mycelium white to yellowish white. Context concolorous with stipe surface, often with red pigments at the very base of the stipital context, blueing instantly and strongly, fading to pale yellow. KOH on pileus dark maroon to black, red elsewhere; FeSO₄ negative, erasing blue stains from flesh.

Basidiospores (11)11.5–15(16) × 4–6 µm, n = 30, Q = 2.4, fusiform, sometimes with a suprahilar depression present. Basidia 25–50 × 8–10 µm, 4-spored, thin-walled, hyaline, clavate to pyriform; sterigmata 1–2 µm, occasionally pigmented like pleurocystidia. Basidioles similarly sized and shaped. Pleurocystidia 30–60 × 7–10 µm, fusoid to ampullaceous, hyaline or sometimes encrusted. Cheilocystidia similar to pleurocystidia. Pileipellis a trichodermium of strongly interwoven, filamentous, sinuous, rarely branched hyphae, erect or repent in most of the terminal elements, collapsing into a cutis, terminal elements cylindrical, apices rounded or somewhat pointed, 20–70 × 5–10 µm, smooth-walled, inamyloid, hyaline to golden-yellow or somewhat brownish in water and 5% KOH. Clamp connections absent.

Habitat and Distribution – Basidiomes typically occurring singly or more rarely gregariously, widely distributed in eastern North America.
Material examined – USA, Florida, Alachua Co., Gainesville, 2 Oct 1949, W.A. Murrill s.n. (FLAS F16163); *ibid.*, lawn under pecan [*Carya illinoinensis*], 7 Nov 1947, W.A. Murrill s.n. (FLAS F40835); *ibid.*, Kelley’s Hammock, 3 Aug 1938, West and W.A. Murrill s.n. (holotype FLAS F17986); *ibid.*, yard at 936 NW 30th Ave., 9 Aug 1980, G.L. Benny s.n. (FLAS F52704); *ibid.*, lawn under laurel oak [*Quercus laurifolia*], 1 Aug 1947, Murrill s.n. (FLAS F19093); *ibid.*, shaded yard, 6 Nov. 1950, R. Bennett s.n. (FLAS F59706); *ibid.*, lawn under hardwoods, 13 Oct 1950, R. Bennett s.n. (FLAS F19647); *ibid.*, 19 × 1950, R. Bennett s.n. (FLAS F 40863); *ibid.*, lawn on 18th block of NW 11 place, Sept 12 1968, J. Kimbrough s.n. (FLAS F48020); *ibid.*, under large live oak [*Quercus virginiana*] 10 mi. SE of Gainesville, on Palatka Rd., 2 Nov 1947, G.F. Weber s.n. (FLAS F40837); Hillsborough Co., Alafia River State Park, 17 Jul 2018, J. Bolin 177 (USF 300090); Hillsborough County, Tampa, University of South Florida Tampa Campus, entrance area off of Leroy Collins Boulevard, 11 Jun 2016, A. Farid 340 (USF 288424); USF campus, 22 May 2018, Franck 4352 (USF 297911); Tampa, Learning Gate Community grounds, 4 May 2019, A. Farid 920 (epitype here designated USF 301499); Palm Beach Co., Frenchman’s Reserve, 1 III 2019, J. Bolin 324 (USF 300081); Prosperity Oaks, 2 Mar 2019, J. Bolin 325 (USF 300080). OHIO: Hocking Co., 4 Aug 2018, J. Bolin 185 (USF 300091); Vinton Co. 5 Aug 2018, J. Bolin 184 (USF 300085). TENNESSEE: Knox Co., Knoxville, Tobler Rd., 4 Sept 1949, A.J. Sharps s.n. with L.R. Hesler (FLAS-F-53755).

Figure 4 – Field photographs of *Cyanoboletus cyaneitinctus*. A A. Farid 920. B A. Farid 340. Macrochemical tests of basidiomes are labelled. The scale in the top is in centimeters. C JAB 324. D JAB 389. Photo credits: J.A. Bolin.

Notes – *Cyanoboletus* Gelardi, Vizzini, & Simonini is in the *Pulveroboletus* clade, and is comprised of eight species. *Cyanoboletus* was described in 2014 (Vizzini 2014) with *Boletus pulverulentus* Opat. as the type species for the genus. Although no molecular analysis was provided in the protologue, previous molecular analyses demonstrated several species (now in *Cyanoboletus*)
were not related to *Boletus* L. s. str. (Gelardi et al. 2013, Wu et al. 2014). *Cyanoboletus* is distinguished from other boletoid genera by its yellowish brown to dark brown pileus, rapidly blueing context and hymenophore, and smooth basidiospores.

*Cyanoboletus cyaneitinctus* is very similar to the closely related *C. pulverulentus* (Opat.) Gelardi, Vizzini & Simonini. Both are boletes with a dark brown pileus, small pores (1–2 per mm), and yellow stipes with brown punctae; all surfaces instantly bruise blue. The European name has historically been applied to this species in North America (Singer 1947, Smith & Thiers 1971, Bessette et al. 2000, 2017), but we are here treating them as separate species based on our molecular analyses (Fig. 1) and morphological studies. The spore quotient Q is lower in *C. cyaneitinctus* at Q = 2.4 (with the Q usually between 2.3–2.5) compared to 2.6–2.9 in *C. pulverulentus* (Gelardi et al. 2013). These two species are geographically separated, with *C. cyaneitinctus* occurring in eastern North America and the latter found in Europe. *Cyanoboletus sinopulverulentus*, which is sister to *C. cyaneitinctus* (Fig. 1) is distinguished from *C. cyaneitinctus* and *C. pulverulentus* by its evenly dark brown stipe (lacking the reddish and yellow tones often present in the other two species), which is more heavily pruinose to scissurate. *Cyanoboletus sinopulverulentus* has predominately 2-spored basidia (4-spored in the other two taxa), and can also be distinguished on the basis of its Q value, which is reported as 2.17–2.45 (Gelardi et al. 2013), smaller than either of the other two *Cyanoboletus* species mentioned here.

*Boletus mutabilis* Morgan is an earlier but illegitimate name for this American species (see Art. 53.1). Thus, the oldest name we have to apply to the North American species is *C. cyaneitinctus*. Singer (1947) treated *C. cyaneitinctus* as a synonym of *C. pulverulentus*. The type of *C. cyaneitinctus* was examined, and matched the other North American collections examined. This type material is quite old and not in good condition; thus, we have designated an epitype, and have included images (Figs 4, 5A–G) as well as published molecular data. *Cyanoboletus cyaneitinctus* and *C. pulverulentus* are difficult to distinguish morphologically.

**Cyanoboletus cyaneitinctus forma reticulatus** (Snell, E.A. Dick & Hesler) A. Farid comb. nov.

Fig. 5H

Mycobank number: MB 840859; Facesoffungi number: FoF 10465
Basionym – *Boletus pulverulentus* f. *reticulatus* Snell, E.A. Dick & Hesler, Mycologia 43(3): 362. 1951.
Typification – USA, Tennesse, Knox County, Knoxville, on an old sod yard near Robinia and Ligustrum and not far from Ulmus but with no accurate indication of mycorrhizal associate, 4 Sept 1949, L.R. Hesler 19314 (holotype TENN-F-019314, isotype SFSU-F-000439).
Material examined – USA, Florida, Hillsborough Co., Brandon, under Quercus laurifolia, 5 Jun 2020, Farid 1035 (USF 301495).

Notes – *Cyanoboletus cyaneitinctus* f. *reticulatus* differs from the type form by the reticulation present over the upper stipe. The protologue states all other macro- and micromorphological characters are consistent, and this is consistent with our observations.

**Hemileccinum floridanum** J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, A. Farid & J.L. Frank sp. nov.

Fig. 6, 10J–L

Mycobank number: MB 840861; Facesoffungi number: FoF 10464
Etymology – A reference to Florida where this species was first collected and described.
Typification – USA, Florida, Lake County, Lake Louisa State Park, 4 Sep 2016, J. A. Bolin 142 (holotype USF 301495).

Diagnosis – Medium-sized to large basidiocarps with a dry to slightly tacky, reddish brown to chestnut brown pileus and a whitish stipe that becomes pale yellow at the apex and has a white basal mycelium. The hymenophore is bright yellow when young, becomes darker brownish yellow as it matures, and does not stain when bruised. It has white context that slowly stains yellow often from the margin toward the center. The basidiospores measure (10-)13-16(-17) × 4.5-6 µm and are elliptical. It fruits on the ground with oak from late spring through fall.
Figure 5 – Field photographs of Cyanoboletus cyaneitinctus. A JAB 325. B JAB 185. C–G JAB 324. H Cyanoboletus cyaneitinctus f. reticulatus Farid 1035. Photo credits: A–G J.A. Bolin, H A Farid.

