Mycosphere 12(1): 1038–1076 (2021) www.mycosphere.org ISSN 2077 7019

Article

Doi 10.5943/mycosphere/12/1/12

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

Farid A[1], Bessette AE[2], Bessette AR[2], Bolin JA[3], Kudzma LV[4], Franck AR[5] and Garey JR[1*]

[1]*Herbarium, Department of Cell, Molecular, and Microbiology, University of South Florida, Tampa, Florida 33620*
[2]*3109 William Penn Ct, Burlington, NC 27215*
[3]*7340 Viale Sonata, Lake Worth, Florida 33467*
[4]*37 Maple Ave., Annandale, NJ 08801*
[5]*University of Florida Herbarium, Florida Museum of Natural History, Gainesville, Florida 32611*

Farid A, Bessette AE, Bessette AR, Bolin JA, Kudzma LV, Franck AR, Garey JR 2021 – Investigations in the boletes (Boletaceae) of southeastern USA: four novel species, and three novel combinations. Mycosphere 12(1), 1038–1076, Doi 10.5943/mycosphere/12/1/12

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 cyaneitinctus* comb. nov., a bolete that is widespread across the eastern USA, *C. cyaneitinctus* f. *reticulatus*, and *Lanmaoa 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

Submitted 25 March 2021, Accepted 10 September 2021, Published 12 October 2021
Corresponding Author: James R. Garey – e-mail – garey@usf.edu

(Singer & Williams 1992, Baroni 1998, Baroni et al. 1998, Ortiz-Santana et al. 2009, 2016, Frank et al. 2017, Crous et al. 2019, Farid et al. 2020).

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 homoplasic. 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, Zangioideae, 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). While much of the genus-level taxonomy has been explored in the Boletoideae, Xerocomoideae, and the *Pulveroboletus* group (Murrill 1909, Halling et al. 2012, Vizzini 2014, Zhao et al. 2014, Gelardi et al. 2015, Wu et al. 2016, Vadthanarat et al. 2019), many species-level taxa are still being described.

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 cyaneitinctus* 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 subluridus* 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 H_2O, 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/fRPB1-Cr (Matheny et al. 2002) were used to amplify a portion of the *RPB1* gene; PCR products were then diluted in nanoPure H_2O in a 1:100 ratio used in a second hemi-nested PCR using one of the original primers gRPB1-Af or fRPB1-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	CGRCATGTYCGCGATCC
RPB1mexR2bol	GGWTCRTCAGYTTCGCA

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, *Hemileccinum* 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, *Cyanoboletus* Gelardi, Vizzini & Simonini, *Lanmaoa* G. Wu & Zhu L. Yang, *Butyriboletus* D. Arora & J.L. Frank, *Suillellus* Murrill, *Gymnogaster* J.W. Cribb, *Chalciporus* 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	**Locus**				
	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

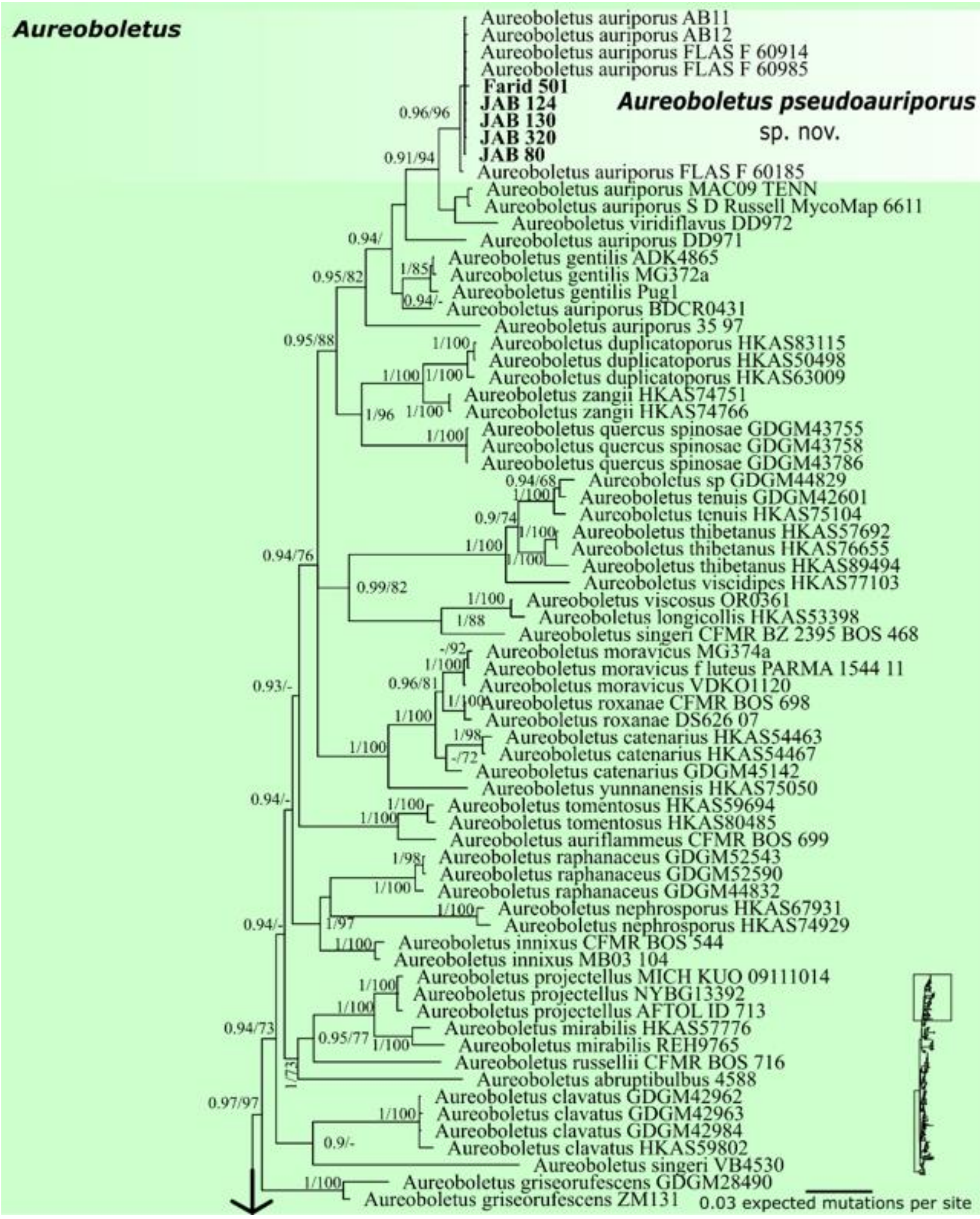

Figure 1 – Phylogram generated from MrBayes based on ITS, 28S, *RPB1*, *RPB2*, and *TEF1* sequence data. Nodes labeled with PP (≥ 0.90) followed by bootstrap replicate support (≥ 70). Colors represent

distinct genera. Specimens with molecular data generated in this study are bolded. Inset phylogeny depicts portion of phylogeny shown in figure.

