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Alessioporus rubriflavus (Boletaceae), a new species from the eastern United States

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Abstract: In 2014 *Alessioporus* was erected as a monotypic genus. Here we describe a new species from the eastern United States, *Alessioporus rubriflavus,* based on unique morphology and molecular data (ITS and LSU nrDNA genes). Ecology, geographical distribution and comparison to other closely allied taxa are discussed.

Key words: Boletaceae, *Alessiosporus rubriflavus*, phylogenetics, new species, taxonomy

Introduction: Smith & Thiers (1971) and Singer (1986) based the definition of genera within the Boletaceae on morphological characters and chemical staining reactions. With continued sampling of bolete taxa in molecular studies, it became obvious that some morphology-based generic concepts did not correspond to monophyletic groups, especially in the larger genera, including *Boletus*. Dentinger et al. (2010) restricted the genus *Boletus* to the "porcini" clade, reducing the number of species in

the genus from approximately 300 to approximately 30 species (Nuhn et al. 2013). Recently many molecular studies have focused on individual genera or placing species into new genera (Arora & Frank 2014; Gelardi et al. 2013; Gelardi et al. 2014; Li et al. 2011; Halling et al. 2012a, 2012b; Halling et al. 2014; Hosen et al. 2012; Wu et al. 2014). Resolving traditional fungal taxonomy based on morphological characteristics continues to require molecular techniques and phylogenetic analyses (Martin, et al. 2011, Yang, 2011).

Alessioporus Gelardi, Vizzini & Simonini was erected in 2014 as a monotypic genus to accommodate the thermo-xerophilic European species *Xerocomus ichnusanus* (Gelardi et al. 2014). The genus was named in honor of the late Carlo Luciano Alessio, who dedicated most of his mycological studies to the investigation of Italian boletes; it is sister to another monotypic genus, described in the same paper, *Pulchroboletus* Gelardi, Vizzini & Simonini. *Alessioporus* species are medium- to large-sized terrestrial boletes that have a dry pileus, yellow to olive-green or olive-brown hymenophores, and solid stipes that are conspicuously reticulate. Their context is whitish to yellow or bright yellow and rapidly stains blue when exposed. The spore print color is olive-brown. They lack a partial veil, annulus, resinous dots and scabers.

In the eastern United States, collections of this unique yellow bolete with reddish brown cap have thus far evaded identification. Now, with molecular techniques, it is possible to determine its phylogenetic position. Here, we use morphological characters and molecular data (ITS and LSU nrDNA genes) to describe a new species, *A. rubriflavus*.

Materials and Methods: *Field and Herbarium collections*.– Fresh material was collected and examined by the authors and observations were recorded on traditional morphological characters of the basidiocarps such as color, surface ornamentation on the pileus and stipe, and staining reactions after cutting or bruising. Each collection was photographed and ecological information, such as potential ectomycorrhizal tree hosts and time of fruiting, was recorded. Spores were examined and measured from the most mature specimen in each collection at 1000× magnification using an Amscope compound microscope and a Leica DMLB compound microscope. Thirty spores from each type collection were measured and then averaged to determine the length-to-width (Q) ratio. Vouchered specimens have been deposited in the fungal herbarium at Florida State University (FLAS).

Molecular Methods.– DNA was extracted, amplified and sequenced from 4 collections. Tissue samples were stored and pulverized with micropestles in buffer (0.1M Tris, 0.3M NaCl, 0.04M EDTA) at 4 C, and extracted in 2 % cetyltrimethyl ammonium bromide (CTAB) with chloroform. DNA was amplified in polymerase chain reactions (PCR) with fungal specific primer ITS1F (5'-ggtcatttagaggaagtaa-3') and universal eukaryote primer TW13 (5'- ggtccgtgtttcaagacg -3') (White et al. 1990; Gardes & Bruns 1993); 20 µl PCR were performed using 0.6 units GoTaq and 4 µL 5x green buffer (Promega), 200 µM each dNTP, 0.3 µM each primer, 2.5 mM $MgCl_2$ and 2 µL undiluted DNA template. An initial 3 min at 93 C was followed by 30 cycles of 30 s at 95 C, 2 min at 54 C, and 3 min at 72 C, with a final cycle for 10 min at 72 C. PCR products were purified with QIAquick PCR Purification kits (Qiagen, Valencia, CA), prepared with BigDye Terminator Ready Reaction Mix v3.1 and sequenced with an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) in the Biotechnology Center at Southern Oregon University. Individual sequences were edited with Chromas 1.45 (McCarthy 1998). Contiguous sequences were assembled in Sequencher v4.7 (Gene Codes Corp. Ann Arbor, MI) and compared to other fungal ITS and LSU sequences in GenBank with BLAST (Altschul et al. 1990).