Description – Pileus 2.8–12.5 cm wide, convex becoming broadly convex to nearly plane in age; surface dry to slightly tacky, smooth to somewhat wrinkled and uneven, glabrous to finely velvety, sometimes with a whitish bloom when young, reddish brown to chestnut brown, cuticle acidic tasting or not distinctive; margin even or nearly so. Hymenophore tubulose 3–12 mm deep, pore surface bright yellow when young, maturing to darker brownish-yellow, not staining when bruised, depressed near the stipe in age, easily detached from the pileus context; pores angular to irregular, 2–3 per mm. Stipe 4–9.5 cm long × 1–3 cm thick, nearly equal or enlarged in either direction, with a pinched base; surface dry, longitudinally striate, nearly glabrous to very weakly scurfy-punctate, not reticulate; whitish overall on young specimens, becoming pale yellow at the apex with variable reddish tints and streaks over a whitish to pale yellow ground color below, with white basal mycelium. Context in the pileus white, slowly staining yellowish often from the margin toward the center, with a slight pinkish-red coloration beneath the cuticle; in the stipe, white, slowly staining yellowish from the pileus trama just above the hymenophore partly downward along the exterior stipe trama when exposed. Cuticle stains brownish red or light orange sometimes fading to light green with the application of KOH, olive and then orange or amber with a green ring with NH₄OH, and
dark orange-amber to orange with FeSO₄. The context stains pale orange to yellow then fades with KOH, is negative with NH₄OH, and negative or light greyish olive green with FeSO₄. Odor slightly sour to not distinctive; taste not distinctive.

**Basidiospores** olive-brown in fresh deposit, (10–)13–16(–17) × 4.5–6 µm, n = 30, Q = 2.86, elliptical in face view, inequilateral in profile, thin-walled, smooth, lacking an apical pore, grayish yellow in KOH, brownish yellow in Melzer’s. **Basidia** 32–38 × 8.5-10.5 µm, clavate, 4-sterigmate, sometimes 3- or 2-sterigmate, hyaline in KOH, yellow in Melzer’s, with granular, inamylloid contents. **Basidioles** 22–29 × 7.5–8.5 µm, clavate, hyaline in KOH, yellow in Melzer’s. **Pleurocystidia** 25–50 × 6–10 µm, hyaline, ventricose in the middle, ampullaceous at the apex, frequent near the pores. **Pileipellis** a cutis of loosely interwoven cylindric hyphae with markedly inflated, sphaerocyst-like oval to subglobose terminal cells, 11–33 × 15–22 µm, grayish yellow in KOH, yellow to orange-yellow with granular contents in Melzer’s; hyphae of the pileipellis 4-8.5 µm wide, thin-walled, smooth, grayish yellow in KOH, yellow in Melzer’s. **Pileus trama** hyphae loosely interwoven, highly variable, 4–16 µm, with rounded terminal ends, thin-walled, smooth, hyaline in KOH, yellow in Melzer’s, inamylloid. **Hymenophoral trama** boletoid, with lateral elements 5–9 µm, moderately divergent, thin-walled, smooth, hyaline to pale grayish yellow in KOH, pale grayish yellow in Melzer’s. **Stipitipellis** 4–17 µm wide, hyphae subparallel, highly variable, tubular with rounded ends and granular contents, thin-walled, smooth, hyaline in KOH, yellow-brown in Melzer’s, caulocystidia not observed. Stipe trama interwoven, 9–27 µm, highly variable, tubular with rounded ends, thin-walled, smooth, hyaline in KOH, hyaline to pale yellow in Melzer’s. **Clamp connections** absent.

Figure 6 – Field photographs of *Hemileccinum floridanum*. A J.A. Bolin 142. B. J.A. Bolin 454. D J.A. Bolin 157. E J.A. Bolin 201. Photo credits: J.A. Bolin. C, F are SEM images of basidiospores from J.A. Bolin 454 (white bar = 4 µm).

Habit, Habitat and Distribution – Solitary, scattered or in groups on the ground with oak; known from Florida, potentially to North Carolina (Singer 1947).

Material examined – USA, Florida, Hillsborough County, Violet Cuery Nature Preserve, 14 June 2017, A. Farid 625 (USF 301503); University of South Florida Tampa campus, trails near tennis courts in NE corner of campus, 4 Jun 2018, A. Farid 790 (USF 297572), *ibid.*, 30 Oct 2019, A. Farid
Lanmaoa sublurida (Murrill) A. Farid & A.R. Franck comb. nov.

MycoBank number: MB 840862; Facesoffungi number: FoF 10463

Basionym – Suillellus subluridus Murrill, Mycologia 30(5): 524 (1938).

Typification – USA. FLORIDA: Alachua Co., Gainesville, Murrill (holotype FLAS-F-15869).

Synonyms – Boletus miniato-olivaceus var. subluridus (Murrill) Singer, Mycologia 37(6): 798 (1945); Boletus subluridus (Murrill) Murrill, Mycologia 30(5): 525 (1938).

Description – Pileus 3–14 cm wide, pulvinate when young, margin entire or wrinkled, becoming convex to nearly plane at maturity, smooth, somewhat tacky when wet, bright red to ruby red when young, becoming mixed with various shades of bright red, orange red, or a peach-colored orange, especially at the margins, or sometimes turning brown entirely at maturity, sometimes becoming rimulose, revealing the context color beneath the cuticle. Hymenophore tubulose, 5–12 mm deep at maturity, tubes sulphur yellow, bluing on injury, fading to olive green; pores initially orange when young, stuffed, slowly and unevenly maturing to reveal red pore mouths at maturity. Stipe 5–8 × 1.5–3 cm, equal, tapering upwards, or sometimes ventricose, pale yellow, especially when young, with a small network of reticulation forming isodiametric meshes in a narrow zone to 2 (–5) mm long at the apex of the stipe, but sometimes absent, especially in younger specimens, and the rest of the stipe glabrous when young with fine floccules which develop over the stipe surface as it matures, appearing smooth without a hand lens or without close inspection, at maturity these floccules giving the appearance of a stipe that is red to purplish-red, stipe surface bruising a light blue, especially when young; basal mycelium white to pale yellow. Context yellow throughout, or sometimes yellow only in the stipital context (especially so when mature), not bluing or only very weakly and slowly bluing when young, mature specimens bluing in the stipital and pileal context around the hymenophore. Taste mild, odor disagreeable, fetid, ammonia-like and slightly alliaceous.

Basidiospores (8.7)9.3–10.8(12.6) × (3)3.4–4(4.6) μm, n = 71, Q = 2.8, boletoid thick-walled, ellipsoid-oblong to subcylindric or subfusoid, smooth, melleous. Basidia 20–25 × 6–8 μm, 2- or 4-spored, thin-walled, hyaline, clavate to pyriform; sterigmata 1–2 μm, occasionally pigmented like pleurocystidia. Basidioles similarly sized and shaped. Pleurocystidia 30–35 × 10–15 μm, pigmented a light golden brown in KOH, NH₄OH, H₂O, and Melzer’s, spores generally are clustered onto cystidia. Cheilocystidia 15–50 × 5–10 μm, moderately thin-walled (0.5 μm), usually pigmented like the pleurocystidia, but occasionally hyaline. Hymenophoral trama divergent. Pileipellis elements septate, terminal elements 20–65 × 4–10 μm, thin-walled, cylindrical, with filiform apices that are occasionally clavate, forming an ixotrichodermium of erect elements, occasionally becoming prostrate and forming an ixosubcutis. Pileal trama composed of interwoven hyphae 3–10 μm wide, thin-walled, cylindrical.

Material examined – USA, Florida, Alachua County, Gainesville, Beneath Laurel Oak [Quercus laurifolia], 3 Jul 2020, A. Farid 1058 with R.E. O’Donovan and C. Peyer (USF 301505).
Hillsborough County, Brandon, S of Camden Visconti pond at main entrance, 19 June 2016, A. Farid 343 (USF 288426); ibid., 26 Jun 2017, A. Farid 631 (USF 301506); ibid., 11 Jun 2018, A. Farid 805 (USF 298026); 22 Oct 2019; ibid., 11 Jun; A. Farid 1023 (USF 300104); Lithia, beneath Quercus laurifolia, 15 Jul 2020, A. Farid 1072 (USF 301508); ibid., 16 Jul 2020, A. Farid 1073 (USF 301504).

Notes – *Lanmaoa* G. Wu & Zhu L. Zang is a genus of boletes which is typically distinguished by its thin hymenophore (1/3–1/5 the thickness of the pileal context at a position halfway to the pileal center), which stains blue when bruised, a light-yellow context which stains pale blue slowly when cut, and an interwoven trichodermium to subcutis pileipellis. Although no molecular diagnosis was provided in the paper describing the genus (Wu et al. 2015), the phylogenetic placement was based on the work by Wu et al. (2014). Chai et al. (2018) describe the overlapping features of *Lanmaoa rubriceps* N.K. Zeng & Hui Chai with *Cyanoboletus*, including hymenophore size, and staining features. *Lanmaoa sublurida* is distinguished from similar looking boletes by the combination of its characteristic odor, a pileus that varies in reds and orange that matures to a peach-orange, sometimes brown, yellow tubes with pores that appear yellow and mature to carmine, and a light-yellow stipe with fine floccons that densely cover it at maturity.