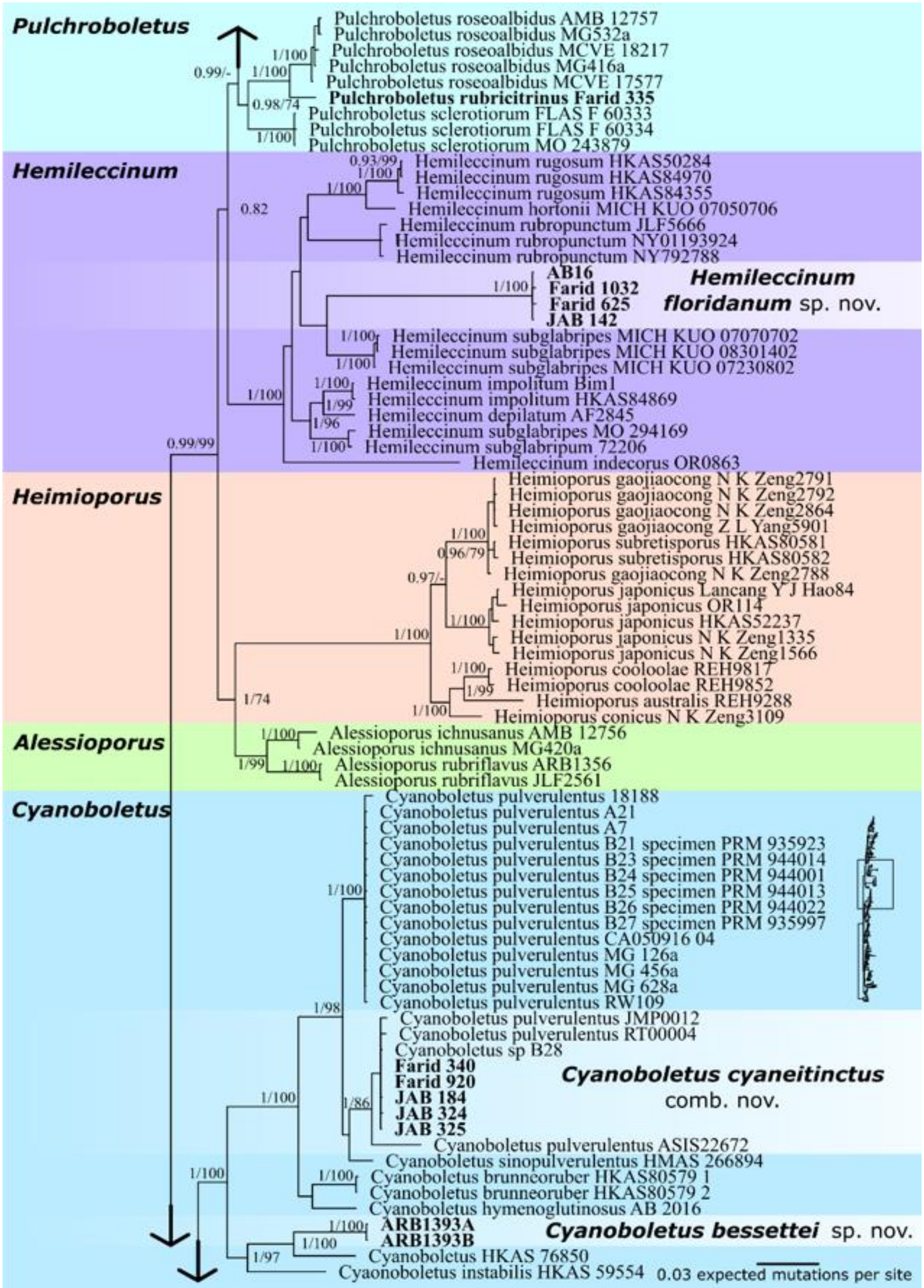

Figure 1 – Continued.

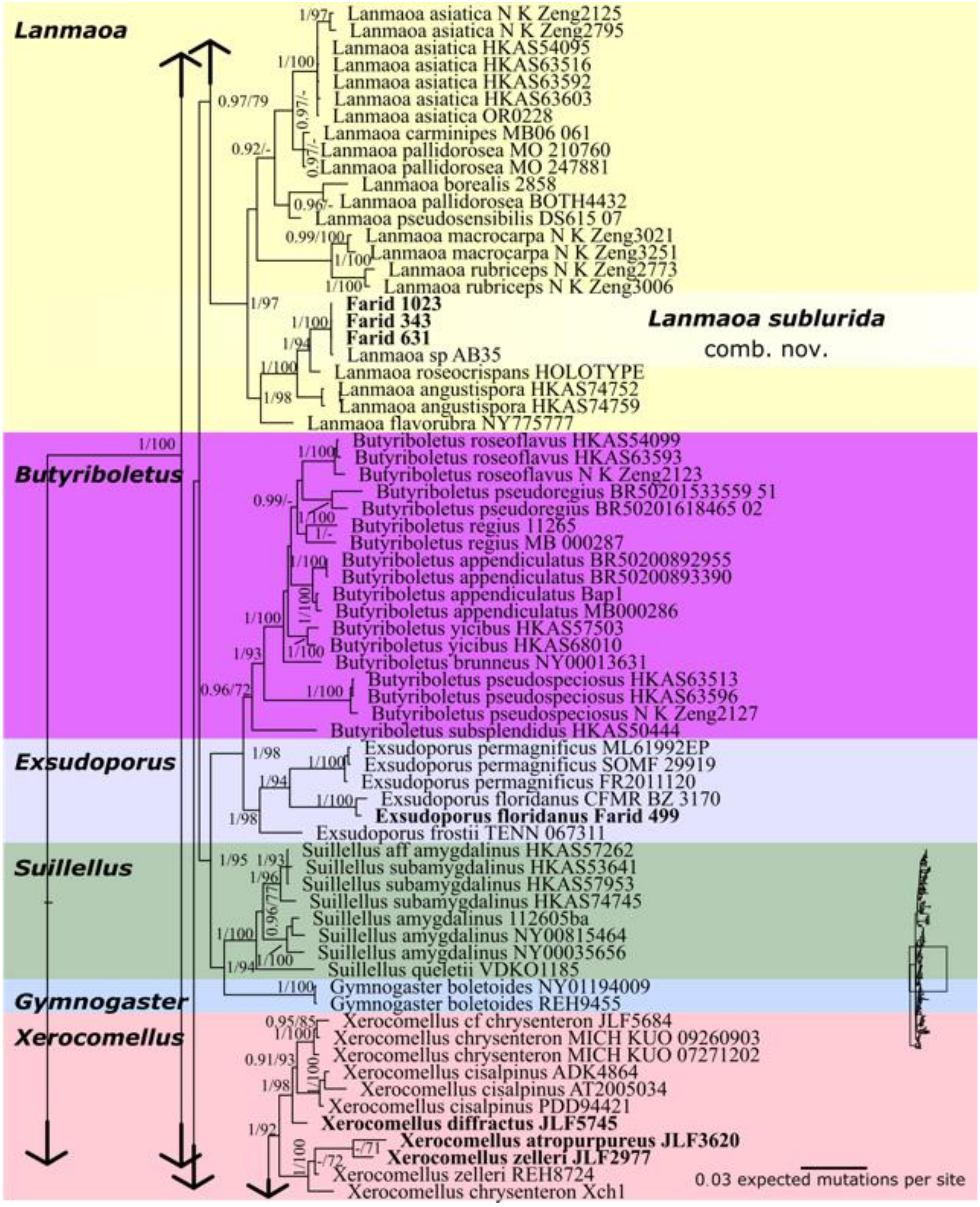

Figure 1 – Continued.