Xerocomus subtomentosus was used as the outgroup for the ITS data set. Alignments were generated using MAFFT and edited manually and visually assessed for regions of homology using Mesquite (Katoh et al. 2002; Maddison & Maddison 2011). Phylogenetic trees, using parsimony with 1000 bootstrap replicates and 1000 jack-knife replicates, and maximum likelihood using a full heuristic search, were generated using PAUP* 4.10b10 (Swofford 2002). Consensus trees with 50% majority-rule were generated using a tree-bisection-reconnection branch swapping algorithm. All characters were given equal weight; gaps were treated as missing. Sequences were deposited in GenBank.

Results: ITS rDNA sequences from 4 collections and LSU rDNA sequences from 2 collections were generated and deposited in GenBank, KC812305-6, KT223008-9 and KU736957-8. We aligned our ITS data with an additional 12 ITS sequences from GenBank; a total of 796 characters were aligned, with 484 remaining constant, 114 variable, and 198 parsimony informative.

A maximum likelihood tree generated from our ITS rDNA data with data from GenBank (FIG. 1) shows *Alessioporus rubriflavus* from the eastern United States most closely related to, and separate from, the European *A. ichnusanus*. Our molecular data and phylogenetic analyses agree with Gelardi et al. (2014) and, with strong bootstrap support, place the genus *Alessioporus* nearest to *Pulchroboletus* and the "Hemileccinum Clade" in the "Hypoboletus Group." Here, we provide a second species to this previously monotypic genus.

Taxonomy

Alessioporus rubriflavus J.L. Frank, A.R. Bessette & A.E. Bessette sp. nov. FIGS. 2-6
Mycobank no.: 812767

Holotype: United States, Georgia, Elbert County, Ruckersville Road: under mixed pine and oak, 15 September 2014, *ARB1262* (FLAS).
Diagnosis: Medium-sized to large basidiocarps with dry yellow pileus with areas of reddish brown. The yellow tubes, becoming yellow to greenish in age, and yellow reticulate stalk bruise blue. The context is whitish to yellow or bright yellow and rapidly stains blue when exposed. Spores subellipsoid to subfusiform, (13)15.5-18(19) x 4-6 µm. Fruiting in summer with oak and pine.

Etymology: *rubr-* (from *ruber*, L.) = red; *flavus* (L.) = yellow, referring to the colors of the fruitbody.

Pileus 5-14 (21) cm wide, convex when young, becoming broadly convex to nearly plane at maturity, margin strongly incurved at first, initially with a narrow band of sterile tissue, becoming even, sometimes conspicuously undulating on older specimens; surface slightly viscid, becoming dry in age, appressed-tomentose to fasiculate-tomentose, older specimens becoming rimose-areolate and fasciculate, dark wine-red on very young buttons, soon developing a yellow ground color covered with streaks and splashes of various shades of wine-red, red-brown and ocher, sometimes retaining wine-red coloration well into maturity, becoming olive to brownish olive over the disc in age, staining greenish blue to bluish black when bruised or handled. Context firm, bright yellow, quickly staining dark blue when exposed; odor not distinctive; taste unpleasant, sour, or sometimes astringent. Pileipellis a suberect trichodermium becoming a cutis of repent, tangled and interwoven tubular, thin-walled hyphae, smooth, hyaline in KOH, yellowish gray in Melzer's, inamyloid, 3.5-5 µm wide. Pileus trama hyphae interwoven, 4-15 µm wide, thin-walled, hyaline in KOH, grayish yellow in Melzer's, inamyloid, with scattered oleiferous elements. Red portions of the pileipellis immediately stain amber with KOH, are negative with $FeSO_4$, and pale amber

with NH_4OH; yellow portions of the pileipellis quickly stain dull brown with KOH, faintly yellow with $FeSO_4$, and slowly dark blue with NH_4OH; context immediately stains orange with KOH, bright yellow with $FeSO_4$ and yellow that slowly develops a persistent and prominent blue ring with NH_4OH.