![Field photographs of *Lanmaoa sublurida*.](image)

**Figure 7** – Field photographs of *Lanmaoa sublurida*. A. Farid 1072. B. Farid 343. C. Farid 1073. Photo credits: A Farid.
There are several species in the southeastern USA that might be confused with *L. sublurida*. *Boletus carminiporus* Bessette, Both & Dunaway, described from Mississippi, could be confused with *L. sublurida*, although *B. carminiporus* differs in the lack of staining in the context at any stage, lacks any distinctive odor, and its stipe is usually redder, and stains olive-brown, olive-green, to olive-yellow. *Lanmaoa borealis* (A.H. Sm. & Thiers) A.E. Bessette, M.E. Nuhn & R.E. Halling is similar, but has larger spores (11–13[15] µm long) and has only been documented from the northeastern USA. The similar *Boletus sensibilis* Peck, found in the eastern USA, bruises similarly on the stipe, but the pore mouths are yellow (never red), the stipe develops a flush of red on the bottom half (never the purplish red that *L. sublurida* develops at maturity), and the context blues more readily throughout.

**Xerocomellus bolinii** J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, J.L. Frank & A. Farid, sp. nov.

MycoBank number: MB 840863; Facesoffungi number: FoF 10462

Typification – USA, Florida: Broward County, Davie, Tree Tops Park, 27 Jan 2017, J.A. Bolin 43 (holotype USF 301496).

Etymology – The epithet bolinii honors Franklin Alexander Bolin, a biologist, naturalist and educator who for more than twenty-five years introduced thousands of students to the fields of mycology, herpetology and lepidoptery. Franklin was born and raised in Northeastern Ohio and attended Ohio State University where he earned a master’s degree in both Field Zoology and Herpetology. He went on to become an Advanced Biology teacher at Grove City High School from 1963–1988. Using his unique and progressive classroom style which immersed students in “hands on learning”, Bolin developed a curriculum for the entire school district known as “The Natural History of Ohio”.

Diagnosis – Small to medium-sized basidiocarps with a dry, blue-staining, appressed-fibrillose to squamulose pileus with pinkish brown fibrils with white to creamy white context visible in the
cracks. The cap context is creamy white or a mixture of creamy white and yellow, becoming yellow to orange in the stipe and rapidly stains blue when exposed. The pore surface is yellow when young, becomes dull yellow at maturity, and rapidly stains blue when bruised. The stipe has reddish brown punctae over a whitish to pale yellow ground color that darkens toward the base and staining blues when bruised. Basidiospores measure (10–)12–13(–14) × 4.5–6 µm. It fruits on the ground with Quercus and Pinus.

Description – Pileus 4-8 cm wide, convex becoming broadly convex to nearly plane in age; pileus appressed-fibrillose to squamulose with pinkish brown fibrils and white to creamy white context visible in the cracks, dry, staining blue, sometimes slowly or weakly; margin incurved at first remaining so well into maturity, sterile, sometimes undulating, becoming conspicuously cracked with age. Hymenophore tubulose, pale yellow, becoming dull yellow in age, quickly staining dark blue; pores 1–2 per mm, angular to irregular or slightly elongated; tubes 2-6 mm deep, rapidly staining blue when exposed. Stipe: 50–90 × 8–15 mm wide, nearly equal or slightly tapered downward, with a pinched based, solid; surface dry, weakly longitudinally striate, with reddish brown punctae over a whitish to pale yellow ground color often with reddish tints extending from the base upward, staining blue when bruised, basal mycelium white to creamy white. Context of pileus creamy white or a mixture of creamy white and yellow to orange in the stipe and staining blues when exposed. Odor and taste not distinctive. Macrochemical Testing: Pileus of mature specimens showed light green fading to yellow with NH₄OH; younger specimens turn orange with faint green outline of stained area, eventually fading to yellow. Orange to amber, fading to brown with KOH. Older specimens light brown and younger specimens light green with FeSO₄. Context in both mature and younger specimens pale orange to NH₄OH, orange to amber fading to brown with the application of KOH and yellow with FeSO₄. Cuticle stains light green, fading to yellow with NH₄OH; younger specimens develop orange with a faint green outline that eventually fades to yellow; KOH produces orange to amber that fades to brown; with FeSO₄ mature specimens turn light brown, and younger specimens light green.

Figure 9 – Field photographs of Xerocomellus bolinii. A J.A. Bolin 238. B J.A. Bolin 274. C J.A. Bolin 232. D J.A. Bolin 208. Photo credits: J.A. Bolin.
Figure 10 – Microscopic structures of the boletes from this study. A–C Cyanoboletus cyaneitinctus. D–F Aureoboletus pseudoauriporus. G–I Cyanoboletus bessettei. J–L Hemileccinum floridanum. M–O Lanmaoa sublurida. P–R Xerocomellus bolinii. A, D, G, J, M, P depict the pileipellis for each species (black bar = 50 µm), D showing a gelatinized pileipellis, B, E, H, K, N, Q depict basidiospores with guttules (black bar = 10 µm), and C, F, I, L, O, R depict basidia, basidioles, and cystidia (black bar = 20 µm), with guttules present. Drawing credits: A. Farid.

Basidiospores light brown to olive-brown in fresh deposit, (10–)12–13(–14) × 4.5–6 µm, n = 30, Q = 2.40, elliptical in face view, obscurely inequilateral in profile, thin-walled, smooth, lacking an apical pore, pale grayish yellow in KOH, dull yellow in Melzer’s. Basidia 32-36 × 9-12 µm, mostly clavate, occasionally cylindro-clavate, 2(4)-sterigmate, hyaline in KOH, grayish yellow in Melzer’s. Basidioles 21.5–30 × 6.5–10 µm, clavate. Hymenophoral trama boletoid, with lateral elements, 5-8 µm wide, moderately divergent, hyaline in KOH, grayish yellow in Melzer’s. Pileipellis a tangled layer or repent tubular hyphae, 5–9.5 µm wide, with cylindrical, rounded to slightly inflated end cells, thin-walled, smooth, hyaline in KOH, yellowish in Melzer’s. Pileipellis a tangled layer or repent tubular hyphae, 5–9.5 µm wide, with cylindrical, rounded to slightly inflated end cells, thin-walled, smooth, hyaline in KOH, yellowish in Melzer’s. Pileipellis a tangle of somewhat interwoven, 5–11 µm wide, smooth, thin-walled, hyaline in KOH or Melzer’s. Cheilocystidia and pleurocystidia scattered, 36–48.5 × 9–11.5 µm, fusoid-ventricose, smooth, thin-walled, hyaline to ochraceous in KOH, ochraceous in Melzer’s. Stipitipellis hyphae mostly parallel, slightly interwoven, 4.5–9.5 µm wide, hyaline to yellowish in KOH or Melzer’s, with fascicles of clavate to distorted caulocystidia 34–52 × 9–21 µm, that are dull yellow to brownish yellow in KOH or Melzer’s. Stipe trama subparallel, interwoven, 5–11.5 µm wide, hyaline to yellowish in KOH or Melzer’s. Clamp connections absent.
Habit, Habitat and Distribution – Solitary or scattered in sandy soil associated with *Quercus* and *Pinus*, along woodland edges, typically near saw palmetto (*Serenoa repens*) and/or cabbage palm (*Sabal palmetto*). Currently only documented from Florida. There are several images that we believe to be *X. bolinii* on the citizen science platform MushroomObserver.org (observation nos.: 430943, 412138, 293427, 289394), but no herbarium samples were made.

Specimens examined – USA, Florida, Broward County, Davie, Tree Tops Park, 14 Oct 2017, J.A. Bolin 124 (USF holotype 301494); Lake County, Lake Louisa State Park, Clermont, 13 Jun 2020, A. Farid 1047 with R.E. O'Donovan, C. Matson, and J.A. Bolin (USF 301498); Palm Beach County, Delray Beach, Morikami Museum and Japanese Gardens, 23 Sep 2017, Jason Bolin 110 (USF 300098); *ibid.*, 17 Oct 2018, J.A. Bolin 232 (USF 300082); *ibid.*, 12 Sep 2018, J.A. Bolin 208 (USF 301486); West Palm Beach, Okeeheelee Park, 20 Nov 2017, J.A. Bolin 133 (USF 300094); *ibid.*, 13 Nov 2018, J.A. Bolin 238 (USF 301485); *ibid.*, 23 Nov 2018, J.A. Bolin 274 (USF 301484).

Notes – *Xerocomellus chrysenteron* is similar but has a dark olive to olive-brown or greyish-brown cracked cap with exposed red to pinkish context, stains slowly or erratically greenish-blue on the hymenophore and cap context and has a more northern distribution. *Xerocomellus zelleri* has a dull black to blackish-brown or dark olive-brown pileus, context that is white to pale yellow that is unchanging or sometimes bluing and is reported from the Pacific Northwest south to California and into Mexico.