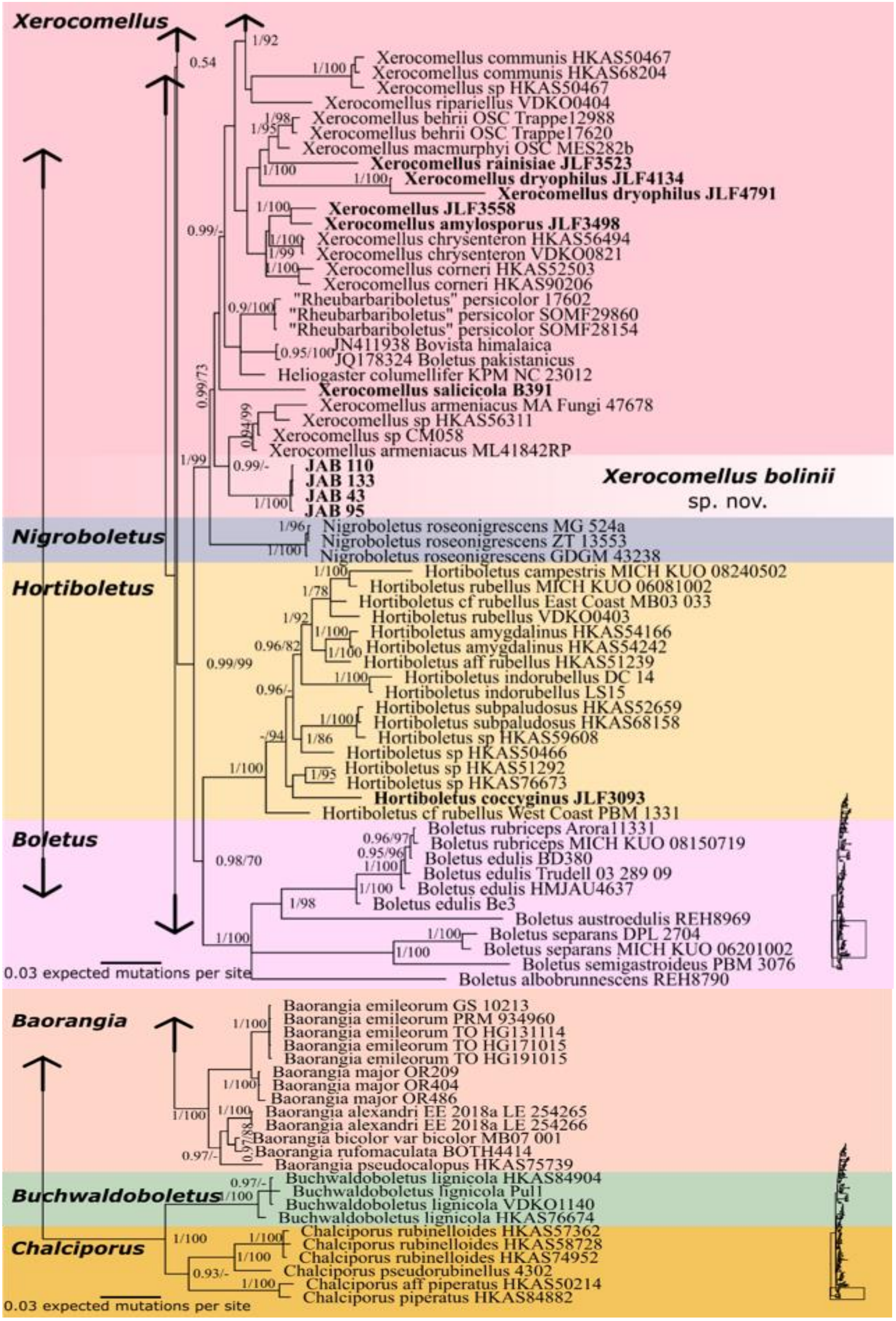

Figure 1 – Continued.

Results

Phylogenetic analyses

The final dataset consisted of 305 specimens comprising 141 ITS, 234 28S, 140 *RPB1*, 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 *Lanmaoa* formed a strongly supported sister clade to *Lanmaoa 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 roseoalbidus* (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. Figs 2, 10D–F

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_4OH, 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_4OH, 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.

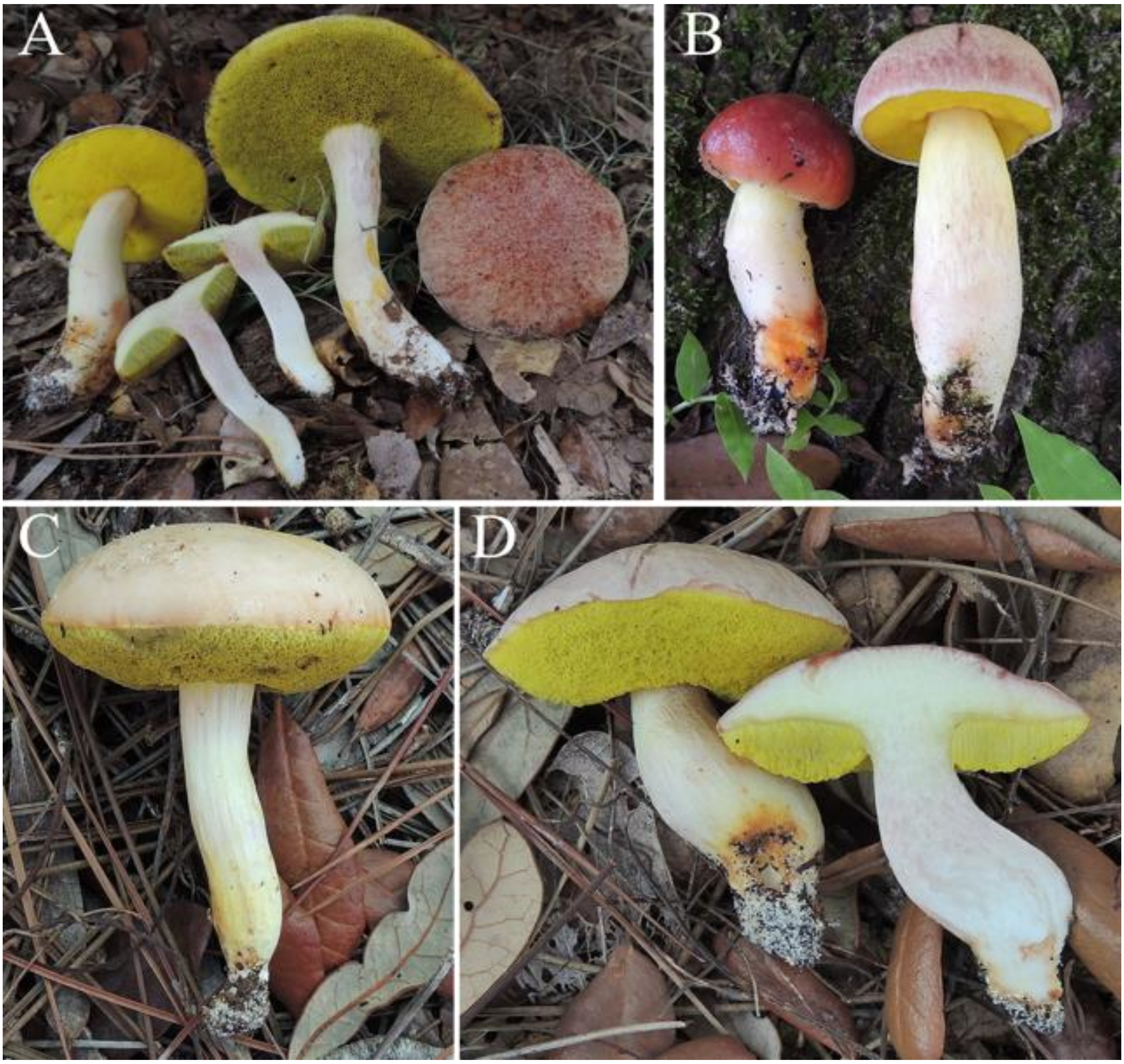

Figure 2 – Field photograph of *Aureoboletus pseudoauriporus.* A J.A. Bolin 488. B J.A. Bolin 157. C J.A. Bolin 124. D J.A. Bolin 130. Photo credit: J.A. Bolin.

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 NH_4OH (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, × = 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 $FeSO_4$, and amber with an expanding blue-green outer ring with NH_4OH. *Context* stains yellow, then pale orange with the application of KOH or NH_4OH and is negative with $FeSO_4$. *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.

Hymenial cystidia abundant, sometimes in fascicles, 36–51 × 8–11 µm, ventricose-rostrate, some with an elongated neck, thin-walled, smooth, yellowish in KOH, non-reactive in Melzer's. *Hymenophoral trama* boletoid, with lateral elements, 3.5–7 µm, wide, moderately divergent, hyaline to grayish yellow in KOH or Melzer's. *Pileipellis* a tangled layer of repent tubular hyphae, 3.5–5.8 µm wide, with cylindrical, rounded end cells, thin-walled, smooth, hyaline to grayish yellow in KOH, yellowish in Melzer's. *Pileus trama* hyphae loosely interwoven, 4.5–13 µm, smooth, thin-walled, hyaline in KOH, yellowish in Melzer's. *Stipitipellis* hyphae mostly parallel, slightly interwoven, 3–6.5 µm, hyaline in KOH, yellowish in Melzer's. *Stipe trama* subparallel, interwoven, 5.2–14 µm, hyaline in KOH, yellowish in Melzer's. *Caulocystidia* none observed. *Clamp connections* absent.