Hymenophore yellow at first, soon becoming pale orange-yellow, then olive-brown and deeply depressed at maturity, quickly bruising blue then slowly reddish brown; pores angular, 1-2 per mm; tubes 8-25 mm deep. Hymenophoral trama boletoid, with lateral elements 4.5-8.5 µm wide, moderately divergent, thin-walled, grayish yellow in KOH, yellowish in Melzer's. Hymenial cystidia present as pleurocystidia, 41-48 x 5.5-8 µm, subcylindric or fusoid-ventricose to ventricose-rostrate, smooth, thin-walled, inamyloid, hyaline in KOH.

Stipe 4.5-11 cm long, 1.5-5 cm thick, enlarged downward, ventricose to clavate, typically with a pinched base, solid; surface dry, conspicuously reticulate over at least the upper half, finely tomentose below, bright yellow near the apex or nearly overall, with dark wine-red to red-brown streaks and splashes below especially near the base, quickly staining blue when bruised or handled, then slowly brownish; reticulation yellow toward the apex, yellow-brown below, darkening when handled; mycelium whitish. Stipitipellis hyphae 4-10.5 µm wide, subparallel to interwoven, hyaline in KOH, grayish yellow in Melzer's, with scattered clavate caulocystidia. Clamp connections absent. Stipe context bright yellow, quickly staining blue when exposed, dark wine-red near the base. Stipe trama parallel to somewhat interwoven, tubular, 4-15.5 µm wide, hyaline in KOH, grayish yellow in KOH.

Spores olive-brown in fresh deposit, (13)15.5-18(19) x 4-6 µm, n = 30, x = 15.45 x 5.02 µm, Q = 3.08, subellipsoid to subfusiform, inamyloid, smooth, thin-walled, pale brownish yellow. Basidia 30-38 x 9-10.5 µm, clavate, 4-sterigmate, hyaline, lacking dextrinoid contents in Melzer's.

Habitat and Distribution*:* Solitary, scattered, or in groups on the ground under mixed pine and oak; Georgia and South Carolina as far north as southern New York, probably farther along the eastern seaboard, complete distribution limits yet to be determined; August - September; in southern range fairly common.

Specimens examined: USA. GEORGIA, Elbert County, Ruckersville Road, 15 September, 2014, *ARB1262* (Holotype: FLAS, GenBank KT223008-9); SOUTH CAROLINA, Oconee County, Highway 59, 15 September, 2014, *ARB1356* (FLAS, GenBank KU736957); NEW YORK, Suffolk County, Fresh Pond Road, 19 August, 2012, *JLF2561* (FLAS, GenBank KC812305-6); *JLF2561b* (GenBank KU736958).

Discussion: *Boletus flavissimus* (Murrill) Murrill, originally described from Florida, is similar but its pileus is bright yellow, lacks red coloration in all stages of development, does not stain when bruised and its stipe lacks reticulation. *Butyriboletus brunneus* (Peck) D. Arora & J. L. Frank is also similar but it has a reddish brown or yellow-brown to olive-brown pileus, pale yellow context that quickly stains blue, a yellow, finely reticulated stipe with red on the lower portion, and smaller spores, 10-15 x 3-5.5 µm. *Butyriboletus roseopurpureus* (Both, Bessette & Roody) K. Zhao, Z.L. Yang & Halling has a pinkish purple to dark purplish red pileus and smaller spores, 9.4-13 x 2.7-3.5 µm. *Boletus speciosus* Frost has a bright rose red to rose pink pileus that becomes orange-red to pinkish brown in age, and smaller spores, 11-15 x 3-5.5 µm. *Neoboletus pseudosulphureus* (Kallenb.) W. Klofac has a bright yellow pileus that becomes duller yellow to tawny at maturity and typically develops brown to brownish red tints, especially over the disc in age. It has a yellow stipe that sometimes develops reddish tints, especially near