Discussion

**Contextualizing the species treated in this study**

The species treated in this paper further our understanding of the boletes, both in terms of biodiversity and systematics. Our analyses (Fig. 1) of *Aureoboletus pseudoauriporus* and its allies indicate that *A. auriporus* (Peck) Pouzar represents a species complex. *Aureoboletus auriporus* was described from New York as *Boletus auriporus* Peck (Peck 1873), with the protologue indicating a grayish-brown, sometimes tinged with red pileus color. The name has been applied widely to specimens across eastern North America, but specimens sequenced from Florida differ phylogenetically from specimens in the northeast. The pileus in *A. pseudoauriporus* is pinkish-tan, which differs from the grayish colors described in the protologue of *Boletus auriporus*. Our phylogenetic analyses placed *A. pseudoauriporus* sister to a clade containing two specimens of *A. auriporus* from Indiana and Tennessee and *A. viridiflavus* from North Carolina. Three other specimens of *A. auriporus* (from Massachusetts, North Carolina, and Costa Rica) fell separately outside of this group (see Fig. 1). *Aureoboletus viridiflavus*, described from North Carolina, is a rarely documented species that is often confused for *A. auriporus*. In a monograph of *Aureoboletus*, Klofac (2010) noted most authors took *A. viridiflavus* as *A. auriporus*, but noted the subtle morphological characters separating the two species.

We have expanded our understanding of North American *Cyanoboletus* with the resurrection of *C. cyaneitinctus* as well as the addition of the novel species *C. bessettei*. *Cyanoboletus cyaneitinctus* is widely distributed across North America. Many previous works on North American boletes applied the European name *C. pulverulentus* to the North American species (Singer 1947, Bessette et al. 2017). Phylogenies consistently show significant divergence between specimens from North America and those from Europe (Gelardi et al. 2013, 2015, Braeuer et al. 2018, Fig. 1), supporting the recognition of North American material as the species *C. cyaneitinctus*. So far, the only other *Cyanoboletus* species known from North America now includes *C. bessettei*. *Cyanoboletus bessettei* is only known from the type location in South Carolina, but we expect future studies will better establish its geographical limits. Although briefly treated as a *Cyanoboletus* in a study by Vizzini (2014), molecular analyses by Frank et al. (2020) have since shown *Xerocomellus rainisiae* (Bessette & O.K. Mill.) N. Siegel, C.F. Schwarz & J.L. Frank is not a member of *Cyanoboletus*. Morphological characters that *X. rainisiae* shares with *Xerocomellus Šutara include the pileus that becomes rimose in age, the deep red pigmentation of the basal stipital context (though less extensive than typical *Xerocomellus*), and the bright yellow, blue-staining hymenium.
Similar to Chai et al. 2018, we have found Lanmaoa and Cyanoboletus to be closely related (Fig. 1) and morphologically intergrading, although Cyanoboletus tends to have dull brown colors and Lanmaoa often has bright red or yellow coloration (Wu et al. 2014, 2016, Chai et al. 2018). Cyanoboletus bessettei and C. instabilis both share the 1/3–1/5 hymenophore-to-pileal-context ratio found in Lanmaoa (and some Baorangia). Chai et al. (2018) suggested future research may consider treating Cyanoboletus and Lanmaoa as one genus, in which Cyanoboletus would have priority over Lanmaoa (Art. 11.3 of the Shenzhen Code). This is complicated by Vadthanarat et al.’s (2019) phylogenetic inference of the genus Cacaoporus Raspé & Vadthanarat, which used the loci TEF1, RPB2, atp6, and cox3 to place two named and one unnamed species of Cacaoporus sister to Cyanoboletus, while receiving no phylogenetic support for Lanmaoa and Cyanoboletus as sister genera. Due to the limited overlap of data between our dataset and Vadthanarat et al.’s dataset, sequences of Cacaoporus were not included in our final analyses. We believe the suggestion by Chai et al. (2018) to lump Lanmaoa and Cyanoboletus should be carefully re-considered in future studies of this clade as more data become available.

*Hemileccinum floridanum* forms a well-supported sister clade to *Hemileccinum subglabripes*, the species it most closely resembles (Fig. 1). Using Singer (1947), *Hemileccinum floridanum* keys out to *Leccinum subglabripes* (Peck) Sing. (= *Hemileccinum subglabripes*). Insightfully, under his *L. subglabripus*, Singer (1947) gave a separate description for the Florida collections, which here conform to the new species *H. floridanum*. *Leccinum subglabripes* var. *corrugatoides* Singer was also described in Singer (1947), but differs from *H. floridanum* by a very rugose, “light brownish olive” pileus and a “light brownish olive” spore print (Singer 1947). Our collections do not possess these features, and it remains to be determined if this taxon is distinct from *H. floridanum*. Molecular analyses by Kuo & Ortiz-Santana (2020) revised the concept of *Hemileccinum* to include *H. rubropunctum*, a widespread species in North America which forms tuberculate ectomycorrhizas with *Quercus* spp. (Smith & Pfister 2009). Roots beneath several collections of *Hemileccinum floridanum* were examined for tuberculate ectomycorrhizas, but none were located. Thus far *H. rubropunctum* is unique in its ability to form tuberculate ectomycorrhizas within the Boletaceae, though other Boletales are capable of this (e.g. *Suillus* and *Rhizopogon*).

*Xerocomellus bolinii* is here placed as sister to a clade of Eurasian species, one of which has been considered part of the genus *Rheubarbariboletus* Vizzini, Simonini & Gelardi. Similar to Frank et al. (2020), our phylogenetic analysis finds *Heliogaster* Orihara & K. Iwase and *Rheubarbariboletus* embedded in the *Xerocomellus* lineage and *Nigroboletus* to be sister to this broadly defined *Xerocomellus* lineage. Vizzini (2015) cited the ITS-based phylogeny in Gelardi et al. (2013) and unpublished data as the molecular basis for establishing *Rheubarbariboletus*, differing from *Xerocomellus* by its smooth, non-striate and non-truncate spores, smooth or finely incrusted pileipellis, conglutulous plaques on the hyphal surface, tapered and rooting stipe base, the bright yellow-ochreous to orange-rhubarb and unchangeable context in the stipe base, and the dark blue-green blackish reaction with FeSO₄ on the pileus surface and the base of the stipe context. *Xerocomellus bolinii*, while sharing the non-bluing basal stipital context, smooth, non-truncate spores, and smooth pileipellis of *Rheubarbariboletus*, differs in its reaction to the application of FeSO₄ to the context by only turning light brown to light green (in old and young specimens, respectively), and by lacking a rooting stipe. In light of these molecular and morphological data, it seems best to include *Heliogaster* and *Rheubarbariboletus* within *Xerocomellus* at this time.

We follow Bozok et al. (2019) in recognizing *Exsudoporus* as a genus separate from *Butyriboletus*. Wu et al. (2016) treated the genus as a synonym of *Butyriboletus*, citing the reticulation and interwoven trichodermium to subcutis pileipellis as shared characters with the genus *Butyriboletus*. Bozok et al. (2019) reported on the positive amyloid reaction in the stipe tissues of *E. permagnificus*, a feature not shared with *Butyriboletus*. Our observations of *E. floridanus* show that the stipe base context exhibits a dextrinoid reaction in the stipe base (pers. obs.). Also, *Exsudoporus* species have pores that are discolorous with the tubes, and the basidiomes bruise blue much darker and heavier than *Butyriboletus* species. The guttation on the pores is regularly found, especially in younger specimens of *Exsudoporus* species, and is a useful distinguishing character. Wu et al. (2016)
reported species which were sister to the clade containing *Exsudoporus* and *Butyriboletus*, but these species remain undescribed. Additional analyses and thorough morphological comparison of those undescribed species might justify a broader concept of *Butyriboletus*, however until those analyses are produced, retaining the genus *Exsudoporus* is preferred.

**Conclusions**

This paper updates our understanding of the boletes in southeastern USA. Four novel species are described, as well as resurrecting and applying the name *Cyanoboletus cyaneitinctus* to the *Cyanoboletus* species widespread across North America. Our molecular analyses (Fig. 1) provide a DNA-based approach to aid morphological classification of these boletes and to better understand the distribution of these species. Our analyses also support the many genera found in recent Boletaceae phylogenetic reconstructions (Wu et al. 2014, 2016). By analyzing the protein-coding loci (*RPB1, RPB2, TEF1*) from a collection of *Butyriboletus floridanus* on GenBank, we have also confirmed a disjunct distribution for this tropical species. Inclusion of additional data from the epitype of *Pulchroboletus rubricitrinus* also lends the specimen to broader phylogenetic analyses. We also provide sequences of western USA *Xerocomellus*, which will aid future bolete phylogenetic reconstructions, as many species of *Xerocomellus* from North America lack protein coding loci.

This paper increases the knowledge of biodiversity present in the region. The potential for robust future studies is impeded by a lack of baseline knowledge of biodiversity. As molecular phylogenetic analyses continuously update the taxonomy of our classifications of the boletes, the need for further investigations into the boletes of the southeastern USA becomes readily apparent. Important aspects, such as morphological traits, host-specificity and geographic distribution, have been shown to be incredibly important with regards to boletes. Species-level concepts which were once broadly defined and applied widely across eastern North America have been shown to encompass several species, sometimes with clear morphological characters to distinguish them, as well as cryptic species in which geography seems to play a key role. Increasing and updating our understanding of boletes allows researchers to obtain richer species-level sequence-based identifications in environmental studies (Hibbett et al. 2011, Truong et al. 2017, Xu 2016), which is important for ecological studies, and paramount to better understanding threatened ecosystems in the southeastern USA. Macrofungal species have shown the potential for introduction and spread, e.g. *Favolaschia, Clathrus archeri, Perenniporia ochroleuca*, and the bolete *Aureoboletus projectellus* (Desprez-Loustau et al. 2007, Pringle et al. 2009, Vizzini et al. 2009, Wrzosek et al. 2017, Banasiak et al. 2019). Considering many species of boletes in the southeastern USA are geographically restricted, there is the potential that exotic mycorrhizal fungi may outcompete these endemic species.