Habitat and Distribution – Scattered or in groups in sparsely grassy areas and sandy soil in association with *Quercu*s and *Pinus* in fall (September), in southeastern United States, known only from South Carolina.

Notes – The combination of distinctive staining reactions and very small spore size is distinct. To date, it is known only from the type location in southeastern South Carolina, United States. *Caloboletus inedulis* (Murrill) Vizzini is superficially similar with a pale yellow hymenophore that becomes olive yellow at maturity and stains dark blue then brownish when bruised. It has a reticulated pale-yellow stipe that may have pinkish tones at the apex and/or pinkish tints below, and bitter tasting context.

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 flushes 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; $FeSO_4$ 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 pleruocystidia. *Basidioles* similarly sized and shaped. *Pleurocystidia* 30–60 × 7–10 µm, fusoid to ampullaceous, hyaline or sometimes encrusted. *Cheilocystidia* similar to pleurocystidia. *Pilepellis* 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 der 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, Tennessee, 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 301501).

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. Figs 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 yellow 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 NH_4OH, and dark orange-amber to orange with $FeSO_4$. The context stains pale orange to yellow then fades with KOH, is negative with NH_4OH, and negative or light greyish olive green with $FeSO_4$. *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, inamyloid 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, inamyloid. *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 Cury 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

1032 (USF 301509). Lake County, Lake Louisa State Park, 4 Sept 2017, J.A. Bolin 142; Palm Beach County, Frenchman's Forest Natural Area, Jupiter 7 Nov 20 May 2018, J.A. Bolin 157 (USF 301488), *ibid.,* 7 Nov 2019, J.A. Bolin 142 (USF 301495); *ibid.,* 7 Nov 2019, J.A. Bolin 454 (USF 301491).

Notes – *Hemileccinum subglabripes* (Peck) R. Halling is very similar, but its stipe is furfuraceous to scabrous or fibrillose and pale to bright yellow. It has smaller narrower spores and smaller sphaerocyst-like elements that measure 10–24 µm. *Hemileccinum hortonii* (A.H. Sm. & Thiers) M. Kuo & B. Ortiz is also similar, but it has a conspicuously pitted pileus, a smooth to lightly pruinose stipe that sometimes has delicate reticulation on the upper half, and pores that sometimes bruise blue. *Hemileccinum rubropunctum* (Peck) R. Halling & B. Ortiz has a conspicuously punctate stipe, yellowish context, sometimes with an unpleasant odor reminiscent of stale cigarette butts in an ashtray and larger spores that measure (10) 16–22 × 5.5–7.5 µm. The basidiospores under SEM were smooth, lacking the very minute warts present in some species of *Hemileccinum* (Šutara 2008, Wu et al. 2014).

Lanmaoa sublurida (Murrill) A. Farid & A.R. Franck comb. nov. Figs 7, 8, 10M–O

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 appearing yellow 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 floccons which develop over the stipe surface as it matures, appearing smooth without a hand lens or without close inspection, at maturity these floccons 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 pluerocystidia. *Basidioles* similarly sized and shaped. *Pleurocystidia* 30–35 × 10–15 µm, pigmented a light golden brown in KOH, NH_4OH, H_2O, 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.

Figure 7 – Field photographs of *Lanmaoa sublurida.* A. Farid 1072. B. Farid 343. C. Farid 1073. Photo credits: A Farid.

Figure 8 – Field photographs of *Lanmaoa sublurida.* A Farid 1072. B–C Farid 1078. D Farid 631. E–F Farid 1072, cross-section of younger (E) and older (F) specimens. 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. Figs 9, 10P–R

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 becoming yellow to orange in the stipe and rapidly stains blue when exposed. *Odor* and *taste* not distinctive. *Macrochemical Testing: Pileus* of mature specimens showed light green fading to yellow with NH_4OH; 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 $FeSO_4$. *Context* in both mature and younger specimens pale orange to NH_4OH, orange to amber fading to brown with the application of KOH and yellow with $FeSO_4$. 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 $FeS0_4$ 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.