the base, usually lacks reticulation, but sometimes is reticulate on the upper portion. It has smaller spores that measure 10-16 x 4-6 µm. *Boletus aureissimus* (Murrill) Murrill has a yellow pileus and stipe that lacks red coloration, does not stain blue when bruised, and has smaller spores, 10-14 x 3-5 µm. *Xerocomellus truncatus* (Singer, Snell & E.A. Dick) W. Klofac and *X. chrysenteron* (Bull.) Šutara, sensu eastern United States, are smaller and more slender, and their stipes lack reticulation. Because of its phylogenetic proximity, *Xerocomus subtomentosus* (L.) Quél. was suitable to use as an outgroup in our analysis, but it is easily distinguished in the field by its dull brown cap and non-reticulate stipe.

Fruiting along the eastern seaboard of the United States, sporting a rusty cap and staining dark blue when handled, this striking yellow bolete is occasionally observed by collectors. While its range and population structure remain to be fully determined, presently, *A. rubriflavus* is the only member of this genus known outside of Italy. We expect that as more boletoid fungi from around the world are collected and sequenced, relationships will be further clarified and more species may be included in the genus *Alessioporus*. The close relationship between *Alessioporus* and *Pulchroboletus* also will require more collections to fully support.

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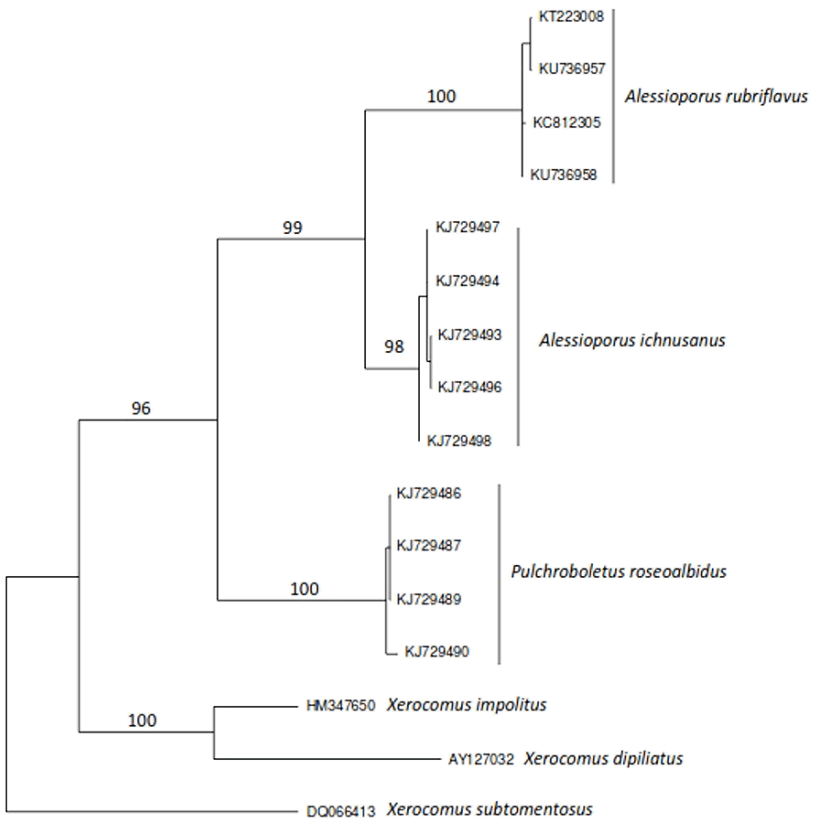

FIGURE 1. Maximum likelihood tree inferred from ITS nrDNA showing placement *of Alessioporus rubriflavus* near to the European *A. ichnusanus*. Bootstrap values (>50% with 1000 replicates) using PAUP* are shown above branches.