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Supplementary Table 1 GenBank accession number and other information of sequences used in phylogenetic analyses in this study. Sequences in bold were generated in this study.

| Species                | GenBank voucher     | Location | ITS       | 28S       | RPB1      | RPB2      | TEF       |
|------------------------|---------------------|----------|-----------|-----------|-----------|-----------|-----------|
| Alessioporus ichnusanus| AMB 12756           | KJ729491 | KJ729504  | –         | –         | –         | KJ729513  |
| Alessioporus ichnusanus| MG420a              | KJ729496 | KJ729509  | –         | –         | –         |           |
| Alessioporus rubriflavus| ARB1356            | KU736957 | MH656696  | –         | –         | –         |           |
| Alessioporus rubriflavus| JLF2561            | KU736958 | KC812306  | –         | –         | –         |           |
| Aureoboletus auriflammeus| CFMR BOS 699     | –        | MK601706  | –         | MK766269  | MK721060  |           |
| Aureoboletus auriporus| 35 97               | –        | DQ534636  | –         | –         | –         |           |
| Aureoboletus auriporus| AB11                | MH796985 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| AB12                | MH796989 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| BDCR0431           | –        | HQ161871  | HQ161840  | –         | –         |           |
| Aureoboletus auriporus| FLAS F 60185       | MH796985 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| FLAS F 60914       | MH211684 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| FLAS F 60985       | MH016931 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| MAC09 TENN         | MF755267 | –         | –         | –         | –         |           |
| Aureoboletus auriporus| S D Russell MycoMap | MK560093 | –         | –         | –         | –         |           |
| Aureoboletus catenarius| GDGM45142         | –        | MN204514  | –         | –         | –         |           |
| Aureoboletus catenarius| HKAS54463         | –        | KT990509  | KT990890  | KT990348  | KT990710  |           |
Supplementary Table 1 Continued.

| Species                          | GenBank voucher | Locus ITS | 28S | RPB1       | RPB2       | TEF       |
|----------------------------------|-----------------|-----------|-----|------------|------------|-----------|
| *Aureoboletus catenarius*        | HKAS54467       | –         | KT990510 | –          | KT990349  | KT990711  |
| *Aureoboletus clavatus*          | GDGM42962       | –         | KR052045 | KR052056   | –          | –         |
| *Aureoboletus clavatus*          | GDGM42963       | –         | KR052046 | KR052057   | –          | KR052054  |
| *Aureoboletus clavatus*          | GDGM42984       | –         | KR052047 | –          | –          | KR052055  |
| *Aureoboletus clavatus*          | HKAS59802       | –         | KR052044 | –          | –          | KR052053  |
| *Aureoboletus duplicatoporus*    | HKAS63009       | –         | KT990511 | KT990891   | KT990350   | KT990712  |
| *Aureoboletus duplicatoporus*    | HKAS83115       | –         | KT990512 | KT990892   | KT990351   | KT990713  |
| *Aureoboletus gentilis*          | ADK4865         | –         | –      | –          | KT823994   | KT824027  |
| *Aureoboletus gentilis*          | MG372a          | –         | KF112344 | KF112557   | KF112741   | KF134014  |
| *Aureoboletus gentilis*          | Pug1            | –         | DQ534635 | –          | –          | KF030399  |
| *Aureoboletus griseorufescens*   | GDGM28490       | –         | MH670278 | –          | MH700241   | –         |
| *Aureoboletus griseorufescens*   | ZM131           | –         | MH670279 | MH700220   | MH700242   | –         |
| *Aureoboletus innixus*           | CFMR BOS 544    | –         | MK601707 | –          | MK766270   | MK721061  |
| *Aureoboletus innixus*           | MB03 104        | –         | KF030239 | –          | –          | KF030400  |
| *Aureoboletus mirabilis*         | HKAS57776       | –         | KF112360 | KF112624   | KF112743   | KF112229  |
| *Aureoboletus mirabilis*         | REH9765         | –         | KP327661 | –          | –          | KP327709  |
| *Aureoboletus moravicus*         | MG374a          | –         | KF112421 | KF112559   | KF112745   | KF112232  |
| *Aureoboletus moravicus*         | VDKO1120        | –         | –      | –          | MG212615   | MG212573  |
| *Aureoboletus moravicus f luteus*| PARMA 1544 11   | KJ676960  | KJ676958 | –          | –          | KJ676959  |
| *Aureoboletus nephrosporus*      | HKAS67931       | –         | KT990516 | KT990895   | KT990357   | KT990720  |
| *Aureoboletus nephrosporus*      | HKAS74929       | –         | KT990517 | KT990896   | KT990358   | KT990721  |
| *Aureoboletus projectellus*      | MICH KUO 09111014 | –     | MK601708 | –          | MK766271   | MK721062  |
| *Aureoboletus projectellus*      | NYBG13392       | –         | KP327622 | –          | –          | KP327675  |
| *Aureoboletus quercus*           | spinosae        | KY039954  | KY039967 | KY039963   | KY039958   | –         |
| *Aureoboletus quercus*           | GDGM43755       | KY039955  | KY039968 | KY039964   | KY039959   | –         |
| *Aureoboletus quercus*           | GDGM43758       | –         | KY039969 | KY039965   | KY039960   | –         |
| *Aureoboletus raphanaceus*       | GDGM44832       | –         | MH670268 | MH700218   | MH700236   | MH700194  |
| Species                | GenBank voucher | Locus   | Locus   | Locus   | Locus   |
|-----------------------|-----------------|---------|---------|---------|---------|
|                        |                 | ITS     | 28S     | RPB1    | RPB2    | TEF     |
| Aureoboletus raphanaceus | GDGM52543      | MH670271| –       | MH700237| –       |
| Aureoboletus raphanaceus | GDGM52590      | MH670272| MH700219| MH700238| MH700193|
| Aureoboletus roxanae   | CFMR BOS 698   | –       | MK601709| –       | MK766272| MK721063|
| Aureoboletus roxanae   | DS626 07       | –       | KF030311| KF030381| –       | KF030402|
| Aureoboletus russellii | CFMR BOS 716   | –       | MK601710| –       | MK766273| MK721064|
| Aureoboletus sp tenuis | CFMR BZ 2395 BOS 468 | MN250221| MK601711| –       | MK766274| MK721065|
| Aureoboletus sp tenuis | GDGM44829      | –       | KY039970| –       | KY039961| –       |
| Aureoboletus sp tenuis | GDGM42601      | KF265358| KF534789| –       | KT291754| KT291745|
| Aureoboletus tenuis    | HKAS75104      | –       | KT990518| KT990897| KT990359| KT990722|
| Aureoboletus thibetanus| HKAS57692      | –       | KT990524| KT990901| KT990365| KT990728|
| Aureoboletus thibetanus| HKAS76655      | –       | KF112420| KF112626| KF112752| KF112236|
| Aureoboletus thibetanus| HKAS89494      | –       | KT990525| KT990902| KT990366| KT990729|
| Aureoboletus tomentosus| HKAS59694      | –       | KT990513| KT990893| KT990352| KT990714|
| Aureoboletus tomentosus| HKAS80485      | –       | –       | KT990894| KT990353| KT990715|
| Aureoboletus viridiflavus| –              | AY612805| –       | –       | –       | –       |
| Aureoboletus viscidipes| HKAS77103      | –       | KT990519| –       | KT990360| KT990723|
| Aureoboletus viscosus  | OR0361         | –       | –       | –       | MH614751| MH614703|
| Aureoboletus yunnanensis| HKAS75050     | –       | KT990520| KT990898| KT990361| KT990724|
| Aureoboletus zangii    | HKAS74751      | –       | KT990521| KT990899| KT990362| KT990725|
| Aureoboletus zangii    | HKAS74766      | –       | KT990522| KT990900| KT990363| KT990726|
| Baorangia alexandri   | EE 2018a LE 254265 | MH043612| MH036170| –       | –       | –       |
| Baorangia alexandri   | EE 2018a LE 254266 | MH043611| MH036169| –       | –       | –       |
| Baorangia bicolor      | MB07 001 GS 10213 | –       | KF030246| KF030370| –       | KF030405|
| Baorangia emileorum    | PRM 934960     | MH043616| MH036174| –       | –       | –       |
| Baorangia emileorum    | TO HG131114    | MH043617| MH036175| –       | –       | –       |
| Baorangia emileorum    | TO HG171015    | MH043615| MH036173| –       | –       | –       |
| Baorangia emileorum    | TO HG191015    | MH043614| MH036172| –       | –       | –       |
| Baorangia major        | OR209          | –       | –       | –       | MG897441| MG897431|
| Baorangia sp           | GDGM44829      | –       | –       | –       | –       | –       |
| Baorangia sp           | GDGM42601      | KF265358| KF534789| –       | KT291754| KT291745|
| Baorangia sp           | HKAS75104      | –       | KT990518| KT990897| KT990359| KT990722|
| Baorangia sp           | HKAS57692      | –       | KT990524| KT990901| KT990365| KT990728|
| Baorangia sp           | HKAS76655      | –       | KF112420| KF112626| KF112752| KF112236|
| Baorangia sp           | HKAS89494      | –       | KT990525| KT990902| KT990366| KT990729|
| Baorangia sp           | HKAS59694      | –       | KT990513| KT990893| KT990352| KT990714|
| Baorangia sp           | HKAS80485      | –       | –       | KT990894| KT990353| KT990715|
| Baorangia sp           | –              | AY612805| –       | –       | –       | –       |
### Supplementary Table 1 Continued.