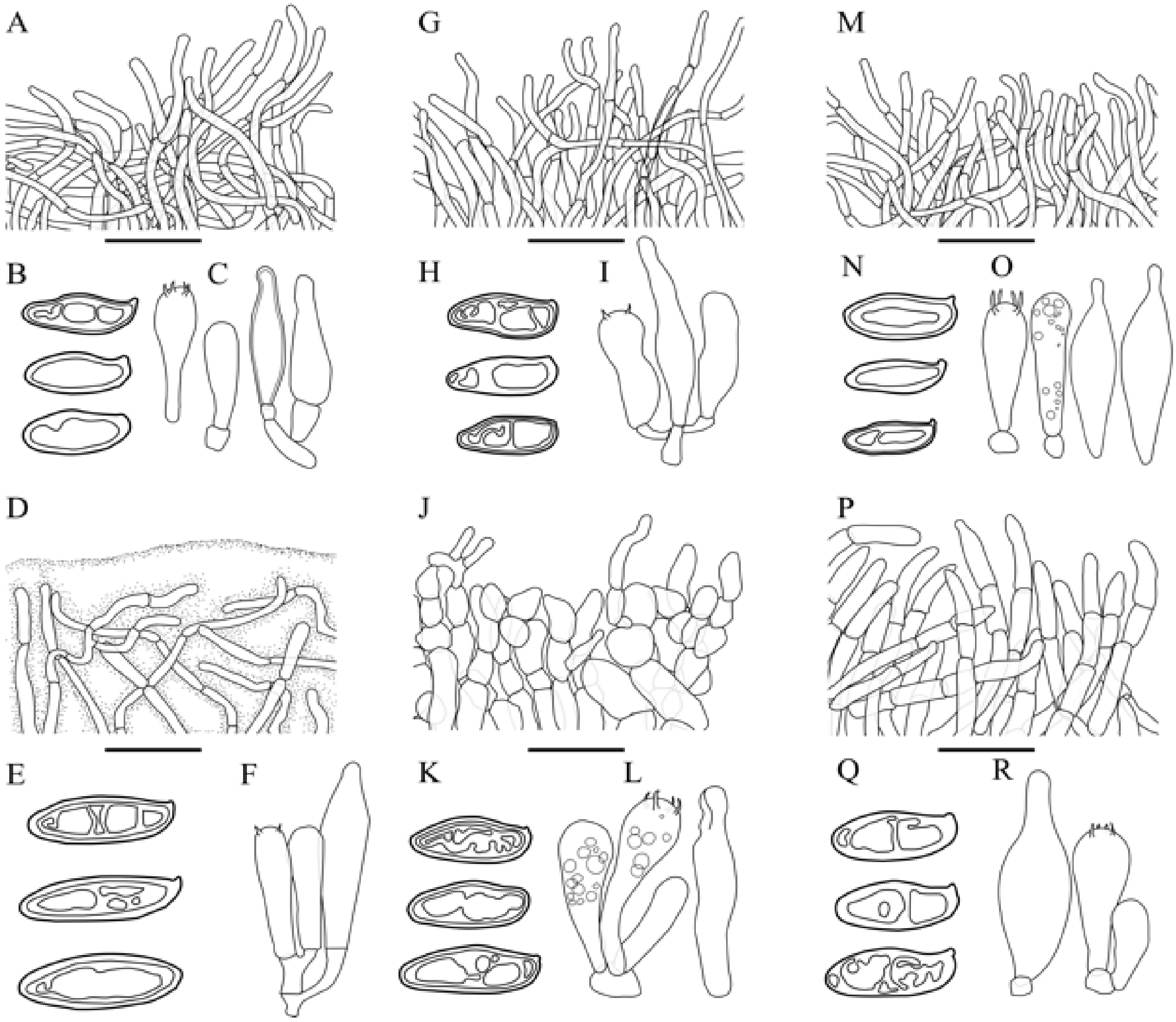

Figure 10 – Microscopic structures of the boletes from this study. A–C *Cyanoboletus cyaneitinctus*. D–F *Aureoboletus pseudoauriporus.* G–I *Cyanoboletus bessettei*. J–L *Hemileccinum floridanum.* M–O *Lanmaoa sublurida.* P–R *Xerocomellus bolinii*. A, D, G, J, M, P depict the pileipellis for each species (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. *Pileus trama* hyphae loosely 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 Tennesssee 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 subglrabipes*, the species it most closely resembles (Fig. 1). Using Singer (1947), *Hemileccinum floridanum* keys out to *Leccinum subglabripum* (Peck) Sing. (= *Hemileccinum subglabripes*). Insightfully, under his *L. subglabripum*, Singer (1947) gave a separate description for the Florida collections, which here conform to the new species *H. floridanum*. *Leccinum subglapripes* 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 ectomycorrhizae with *Quercus* spp. (Smith & Pfister 2009). Roots beneath several collections of *Hemileccinum floridanum* were examined for tuberculate ectomycorrhizae, but none were located. Thus far *H. rubropunctum* is unique in its ability to form tuberculate ectomycorrhizae 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, congophilous plaques on the hyphal surface, tapered and rooting stipe base, the bright yellow-ochraceous to orange-rhubarb and unchangeable context in the stipe base, and the dark blue-green blackish reaction with $FeSO_4$ 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 $FeSO_4$ 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.

Acknowledgements

This work was funded by the Cooley and Lakela funds at USF. The authors would like to thank the herbaria staff and faculty who assisted in facilitating loans for this study. We would also like to thank Hillsborough County Conservation and Environmental Lands Management, the Southwest Florida Water Management District, the Florida Park Service's Bureau of Natural & Cultural Resources, and the Everglades National Park for granting us research permits that enabled us to sample fungi from their respective parks and are grateful to the park managers and staff for their support and assistance. We would also like to thank Jonathan Frank for sending USF specimens of western *Xerocomellus* for our phylogenetic analyses, and Clare Dennison for her help at the SEM lab at USF.

References

Banasiak Ł, Pietras M, Wrzosek M, Okrasińska A et al. 2019 – *Aureoboletus projectellus* (Fungi, Boletales) – an American bolete rapidly spreading in Europe as a new model species for studying expansion of macrofungi. Fungal Ecology 39, 94-9.

Baroni TJ. 1998 – *Boletus aurantiosplendens* sp. nov. from the southern Appalachian Mountains with notes on *Pulveroboletus auriflammeus*, *Pulveroboletus melleouluteus* and *Boletus auripes*. Bull Buff Soc Nat Sci 36, 245–255.
Baroni TJ. 2017 – Mushrooms of the northeastern United States and eastern Canada. Timber Press. 600 p.
Baroni TJ, Bessette AE, Roody WC. 1998 – *Boletus patrioticus* – A new species from the eastern United States. Bull Buff Soc nat Sci 36, 265–268.
Berkeley MJ, Curtis MA. 1853 – Centuries of North American Fungi. Annals and Magazine of Natural History 12(72), 417–435.
Bessette AE, Bessette AR, Roody WC. 2000 – North American Boletes: a color guide to the fleshy pored mushrooms. Syracuse University Press. 146 p.
Bessette AE, Roody WC, Bessette AR. 2017 – Boletes of Eastern North America. Syracuse University Press, Syracuse.
Both EE. 1993 – The Boletes of North America: A compendium. Buffalo Society of Natural Sciences, New York.
Both EE. 1998 – New taxa of boletes and two boletes with identity problems. Bulletin of the Buffalo Society of Natural Sciences, 36, 215–232.
Both EE, Ortiz-Santana B. 2010 – Clinton, Peck and Frost: The dawn of North American boletology. Bulletin of the Buffalo Society of Natural Sciences, 39, 11–28.
Bozok F, Assyov B, Taşkin H. 2019 – First records of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* (Boletales) in association with non-native Fagaceae, with taxonomic remarks. Phytologia Balcanica 25(1), 13–27.
Braeuer S, Goessler W, Kameník J, Konvalinková T et al. 2018 – Arsenic hyperaccumulation and speciation in the edible ink stain bolete (*Cyanoboletus pulverulentus*). Food chemistry 242, 225–31.
Castellano MA, Elliott TF, Truong C, Séné O et al. 2016 – *Kombocles bakaiana* gen. sp. nov. (Boletaceae), a new sequestrate fungus from Cameroon. IMA fungus 7(2), 239.
Chai H, Liang ZQ, Jiang S, Fu XL, Zeng NK. 2018 – *Lanmaoa rubriceps*, a new bolete from tropical China. Phytotaxa 347(1), 71–80.
Coker WC, Beers AH. 1943 – The boleti of North Carolina. Dover, New York. (1971 reprint.)
Crous PW, Wingfield MJ, Lombard L, Roets F et al. 2019 – Fungal Planet description sheets: 951–1041. Persoonia: Molecular Phylogeny and Evolution of Fungi 43, 223–425.
Darriba D, Taboada GL, Doallo R, Posada D. 2012 – jModelTest 2: more models, new heuristics and parallel computing. Nature methods 9(8), 772.
Desprez-Loustau ML, Robin C, Buee M, Courtecuisse R et al. 2007 – The fungal dimension of biological invasions. Trends in Ecology & Evolution 22(9), 472–80.
Farid A, Franck AR, Bolin J, Garey JR. 2020 – Expansion of the genus *Imleria* in North America to include *Imleria floridana*, sp. nov., and *Imleria pallida*, comb. nov. Mycologia 112(2), 423–37.
Farid A, Franck AR, Garey JR. 2017 – *Boletus rubricitrinus* belongs in *Pulchroboletus* (Boletaceae). Czech Mycology 69(2), 143–162.
Farid A, Gelardi M, Angelini C, Franck AR et al. 2018 – *Phylloporus* and *Phylloboletellus* are no longer alone: *Phylloporopsis* gen. nov. (Boletaceae), a new smooth-spored lamellate genus to accommodate the American species *Phylloporus boletinoides*. Fungal Systematics and Evolution 2(1), 341–359.
Frank J, Bessette AR, Bessette AE. 2017 – *Alessioporus rubriflavus* (Boletaceae), a new species from the eastern United States. North American Fungi 28 12, 1–8.
Frank JL, Siegel N, Schwarz CF, Araki B, Vellinga EC. 2020 – *Xerocomellus* (Boletaceae) in western North America. Fungal Systematics and Evolution 5(1), 265–288.
Felsenstein J, Felenstein J. 2004 – Inferring phylogenies. Sinauer associates, Sunderland, Massachussetts.

Gelardi M, Simonini G, Ercole E, Davoli P, Vizzini A. 2015 – *Cupreoboletus* (Boletaceae, Boletineae), a new monotypic genus segregated from *Boletus* sect. *Luridi* to reassign the Mediterranean species *B. poikilochromus*. Mycologia 107(6), 1254–1269.

Gelardi M, Vizzini A, Ercole E, Voyron S et al. 2013 – *Boletus sinopulverulentus*, a new species from Shaanxi Province (central China) and notes on *Boletus* and *Xerocomus*. Sydowia 65(1), 45–57.

Gardes M, Bruns TD. 1993 – ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular ecology 2(2), 113–138.

Grand LF. 1970a – Notes on North Carolina Boletes. I. Species of *Boletellus, Phylloporus, Strobilomyces*, *Tylopilus*, and *Xanthoconium*. Journal of the Elisha Mitchell Scientific Society 1, 49–56.