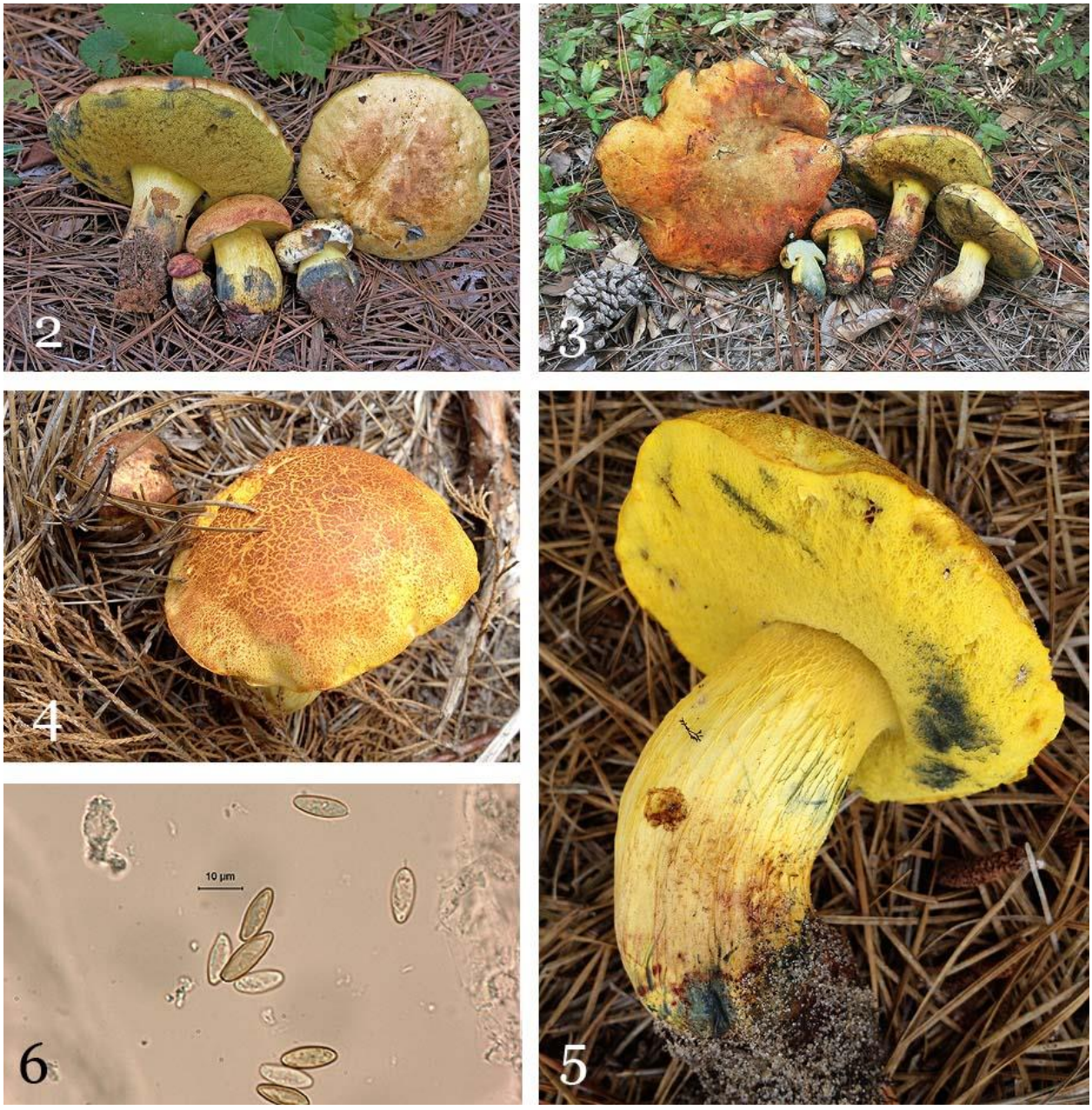

FIGURE 2. *Alessioporus rubriflavus* (ARB1262). FIGURE 3. *Alessioporus rubriflavus* (ARB1262). FIGURE 4. *Alessioporus rubriflavus* (JLF2561) in situ showing the red and yellow coloration of the pileus. FIGURE 5. *Alessioporus rubriflavus* (JLF2561) showing the yellow hymnophore and reticulate yellow stipe both staining blue. FIGURE 6. *Alessioporus rubriflavus* (JLF2561) spores.