| Species                      | GenBank voucher | Locus  | ITS | 28S | RPB1 | RPB2 | TEF   |
|------------------------------|----------------|--------|-----|-----|------|------|-------|
| *Baorangia major*            |                |        |     |     |      |      |       |
| OR404                        |                |        |     |     |      |      |       |
| OR486                        |                |        |     |     |      |      |       |
| *Baorangia pseudocalopus*    | HKAS75739      |        |     |     |      |      |       |
| *Boarangia rufomaculata*     | BOTH4411       |        |     |     |      |      |       |
| *Boletellus longicollis*     | HKAS53398      |        |     |     |      |      |       |
| *Boletellus projectellus*    | AFTOL ID 713   |        |     |     |      |      |       |
| *Boletellus singeri*         | VB4530         |        |     |     |      |      |       |
| 4588                         |                |        |     |     |      |      |       |
| *Boletus abruptibulbus*      | HKAS57262      |        |     |     |      |      |       |
| *Boletus aff amygda linus*   | REH8790        |        |     |     |      |      |       |
| *Boletus melobr unnecens*    | 112605ba       |        |     |     |      |      |       |
| *Boletus amygda linus*       | REH8969        |        |     |     |      |      |       |
| *Boletus austroedulis*       | BD380          |        |     |     |      |      |       |
| *Boletus edulis*             | Be3            |        |     |     |      |      |       |
| *Boletus edulis*             | HMJAU4637      |        |     |     |      |      |       |
| *Boletus edulis*             | Trudell 03 289 |        |     |     |      |      |       |
| *Boletus rubriceps*          | Arora11331     |        |     |     |      |      |       |
| *Boletus rubriceps*          | MIC KUO 081507 |        |     |     |      |      |       |
| *Boletus semigastroideus*    | PBM 3076       |        |     |     |      |      |       |
| *Boletus separans*           | DPL 2704       |        |     |     |      |      |       |
| *Boletus separans*           | MIC KUO 06201002 |       |     |     |      |      |       |
| *Buchwaldoboletus lignicola* | HKAS76674      |        |     |     |      |      |       |
| *Buchwaldoboletus lignicola* | HKAS84904      |        |     |     |      |      |       |
| *Buchwaldoboletus lignicola* | Pul1           |        |     |     |      |      |       |
| *Buchwaldoboletus lignicola* | VDKO1140       |        |     |     |      |      |       |
| *Butyriboletus appendiculatus* | BR502008929  |        |     |     |      |      |       |
| 55                           |                |        |     |     |      |      |       |
| *Butyriboletus appendiculatus* | BR502008933  |        |     |     |      |      |       |
| 90                           |                |        |     |     |      |      |       |
| *Butyriboletus appendiculatus* | Bap1           |        |     |     |      |      |       |
| *Butyriboletus appendiculatus* | MB0000286     |        |     |     |      |      |       |
| *Butyriboletus brunneus*     | NY00013631     |        |     |     |      |      |       |
| *Butyriboletus pseudoregius* | BR502015335    |        |     |     |      |      |       |
| 59 51                        |                |        |     |     |      |      |       |
### Supplementary Table 1

Continued.

| Species                        | GenBank voucher | **Locus** | **ITS** | **28S** | **RPB1** | **RPB2** | **TEF** |
|--------------------------------|-----------------|-----------|---------|---------|----------|----------|---------|
| *Butyriboletus pseudoregius*   | BR502016184 65 02 | KT002602 | KT002613 | KT002625 | –         |         | KT002637 |
| *Butyriboletus pseudospeciosus* | HKAS63513       | –         | KT990541 | KT990909 | KT990380 | KT990743 |
| *Butyriboletus pseudospeciosus* | HKAS63596       | –         | KT990542 | KT990910 | KT990381 | KT990744 |
| *Butyriboletus pseudospeciosus* | N K Zeng2127    | MH885349 | MH879687 | –        | –        | MH879716 |
| *Butyriboletus regius*         | 11265           | –         | –        | –        | –        | –        |         |
| *Butyriboletus regius*         | MB 000287       | KT002605 | KT002616 | KT002628 | –         | –        | KT002640 |
| *Butyriboletus roseoflavus*    | HKAS54099       | KJ909519 | KY418892 | KF739741 | KF739703 | KF739779 |
| *Butyriboletus roseoflavus*    | HKAS63593       | KJ909517 | KJ184559 | –        | –        | KJ184571 |
| *Butyriboletus subsplendidus*  | HKAS50444       | –         | KT990540 | KT990908 | KT990379 | KT990742 |
| *Butyriboletus yicibus*        | HKAS57503       | KT002608 | KT002620 | KT002632 | –         | –        | KT002644 |
| *Butyriboletus yicibus*        | HKAS68010       | –         | KT002619 | KT002631 | –         | –        | KT002643 |
| *Chalciporus aff piperatus*    | HKAS50214       | JQ928610 | JQ928621 | JQ928594 | –         | –        |         |
| *Chalciporus piperatus*        | HKAS84882       | –         | KT990562 | –        | KT990397 | KT990758 |
| *Chalciporus pseudorubinellus* | 4302            | –         | KF030284 | –        | –        | KF030441 |
| *Chalciporus rubinelloides*    | HKAS57362       | –         | KT990563 | –        | KT990398 | KT990759 |
| *Chalciporus rubinelloides*    | HKAS58728       | –         | KT990564 | –        | KT990399 | KT990760 |
| *Chalciporus rubinelloides*    | HKAS74952       | –         | KT990565 | –        | KT990400 | KT990761 |
| *Corneroboletus indecorus*     | OR0863          | –         | –        | –        | MH614772 | MH614726 |
| *Cyanoboletus*                 | HKAS76850       | –         | KF112343 | KF112527 | KF112697 | KF112187 |
| *Cyanoboletus brunneoruber*    | HKAS80579 1     | –         | KT990568 | KT990926 | KT990401 | –        |
| *Cyanoboletus brunneoruber*    | HKAS80579 2     | –         | KT990569 | KT990927 | KT990764 | –        |
| *Cyanoboletus hymenoglutinosus*| AB 2016         | KT860060 | –        | –        | –        | –        |
| *Cyanoboletus pulverulentus*   | 18188           | –         | –        | –        | –        | –        |
| *Cyanoboletus pulverulentus*   | A21             | JX434686 | –        | –        | –        | –        |
| *Cyanoboletus pulverulentus*   | A7              | JX434685 | –        | –        | –        | –        |
| *Cyanoboletus pulverulentus*   | ASIS22672       | KP004920 | –        | –        | –        | –        |
| *Cyanoboletus pulverulentus*   | B21 specimen PRM 935923 | – | – | – | – | – |
Supplementary Table 1 Continued.