Grand LF. 1970b – Notes on North Carolina boletes. II. Species of *Gyrodon*, *Gyroporus*, *Xerocomus*, and *Leccinum*. Journal of the Elisha Mitchell Scientific Society 1, 57–61.

Grand LF. 1970c – Notes on North Carolina boletes. III. Species of *Suillus*. Journal of the Elisha Mitchell Scientific Society 1, 209–213.

Guindon S, Gascuel O. 2003 – A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systeatic biology 52(5), 696–704.

Halling RE. 1983 – Boletes described by Charles C. Frost. Mycologia 75(1), 70–92.

Halling RE. 1986 – An annotated index to species and infraspecific taxa of Agaricales and Boletales described by William A. Murrill. Memoirs of the New York Botanical Garden, New York.

Halling RE, Nuhn M, Fechner NA, Osmundson TW et al. 2012 – *Sutorius*: a new genus for *Boletus eximius*. Mycologia 104(4), 951–961.

Hibbett DS, Ohman A, Glotzer D, Nuhn M et al. 2011 – Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal biology reviews 25(1), 38–47.

Katoh K, Standley DM. 2013 – MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution 30(4), 772–80.

Klofac W. 2010 – The genus *Aureoboletus*, a world-wide survey. a contribution to a monographic treatment. Österreichische Zeitschrift für Pilzkunde 2010(19), 133–174.

Kuo M, Ortiz-Santana B. 2020 – Revision of leccinoid fungi, with emphasis on North American taxa, based on molecular and morphological data. Mycologia 112(1), 197–211.

Matheny PB, Liu YJ, Ammirati JF, Hall BD. 2002 – Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). American Journal of Botany 89(4), 688–698.

Miller MA, Pfeiffer W, Schwartz T. 2010 – Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 gateway computing environments workshop (GCE). IEEE. New Orleans.

Murrill WA. 1909 – The Boletaceae of North America – I. Mycologia 1(1), 4–18.

Murrill WA. 1948 – Florida Boletes. Lloydia 11, 21–35.

Nuhn ME, Binder M, Taylor AF, Halling RE, Hibbett DS. 2013 – Phylogenetic overview of the Boletineae. Fungal Biology 117(7–8), 479–511.

Ortiz-Santana B, Bessette AE, McConnell OL. 2016 – *Boletus durhamensis* sp. nov. from North Carolina. Mycotaxon 131(3),703–715.

Ortiz-Santana B, Roody WC, Both EE. 2009 – A new arenicolous Boletus from the Gulf Coast of northern Florida. Mycotaxon 107, 243–247.

Peck CH. 1873 – Report of the Botanist (1869). Annual Report on the New York State Museum of Natural History 23, 27–135.

Peck CH. 1889 – Boleti of the United States. Bulletin of the New York State Museum 2(8). 73–166.

Pringle A, Adams RI, Cross HB, Bruns TD. 2009 – The ectomycorrhizal fungus *Amanita phalloides* was introduced and is expanding its range on the west coast of North America. Molecular Ecology 18(5), 817–833.

R Core Team. 2017 – R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. URL https://www.R-project.org/.
Rambaut A. 2007 – FigTree, a graphical viewer of phylogenetic trees. Institute of Evolutionary Biololgy, University of Edinburgh.
Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018 – Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic biology 67(5), 901–904.
Ronquist F, Teslenko M, Van Der Mark P, Ayres DL et al. 2012 – MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology 61(3), 539–542.
von Schweinitz LD. 1822 – Synopsis fungorum Carolinae superioris secundum observationes Ludovici Davidis de Schweinitz. Leipzig: Johann Ambrosius Barth.
Singer R. 1945a – The Boletineae of Florida with notes on extralimital species I. The Strobilomycetaceae. Farlowia 2, 97–141.
Singer R. 1945b – The Boletineae of Florida with notes on extralimital species. II. The Boletaceae. Farlowia 2, 223–303
Singer R. 1947 – The Boletoideae of Florida with Notes on Extralimital Species III. The American Midland Naturalist 37(1), 1–135.
Singer R. 1986 – The Agaricales in modern taxonomy. 4 edn. Vaduz, Germany: Koeltz. 726 p.
Singer R, Williams R. 1992 – Some boletes from Florida. Mycologia 84(5), 724-728.
Smith AH, Thiers HD. 1971 – Boletes of Michigan. University of Michigan Press, Ann Arbor.
Smith ME, Amses KR, Elliott TF, Obase K et al. 2015 – New sequestrate fungi from Guyana: *Jimtrappea guyanensis* gen. sp. nov., *Castellanea pakaraimophila* gen. sp. nov., and *Costatisporus cyanescens* gen. sp. nov. (Boletaceae, Boletales). IMA fungus 6(2), 297–317.
Smith ME, Pfister DH. 2009 – Tuberculate ectomycorrhizae of angiosperms: the interaction between *Boletus rubropunctus* (Boletaceae) and *Quercus* species (Fagaceae) in the United States and Mexico. American Journal of Botany, 96(9), 1665–1675.
Stamatakis A. 2014 – RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312–1313.
Šutara J. 2008 – *Xerocomus* s. l. in the light of the present state of knowledge. Czech Mycol 60(1): 29–62.
Thiers HD. 1963 – The bolete flora of the Gulf coastal plain. I. The Strobilomyceteae. Journal of the Elisha Mitchell Scientific Society, 79(1), 32–41.
Truong C, Mujic AB, Healy R, Kuhar F et al. 2017 – How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New Phytologist 214(3), 913–9.
Vizzini A. 2014 – Nomenclatural novelties. Index Fungorum 176, 1.
Vizzini A. 2015 – Nomenclatural novelties. Index Fungorum 244, 1.
Vizzini A, Zotti M, Mello A. 2009 – Alien fungal species distribution: the study case of *Favolaschia calocera*. Biological invasions 11(2), 417–429.
Vadthanarat S, Lumyong S, Raspé O. 2019 – *Cacaoporus*, a new Boletaceae genus, with two new species from Thailand. MycoKeys 54, 1–29.
Vadthanarat S, Raspé O, Lumyong S. 2018 – Phylogenetic affinities of the sequestrate genus *Rhodactina* (Boletaceae), with a new species, *R. rostratispora* from Thailand. MycoKeys 2018(29), 63–80.
Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of bacteriology 172(8), 4238–4246.
Walter T. 1788 – Flora Caroliniana. Flora caroliniana. J. Fraser, London. 263 p.
Weber GF. 1961 – William Alphonso Murrill. Mycologia 53(6), 543–557.
White TJ, Bruns T, Lee SJ, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1), 315–322.

Wrzosek M, Motiejūnaitė J, Kasparavičius J, Wilk M et al. 2017 – The progressive spread of *Aureoboletus projectellus* (Fungi, Basidiomycota) in Europe. Fungal Ecology 27:134–136.
Wu G, Lee SM, Horak E, Yang ZL. 2018 – *Spongispora temasekensis*, a new boletoid genus and species from Singapore. Mycologia 110(5), 919–929.
Wu G, Zhao K, Li YC, Zeng NK et al. 2016 – Four new genera of the fungal family Boletaceae. Fungal Diversity 81(1), 1–24.
Wu G, Feng B, Xu J, Zhu XT et al. 2014 – Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Diversity 69(1) 93–115.
Xu J. 2016 – Fungal DNA barcoding. Genome 59(11) 913–932.
Yang ZL, Trappe JM, Binder M, Sanmee R et al. 2006 – The sequestrate genus *Rhodactina* (Basidiomycota, Boletales) in northern Thailand. Mycotaxon 96, 133–140.
Zhang M, Li TH. 2018 – *Erythrophylloporus* (Boletaceae, Boletales), a new genus inferred from morphological and molecular data from subtropical and tropical China. Mycosystema 37(9), 1111–1126.
Zhao K, Wu G, Yang ZL. 2014 – A new genus, *Rubroboletus*, to accommodate *Boletus sinicus* and its allies. Phytotaxa 188(2), 61–77.

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**	**Locus**				
		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 6611	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 GDGM43755	KY039954	KY039967	KY039963	KY039958	–
Aureoboletus quercus	spinosae GDGM43758	KY039955	KY039968	KY039964	KY039959	–
Aureoboletus quercus	spinosae GDGM43786	–	KY039969	KY039965	KY039960	–
Aureoboletus raphanaceus	GDGM44832	–	MH670268	MH700218	MH700236	MH700194

Supplementary Table 1 Continued.

Species	GenBank voucher	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 singeri	CFMR BZ 2395 BOS 468	MN250221	MK601711	–	MK766274	MK721065
Aureoboletus sp	GDGM44829	–	KY039970	–	KY039961	–
Aureoboletus 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	–	KF030246	KF030370	–	KF030405
Baorangia emileorum	GS 10213	MH043613	MH036171	–	–	–
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

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		ITS	*28S*	*RPB1*	*RPB2*	TEF
Baorangia major	OR404	–	–	–	MG897442	MG897432
Baorangia major	OR486	–	–	–	MG897443	MG897433
Baorangia pseudocalopus	HKAS75739	–	KJ184558	KJ184564	KM605179	KJ184570
Baorangia rufomaculata	BOTH4414	–	KF030248	KF030369	MG897435	KF030406
Boletellus longicollis	HKAS53398	–	KF112376	KF112625	KF112755	KF112238
Boletellus projectellus	AFTOL ID 713	AY789082	AY684158	–	AY787218	AY879116
Boletellus singeri	VB4530	–	KP327669	–	–	KP327713
Boletus abruptibulbus	4588	–	KF030302	KF030388	–	KF030401
Boletus aff amygdalinus	HKAS57262	–	KF112316	KF112501	KF112660	KF112174
Boletus albobrunnescens	REH8790	KF668279	HQ161879	HQ161877	–	–
Boletus amygdalinus	112605ba	–	JQ326996	KF030360	–	JQ327024
Boletus austroedulis	REH8969	JN020990	HQ161847	HQ161816	–	–
Boletus edulis	BD380	EU231984	HQ161848	HQ161817	–	–
Boletus edulis	Be3	–	KF030282	GU187444	GU187774	GU187682
Boletus edulis	HMJAU4637	–	KF112455	KF112586	KF112704	KF112202
Boletus edulis	Trudell 03 289 09	EU231983	EU232006	EU231999	–	–
Boletus rubriceps	Arora11331	KC900403	KC900404	–	–	–
Boletus rubriceps	MICH KUO 08150719	–	MK601722	–	MK766284	MK721076
Boletus semigastroideus	PBM 3076	JX258840	KF030352	KF030384	–	KF030430
Boletus separans	DPL 2704	–	KF030329	KF030385	–	KF030431
Boletus separans	MICH KUO 06201002	–	MK601723	–	MK766285	MK721077
Buchwaldoboletus lignicola	HKAS76674	–	KF112350	KF112642	KF112819	KF112277
Buchwaldoboletus lignicola	HKAS84904	–	KT990538	–	KT990377	KT990740
Buchwaldoboletus lignicola	Pul1	–	JQ326997	–	–	JQ327040
Buchwaldoboletus lignicola	VDKO1140	–	–	–	MH614756	MH614710
Butyriboletus appendiculatus	BR502008929 55	KJ605668	KJ605677	KJ619481	–	–
Butyriboletus appendiculatus	BR502008933 90	KT002598	KT002609	KT002621	–	KT002633
Butyriboletus appendiculatus	Bap1	–	AF456837	KF030359	–	JQ327025
Butyriboletus appendiculatus	MB000286	KT002599	KT002610	KT002622	–	KT002634
Butyriboletus brunneus	NY00013631	KT002600	KT002611	KT002623	–	KT002635
Butyriboletus pseudoregius	BR502015335 59 51	KT002603	KT002614	KT002626	–	KT002638

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	–	KF030267	–	–	KF030411
Butyriboletus regius	MB 000287	KT002605	KT002616	KT002628	–	KT002640
Butyriboletus roseoflavus	HKAS54099	KJ909519	KY418892	KF739741	KF739703	KF739779
Butyriboletus roseoflavus	HKAS63593	KJ909517	KJ184559	–	–	KJ184571
Butyriboletus roseoflavus	N K Zeng2123	MH885348	MH879686	–	–	MH885348
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	JF907794	–	–	–	–
Cyanoboletus pulverulentus	A21	JX434686	–	–	–	–
Cyanoboletus pulverulentus	A7	JX434685	–	–	–	–
Cyanoboletus pulverulentus	ASIS22672	KP004920	–	–	–	–
Cyanoboletus pulverulentus	B21 specimen PRM 935923	LT714704	–	–	–	–

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		ITS	*28S*	*RPB1*	*RPB2*	TEF
Cyanoboletus pulverulentus	B23 specimen PRM 944014	LT714705	–	–	–	–
Cyanoboletus pulverulentus	B24 specimen PRM 944001	LT714706	–	–	–	–
Cyanoboletus pulverulentus	B25 specimen PRM 944013	LT714707	–	–	–	–
Cyanoboletus pulverulentus	B26 specimen PRM 944022	LT714708	–	–	–	–
Cyanoboletus pulverulentus	B27 specimen PRM 935997	LT714709	–	–	–	–
Cyanoboletus pulverulentus	CA050916 04	HM347646	–	–	–	–
Cyanoboletus pulverulentus	JMP0012	EU819453	–	–	–	–
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	LT714710	MF373585	–	–	–
Cyaonoboletus instabilis	HKAS 59554	–	KF112412	KF112528	KF112698	KF112186
Exsudoporus floridanus	CFMR BZ 3170	MN250222	MK601725	–	MK766287	MK721079
Exsudoporus frostii	TENN 067311	KT002601	KT002612	KT002624	–	KT002636
Gymnogaster boletoides	NY01194009	–	KT990572	KT990928	KT990406	KT990768
Gymnogaster boletoides	REH9455	–	JX889673	–	–	JX889683
Heimioporus australis	REH9288	–	KP327652	–	–	KP327703
Heimioporus conicus	N K Zeng3109	MH241052	MH241051	–	–	MH241053.
Heimioporus cooloolae	REH9817	–	KP327664	–	–	KP327710
Heimioporus cooloolae	REH9852	–	KP327665	–	–	KP327711
Heimioporus gaojiaocong	N K Zeng2788	–	MF962380	–	–	MF962410
Heimioporus gaojiaocong	N K Zeng2791	MF962398	MF962383	–	–	MF962412
Heimioporus gaojiaocong	N K Zeng2792	MF962399	MF962384	–	–	MF962413
Heimioporus gaojiaocong	N K Zeng2864	MF962400	MF962385	–	–	MF962415
Heimioporus gaojiaocong	Z L Yang5901	MF962394	MF962377	–	–	MF962409

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		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	–	–	KF030375	–	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 subglabripes	MICH KUO 07070702	–	MK601737	–	MK766299	MK721091
Hemileccinum subglabripes	MICH KUO 07230802	–	MK601738	–	MK766300	MK721092
Hemileccinum subglabripes	MICH KUO 08301402	–	MK601739	–	MK766301	MK721093
Hemileccinum subglabripes	MO 294169	MN128237	MN128238	–	–	–
Hemileccinum subglabripum	72206	–	KF030303	KF030374	–	KF030404
Hortiboletus aff rubellus	HKAS51239	–	KF112425	KF112618	KF112695	KF112184
Hortiboletus amygdalinus	HKAS54166	–	KT990581	KT990933	KT990416	KT990777
Hortiboletus amygdalinus	HKAS54242	–	KT990580	–	KT990415	KT990776
Hortiboletus campestris	MICH KUO 08240502	–	MK601740	–	MK766302	MK721094