| Species     | GenBank voucher | Locus | 28S | RPB1 | RPB2 | TEF |
|-------------|-----------------|-------|-----|------|------|-----|
| Cyanoboletus pulverulentus | B23 specimen PRM 944014 | ITS  | LT714705 | – | – | – |
| Cyanoboletus pulverulentus | B24 specimen PRM 944001 | 28S | LT714706 | – | – | – |
| Cyanoboletus pulverulentus | B25 specimen PRM 944013 | RPB1 | LT714707 | – | – | – |
| Cyanoboletus pulverulentus | B26 specimen PRM 944022 | RPB2 | LT714708 | – | – | – |
| Cyanoboletus pulverulentus | B27 specimen PRM 935997 | TEF | LT714709 | – | – | – |
| Cyanoboletus pulverulentus | CA050916 04 PRM 944013 | – | – | – | – | – |
| Cyanoboletus pulverulentus | JMP0012 | – | – | – | – | – |
| Cyanoboletus pulverulentus | MG 126a KT157053 KT157062 | – | – | – | – | – |
| Cyanoboletus pulverulentus | MG 456a KT157054 KT157063 | – | – | – | – | – |
| Cyanoboletus pulverulentus | MG 628a KT157055 KT157064 KT157069 | – | – | – | – | – |
| Cyanoboletus pulverulentus | RT00004 EU819502 | – | – | – | – | – |
| Cyanoboletus pulverulentus | RW109 | – | – | – | – | KT824013 |
| Cyanoboletus sinopulverulentus | HMAS 266894 KC579402 | – | – | – | – | – |
| Cyanoboletus sp | B28 HKAS 59554 | ITS  | LT714710 | MF373585 | – | – | – |
| Cyanoboletus instabilis | CFMR BZ 3170 MN250222 | 28S | KF1112412 KF112528 | KF112698 KF112186 |
| Exsudoporus floridanus | TENN 067311 | RPB1 | KT002601 KT002612 KT002624 | – | – | – |
| Exsudoporus frostii | NY01194009 | RPB2 | KT990572 KT990928 KT990406 KT990768 |
| Gymnogaster boletoides | REH9455 | – | JX889673 | – | – | JX889683 |
| Gymnogaster boletoides | REH9288 | – | KP327652 | – | – | KP327703 |
| Heimioporus australis | N K Zeng3109 HKAS 59554 | 28S | MH241052 MH241051 | – | – | MH241053 |
| Heimioporus conicus | REH9817 | – | KP327664 | – | – | KP327710 |
| Heimioporus cooloolae | REH9852 | – | KP327655 | – | – | KP327711 |
| Heimioporus cooloolae | N K Zeng2788 HKAS 59554 | RPB1 | MF962380 | – | – | MF962400 |
| Heimioporus gaojiaocong | N K Zeng2791 | RPB2 | MF962398 MF962383 | – | – | MF962412 |
| Heimioporus gaojiaocong | N K Zeng2792 | TEF | MF962399 MF962384 | – | – | MF962413 |
| Heimioporus gaojiaocong | N K Zeng2864 | – | MF962400 MF962385 | – | – | MF962415 |
| Heimioporus gaojiaocong | Z L Yang5901 | – | MF962394 MF962377 | – | – | MF962409 |
| Species                  | GenBank voucher       | ITS     | 28S     | RPB1   | RPB2   | TEF     |
|-------------------------|-----------------------|---------|---------|--------|--------|---------|
| Heimioporus japonicus   | HKAS52237             | –       | KF112347| KF112618| KF112806| KF112228|
| Heimioporus japonicus   | Lancang Y J Hao84     | MF962402| MF962386| –      | –      | MF962416|
| Heimioporus japonicus   | N K Zeng1335          | MF962404| MF962388| –      | –      | MF962418|
| Heimioporus japonicus   | N K Zeng1566          | –       | MF962389| –      | MF962424| MF962419|
| Heimioporus japonicus   | OR114                 | –       | –       | –      | –      | KT824004| KT824037|
| Heimioporus subretisporus| HKAS80581             | –       | KT990573| –      | KT990407| KT990769|
| Heimioporus subretisporus| HKAS80582             | –       | KT990574| –      | KT990409| KT990770|
| Hemileccinum depilatum  | AF2845                | –       | –       | –      | –      | MG212633| MG212591|
| Hemileccinum impolitum  | Bim1                  | –       | –       | –      | –      | JQ327034|
| Hemileccinum impolitum  | HKAS84869             | –       | KT990575| KT990930| KT990410| KT990771|
| Hemileccinum rubropunctum| FH MES116             | FJ480434| –       | –      | –      | –       |
| Hemileccinum rubropunctum| FH MES117             | FJ480433| –       | –      | –      | –       |
| Hemileccinum rubropunctum| JLF5666              | MH190826| MK874830| –      | –      | –       |
| Hemileccinum rubropunctum| NY01193924            | –       | MK601769| –      | MK766328| MK721123|
| Hemileccinum rubropunctum| NY792788              | –       | MK601768| –      | MK766327| MK721122|
| Hemileccinum rugosum    | HKAS50284             | –       | KT990576| –      | KT990411| KT990772|
| Hemileccinum rugosum    | HKAS84355             | –       | KT990578| KT990931| KT990413| KT990774|
| Hemileccinum rugosum    | HKAS84970             | –       | KT990577| –      | KT990412| KT990773|
| Hemileccinum rugosum    | MICHI KUO             | –       | MK601737| –      | MK766299| MK721091|
| Hemileccinum subglabripes| MICH KUO 07070202    | –       | MK601738| –      | MK766300| MK721092|
| Hemileccinum subglabripes| MICH KUO 07230802    | –       | MK601739| –      | MK766301| MK721093|
| Hemileccinum subglabripes| MICH KUO 08301402    | –       | MK601740| –      | MK766302| MK721094|
| Hemileccinum subglabripes| MICH KUO 08240502    | –       | MK601740| –      | MK766302| MK721094|

Supplementary Table 1 Continued.
**Supplementary Table 1** Continued.

| Species                        | GenBank voucher | Locus  | 28S   | RPB1   | RPB2   | TEF   |
|-------------------------------|----------------|--------|-------|--------|--------|-------|
| *Hortiboletus cf rubellus*    | East Coast     |        | –     | –      | KF030371 | –     | KF030419 |
|                               | MB03 033       |        | –     | –      | –       | –     | –     |
| *Hortiboletus cf rubellus*    | West Coast     |        | –     | –      | –       | –     | KF030420 |
|                               | PBM 1331       |        | –     | –      | –       | –     | –     |
| *Hortiboletus indorubellus*   | DC 14          | KT319647 | KU566807 | –      | –      | –     |
| *Hortiboletus indorubellus*   | LS15           | MK002767 | MK002872 | –      | –      | –     |
| *Hortiboletus rubellus*       | MICH KUO 06081002 | –    | MK601741 | –      | MK766303 | MK721095 |
| *Hortiboletus rubellus*       | VDKO0403       | –     | –     | –      | –       | –     | –     |
| *Hymenogaster behrii*         | OSC 12988      | KJ882288 | –     | –      | –       | –     | –     |
| *Hymenogaster behrii*         | OSC 17620      | KJ882290 | –     | –      | –       | –     | –     |
| *Hymenogaster macmurphyi*     | OSC MES282b    | KJ882289 | KJ882291 | –      | –      | –     |
| *Lanmaoa angustispora*        | HKAS74752      | –     | KM605139 | KM605166 | KM605177 | KM605154 |
| *Lanmaoa angustispora*        | HKAS74759      | –     | KM605140 | KM605167 | KM605178 | KM605155 |
| *Lanmaoa asiatica*           | HKAS54095      | –     | KM605141 | KM605164 | KM605174 | KM605151 |
| *Lanmaoa asiatica*           | HKAS63516      | –     | KT990584 | KT990935 | KT990419 | KT990780 |
| *Lanmaoa asiatica*           | HKAS63592      | –     | KM605142 | KM605163 | KM605175 | KM605152 |
| *Lanmaoa asiatica*           | HKAS63603      | –     | KM605143 | KM605165 | KM605176 | KM605153 |
| *Lanmaoa asiatica*           | N K Zeng2125   | MG030477 | MG030470 | –      | –      | MG030481 |
| *Lanmaoa asiatica*           | N K Zeng2795   | MG030469 | MG030469 | –      | –      | MG030480 |
| *Lanmaoa asiatica*           | OR0228         | KM605142 | KM605167 | –      | –      | MG030480 |
| *Lanmaoa borealis*            | 2858           | JQ326998 | –     | –      | –      | JQ327021 |
| *Lanmaoa carminipes*          | MB06 061       | JQ327001 | KM030363 | –      | –      | JQ327022 |
| *Lanmaoa cf borealis*         | borealis AB35  | MH796994 | –     | –      | –      | –     |
| *Lanmaoa flavorubra*          | NY775777       | JQ924339 | –     | –      | –      | –     |
| *Lanmaoa macrocarpa*          | N K Zeng3021   | –     | –     | –      | –      | MH879713 |
| *Lanmaoa macrocarpa*          | N K Zeng3251   | MH885347 | MH879685 | –      | –      | MH885347 |
| *Lanmaoa pallidorosea*        | BOTH4432       | –     | –     | –      | –      | MG897437 |
| *Lanmaoa pallidorosea*        | MO 210760      | –     | MH216001 | –      | –      | MH318610 |
| *Lanmaoa pallidorosea*        | MO 247881      | MH234471 | MH230088 | –      | –      | MH337278 |
| *Lanmaoa pseudosensibilis*    | DS615 07       | KF030257 | –     | –      | –      | KF030407 |
| *Lanmaoa roseocrispans*       | HOLOTYPE       | MH036169 | –     | –      | –      | KP327616 |
| *Lanmaoa rubriceps*           | N K Zeng2773   | MG030475 | MG030468 | –      | –      | MG030479 |
| Species                        | GenBank voucher | Locus    | 28S       | RPB1     | RPB2     | TEF     |
|-------------------------------|-----------------|----------|-----------|----------|----------|---------|
| *Lanmaoa rubriceps*           | N K Zeng3006    | ITS      | MH885346  | MH879683 | –        | –       |
| *Nigroboletus roseonigrescens*| GDGM 43238      | 28S      | KT220584  | KT220588 | KT220591 | –       |
| *Nigroboletus roseonigrescens*| MG 524a         | RPB1     | KT220586  | KT220590 | KT220593 | –       |
| *Nigroboletus roseonigrescens*| ZT 13553        | RPB2     | KT220585  | KT220589 | KT220592 | KT220594|
| *Pulchroboletus roseoalbidus*  | AMB 12757       | TEF      | –         | –        | –        | –       |
| *Pulchroboletus roseoalbidus*  | MCVE 17577      |          | KJ729490  | KJ729503 | –        | –       |
| *Pulchroboletus roseoalbidus*  | MCVE 18217      |          | KJ729488  | KJ729501 | –        | –       |
| *Pulchroboletus roseoalbidus*  | MG416a          |          | KJ729489  | KJ729502 | –        | –       |
| *Pulchroboletus roseoalbidus*  | MG532a          |          | KJ729487  | KJ729500 | –        | –       |
| *Pulchroboletus sclerotorum*   | FLAS F 60333    |          | MF098659  | MF614166 | MF614168 | MF614167|
| *Pulchroboletus sclerotorum*   | FLAS F 60334    |          | MF098660  | –         | –        | MF614164|
| *Pulchroboletus sclerotorum*   | MO 243879       |          | MH257545  | –         | –        | MH337281|
| *Pulveroboletus auriporus*     | DD971           |          | –         | –        | –        | –       |
| *Sinoboletus duplicatoporus*   |                 |          | –         | –        | –        | –       |
| *Suillellus amygdaлинus*       |                 |          | –         | –        | –        | –       |
| *Suillellus amygdaлинus*       |                 |          | –         | –        | –        | –       |
| *Suillellus queletii*          |                 |          | –         | –        | –        | –       |
| *Suillellus subamygdalinus*     |                 |          | –         | –        | –        | –       |
| *Suillellus subamygdalinus*     |                 |          | –         | –        | –        | –       |
| *Suillellus subamygdalinus*     |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
| *Xerocomellus armeniacus*      |                 |          | –         | –        | –        | –       |
### Supplementary Table 1