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		ITS	*28S*	*RPB1*	*RPB2*	TEF
Hortiboletus cf rubellus	East Coast MB03 033	–	–	KF030371	–	KF030419
Hortiboletus cf rubellus	West Coast PBM 1331	–	–	–	–	KF030420
Hortiboletus indorubellus	DC 14	KT319647	KU566807	–	–	–
Hortiboletus indorubellus	LS15	MK002767	MK002872	–	–	–
Hortiboletus rubellus	MICH KUO 06081002	–	MK601741	–	MK766303	MK721095
Hortiboletus rubellus	VDKO0403	–	–	–	MH614774	–
Hortiboletus subpaludosus	HKAS52659	–	KT990582	–	KT990417	KT990778
Hortiboletus subpaludosus	HKAS68158	–	KT990583	KT990934	KT990418	KT990779
Hymenogaster behrii	OSC Trappe12988	KJ882288	–	–	–	–
Hymenogaster behrii	OSC Trappe17620	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	–	–	MG030480
Lanmaoa asiatica	OR0228	–	–	–	MH614777	MH614730
Lanmaoa borealis	2858	–	JQ326998	–	–	JQ327021
Lanmaoa carminipes	MB06 061	–	JQ327001	KF030363	–	JQ327022
Lanmaoa cf	borealis AB35	MH796994	–	–	–	–
Lanmaoa flavorubra	NY775777	–	JQ924339	–	KF112681	KF112160
Lanmaoa macrocarpa	N K Zeng3021	–	–	–	–	MH879713
Lanmaoa macrocarpa	N K Zeng3251	MH885347	MH879685	–	–	MH885347
Lanmaoa pallidorosea	BOTH4432	–	–	–	MG897437	MG897427
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

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		ITS	*28S*	*RPB1*	*RPB2*	TEF
Lanmaoa rubriceps	N K Zeng3006	MH885346	MH879683	–	–	MH879712
Nigroboletus roseonigrescens	GDGM 43238	KT220584	KT220588	KT220591	–	KT220588
Nigroboletus roseonigrescens	MG 524a	KT220586	KT220590	KT220593	–	–
Nigroboletus roseonigrescens	ZT 13553	KT220585	KT220589	KT220592	KT220594	KT220596
Pulchroboletus roseoalbidus	AMB 12757	KJ729486	NG_060126	–	–	KJ729512
Pulchroboletus roseoalbidus	MCVE 17577	KJ729490	KJ729503	–	–	–
Pulchroboletus roseoalbidus	MCVE 18217	KJ729488	KJ729501	–	–	–
Pulchroboletus roseoalbidus	MG416a	KJ729489	KJ729502	–	–	–
Pulchroboletus roseoalbidus	MG532a	KJ729487	KJ729500	–	–	–
Pulchroboletus sclerotiorum	FLAS F 60333	MF098659	MF614166	MF614168	MF614169	MF614167
Pulchroboletus sclerotiorum	FLAS F 60334	MF098660	–	–	MF614164	MF614165
Pulchroboletus sclerotiorum	MO 243879	–	MH257545	–	–	MH337281
Pulveroboletus auriporus	DD971	–	AY612819	–	–	–
Sinoboletus duplicatoporus	HKAS50498	–	KF112361	KF112561	KF112754	KF112230
Suillellus amygdalinus	NY00035656	–	KT990650	KT990990	KT990477	KT990840
Suillellus amygdalinus	NY00815464	–	KT990659	KT990997	KT990484	KT990848
Suillellus queletii	VDKO1185	–	–	–	MH645604	MH645598
Suillellus subamygdalinus	HKAS53641	–	KT990651	KT990991	KT990478	KT990841
Suillellus subamygdalinus	HKAS57953	–	KT990652	KT990992	–	KT990842
Suillellus subamygdalinus	HKAS74745	–	KT990653	KT990993	KT990479	KT990843
Xerocomellus armeniacus	MA Fungi 47678	AJ419221	–	–	–	–
Xerocomellus armeniacus	CM058	KP826760	–	–	–	–
Xerocomellus armeniacus	ML41842RP	MH011927	–	–	–	–
"Rheubarbariboletus" persicolor	17602	JF908795	–	–	–	–
"Rheubarbariboletus" persicolor	SOMF 29860	MH011931	–	–	–	–
"Rheubarbariboletus" persicolor	SOMF 298154	MH011932	–	–	–	–
Xerocomellus chrysenteron	HKAS56494	–	KF112357	KF112526	KF112685	KF112172
Xerocomellus chrysenteron	MICH KUO 07271202	–	–	–	MK766373	MK721171

Supplementary Table 1 Continued.

Species	GenBank voucher	Locus				
		ITS	*28S*	*RPB1*	*RPB2*	TEF
Xerocomellus chrysenteron	MICH KUO 09260903	–	–	–	MK766374	MK721172
Xerocomellus chrysenteron	VDKO0821	–	–	–	KT824017	KT824050
Xerocomellus chrysenteron	Xch1	–	–	KF030365	–	KF030415
Xerocomellus cisalpinus	ADK4864	–	–	–	KT823993	KT824026
Xerocomellus cisalpinus	AT2005034	–	–	KF030367	–	KF030417
Xerocomellus cisalpinus	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	KT990494	KF112183
Xerocomellus sp	HKAS50467	–	KF112489	KT991008	KF112770	KF112173
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
Xerocomus hortonii	MICH-KUO 07050706	–	MK601821	–	MK766377	MK721175
Xerocomellus armeniacus	MA-Fungi 47678	AJ419221	–	–	–	–
Xerocomellus persicolor	ML41842RP	MH011927	–	–	–	–
Boletus pakistanicus		JQ178324	–	–	–	–
Bovista himalaica		JN411938	–	–	–	–
Xerocomellus	17602	JF908795	–	–	–	–
Xerocomellus	SOMF12854	MH011931	–	–	–	–
Xerocomellus	SOMF29860	MH011932	–	–	–	–
Xerocomellus	CM058	KP823760	–	–	–	–
Aureoboletus pseudoauriporus	Farid 501	**MW675741**	**MW662576**	**MW737500**	**MW737463**	–
Aureoboletus pseudoauriporus	JAB 124	**MW675754**	–	–	–	–
Aureoboletus pseudoauriporus	JAB 130	**MW67572**5	**MW662581**	–	–	–
Aureoboletus pseudoauriporus	JAB 320	**MW675726**	**MW662585**	**MW737508**	**MW737468**	**MW737489**

Supplementary Table 1 Continued.

Species	**GenBank voucher**	**Locus**				
		ITS	***28S***	***RPB1***	***RPB2***	**TEF**
Aureoboletus pseudoauriporus	JAB 80	**MW675723**	**MW662588**	**MW737510**	**MW737471**	**MW737490**
Cyanoboletus bessettei	ARB 1393A	**MW675734**	**MW662571**	–	**MW737457**	**MW737482**
Cyanoboletus bessettei	ARB 1393B	**MW675735**	–	–	**MW737458**	**MW737483**
Cyanoboletus cyaneitinctus	JAB 324	**MW675732**	**MW662586**	**MW737505**	**MW737469**	–
Cyanoboletus cyaneitinctus	JAB 325	**MW675733**	–	**MW737506**	**MW737470**	–
Cyanoboletus cyaneitinctus	Farid 340	**MW675739**	**MW662574**	**MW737502**	**MW737461**	–
Cyanoboletus cyaneitinctus	Farid 920	**MW675744**	**MW662579**	**MW737503**	**MW737465**	–
Cyanoboletus cyaneitinctus	JAB 184	**MW675731**	**MW662584**	**MW737504**	**MW737467**	–
Cyanoboletus cyaneitinctus f. reticulatus	Farid 1035	**MZ746113**	–	–	–	–
Exsudoporus floridanus	Farid 499	–	–	**MW737497**	**MW737459**	**MW737484**
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**	**Locus**				
		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**	–	–