| Species               | GenBank voucher | Locus  |          |          |          |          |
|-----------------------|-----------------|--------|----------|----------|----------|----------|
| Xerocomellus chrysenteron | MICH KUO 09260903 | ITS    | 28S      | RPB1     | RPB2     | TEF      |
| Xerocomellus chrysenteron | VDKO0821 | –      | –        | –        | KT824017 | KT824050 |
| Xerocomellus chrysenteron | Xch1     | –      | –        | KF030365 | –        | KF030415 |
| Xerocomellus citalpinus  | ADK4864  | –      | –        | –        | KT823993 | KT824026 |
| Xerocomellus citalpinus  | AT2005034 | –      | –        | KF030367 | –        | KF030417 |
| Xerocomellus citalpinus  | PDD94421 | –      | JQ924322 | KF112525 | KF112686 | KF112171 |
| Xerocomellus communis   | HKAS50467 | –      | KT990670 | KT991008 | KT990494 | KT990858 |
| Xerocomellus communis   | HKAS68204 | –      | –        | KT991009 | KT990495 | KT991009 |
| Xerocomellus corneri     | HKAS52503 | –      | KT990668 | KT991006 | KT990492 | KT990856 |
| Xerocomellus corneri     | HKAS90206 | –      | KT990669 | KT991007 | KT990493 | KT990857 |
| Xerocomellus porosporus  | VDKO0311  | –      | –        | –        | MH614773 | MH614727 |
| Xerocomellus ripariellus | VDKO0404  | –      | –        | –        | MH614793 | MH614746 |
| Xerocomellus sp          | HKAS50466 | –      | KF112372 | KF112549 | KF112049 | KF112183 |
| Xerocomellus sp          | HKAS50467 | –      | KF112489 | KT991008 | KT12770  | KT12173  |
| Xerocomellus sp          | HKAS51292 | –      | KF112369 | KF112547 | KF112692 | KF112181 |
| Xerocomellus sp          | HKAS56311 | –      | KF112340 | KF112524 | KF112684 | KF112170 |
| Xerocomellus sp          | HKAS59608 | –      | KF112371 | KF112551 | KF112696 | KF112185 |
| Xerocomellus sp          | HKAS76673 | –      | KF112370 | KF112548 | KF112693 | KF112182 |
| Xerocomellus zelleri     | JLF2977   | KM213666 | KU144799 | –        | –        | –        |
| Xerocomellus zelleri     | REH8724   | –      | KF030271 | KF030366 | –        | KF030416 |
| Xerocominus               | MICH-KUO 07050706 | ITS    |          | MK601821 | MK766377 | MK721175 |
| Xerocomellus              | MA-Fungi   | AJ419221 |          | –        | –        | –        |
| Xerocomellus              | 47678     | –      | –        | –        | –        | –        |
| Xerocomellus              | ML41842RP | MH011927 |          | –        | –        | –        |
| Boletus                   | JQ178324  | –      | –        | –        | –        | –        |
| Bovista himalaica         | JN411938  | –      | –        | –        | –        | –        |
| Xerocomellus              | 17602     | JF908795 |          | –        | –        | –        |
| Xerocomellus              | SOMF12854 | MH011931 |          | –        | –        | –        |
| Xerocomellus              | SOMF29860 | MH011932 |          | –        | –        | –        |
| Xerocomellus              | CM058     | KP823760 |          | –        | –        | –        |
| Aureoboletus              | Farid 501 | MW675741 | MW662576 | MW737500 | MW737463 | –        |
| Aureoboletus              | JAB 124   | MW675754 | –        | –        | –        | –        |
| Aureoboletus              | JAB 130   | MW675725 | MW662581 | –        | –        | –        |
| Aureoboletus              | JAB 320   | MW675726 | MW662585 | MW737508 | MW737468 | MW737489 |
| Species                          | GenBank voucher | Locus  | Locus  | Locus  | Locus  |
|---------------------------------|----------------|--------|--------|--------|--------|
| aureoboletus pseudoauriporus    | JAB 80         | ITS    | 28S    | RPB1   | RPB2   | TEF    |
| cyanoboletus bessettei          | ARB 1393A      | MW675734 | MW662571 | –       | MW737457 | MW737482 |
| cyanoboletus bessettei          | ARB 1393B      | MW675735 | –      | –       | MW737458 | MW737483 |
| cyanoboletus cyanitinctus       | JAB 324        | MW675732 | MW662586 | MW737505 | MW737469 | –       |
| cyanoboletus cyanitinctus       | JAB 325        | MW675733 | –      | MW737506 | MW737470 | –       |
| cyanoboletus cyanitinctus       | Farid 340      | MW675739 | MW662574 | MW737502 | MW737461 | –       |
| cyanoboletus cyanitinctus f. reticulatus | Farid 1035 | MZ746113 | – | – | – | – |
| exsudoporus floridanus          |                |        |        |        |        |        |
| hemileccinum floridanum         | AB16           | MW675745 | MW662570 | – | – | MW737481 |
| hemileccinum floridanum         | Farid 1032     | MW675746 | MW662573 | – | – | – |
| hemileccinum floridanum         | Farid 625      | MW675742 | MW662577 | – | – | – |
| hemileccinum floridanum         | JAB 142        | MW675730 | MW662583 | – | – | MW737488 |
| lanmaoa sublurida               | Farid 1023     | MW675736 | MW662572 | MW737498 | MW737460 | MW737485 |
| lanmaoa sublurida               | Farid 343      | MW675740 | MW662575 | MW737499 | MW737462 | MW737486 |
| lanmaoa sublurida               | Farid 631      | MW675743 | MW662578 | MW737501 | MW737464 | MW737487 |
| pulchroboletus rubricitrinus     | Farid 335      | MF193884 | MG026638 | MW737512 | MW737466 | – |
| xerocomellus bolinii            | JAB 110        | MW675728 | MW662580 | MW737507 | – | – |
| xerocomellus bolinii            | JAB 43         | MW675734 | MW662587 | MW737509 | – | – |
| xerocomellus bolinii            | JAB 133        | MW675729 | MW662582 | – | – | – |
| xerocomellus bolinii            | JAB 95         | MW675735 | MW662589 | MW737511 | MW737472 | MW737491 |
| xerocomellus salicicola         | B391           | MK552408 | MW662569 | MW737496 | – | – |
| hortiboletus coccyginus          | JLF 3093       | KU144805 | – | MW737513 | MW737473 | – |
| xerocomellus amylosporus         | JLF 3498       | KU144743 | – | MW737514 | MW737474 | MW737492 |
| xerocomellus rainisiae           | JLF 3523       | KU144789 | KU144790 | MW737515 | MW737475 | – |
| xerocomellus                    | JLF 3558       | KU144785 | KU144786 | MW737516 | MW737476 | – |
**Supplementary Table 1** Continued.

| Species                  | GenBank voucher | ITS      | 28S      | RPB1     | RPB2     | TEF     |
|--------------------------|-----------------|----------|----------|----------|----------|---------|
| *Xerocomellus atropurpureus* | JLF 3620        | KU144749 | KU144750 | MW737517 | MW737477 | MW737495 |
| *Xerocomellus dryophilus*  | JLF 4134        | KX534076 | KY659593 | –        | MW737478 | MW737493 |
| *Xerocomellus dryophilus*  | JLF 4791        | –        | –        | –        | MW737479 | MW737494 |
| *Xerocomellus mendocinensis* | JLF 5684      | MH168533 | MN294419 | MW737518 | MW737480 | –       |
| *Xerocomellus diffractus*  | JLF 5745        | MH168534 | –        | MW737519 | –        | –       |