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Boletus rubriceps, a new species of porcini from the southwestern USA

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Abstract: The porcini (*Boletus* s.s.) are an economically important group of ectomycorrhizal fungi whose basidiocarps have a white tube layer at first, reticulate stipe, and white flesh. The type species, *B. edulis*, is widespread and morphologically variable, with very little genetic variation from Eurasia to North America. Here we describe a new species of porcini, *Boletus rubriceps,* from the southwestern USA. Morphological characters and molecular data (ITS and LSU) distinguish this species from the Eurasian *B. edulis* and North American *B. edulis* var. *grandedulis*.

Key words: Boletaceae, *Boletus rubriceps,* molecular phylogenetics, new species, taxonomy

Introduction:

The genus *Boletus* (Boletales, Boletaceae) has traditionally included a wide range of medium-sized to large mushrooms with olive-brown spores and hymenophore composed of tightly packed tubes. However, the genus has been shown to be polyphyletic and better restricted to those species with a white hymenophore when young in which the individual tubes are initially stuffed with hyphae (Dentinger et al. 2010). The genus *Boletus* s.s. is thus restricted to *B. edulis* Bull. and its closest relatives, collectively known as king boletes or porcini. The genus is typified by *B. edulis*, originally described from Europe, but even this species is poorly understood; some consider it to be a widespread variable species, while others have described several new taxa with very little molecular distinction.

Despite this more restricted definition, the *Boletus* spp. of North America remain incompletely known. Arora (2008) described three new taxa of *Boletus* s.s. from the west coast of North America, *B. edulis* var. *grandedulis*, *B. rex-veris* and *B. regineus*. Since the 1970s, the senior author has also collected and observed a common, reddish-capped king bolete in the montane spruce forests of the American Southwest. It differs in several respects from other western porcini, but up until now has been reported as *B. edulis* (Arora 1986; States 1990; Evenson 1997) or as *B. pinophilus* Pilát & Dermek. This southwestern bolete is commonly sold in the local farmers' markets of Colorado, New Mexico and Arizona, is served in some restaurants, is dried on a commercial scale and is also sought avidly by many people for their own use. In this study, we compared the macromorphology, micromorphology and ITS and LSU nrDNA sequences of this southwestern red-capped bolete to those of similar species in *Boletus* s. s. and conclude that it merits recognition as a distinct species.

Materials and Methods:

Field and Herbarium collections.– Fresh material was examined by the authors and observations were recorded on traditional morphological characters of the basidiocarps. The senior author had collected the reddish-capped king bolete in Colorado, Arizona and New Mexico for over three decades, and revisited sites to collect specimens during 2011 and 2012. Spores were examined and measured from the most mature specimen in each collection and the average length-width ratio (Q) was calculated for 30 spores. Spores were photographed using a Leica DMLB compound microscope. Vouchered specimens have been deposited in the herbarium at San Francisco State University (SFSU).

Molecular Methods.– DNA was extracted from 15 collections of *Boletus rubriceps*. 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 electrophoresced on 1.5% agarose gels, stained with GelRed nucleic acid stain (1 mg/mL) (Biotium), and visualized using a Kodak EDAS 290 UV transilluminator.

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. Molecular data were obtained by sequencing the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2, and part of the 28S ribosomal gene, with forward primers ITS1F, ITS1 (5'-tccgtaggtgaacctgcgg-3'), ITS3 (5'-gcatcgatgaagaacgcagc-3') and ITS4r (5'-gcaatatcaataagcggagga-3'), and reverse primers ITS2 (5'- gctgcgttcttcatcgatgc-3'), ITS4 and TW13.

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 each other and to other fungal ITS and LSU sequences in GenBank with BLAST (Altschul et al 1990). DNA sequences were deposited in GenBank. We used 15 sequences from our proposed new taxon to establish confidence in a consensus sequence which we then compared pairwise to consensus sequences for the core *B. edulis* from Eurasia, using 20 sequences from GenBank.

Results:

The spruce-associated southwestern bolete alluded to above has a differently colored pileus than that of other porcini in western North America: dark reddish in most stages of development though fading when exposed to strong sunlight. Its spores are 19-23 µm long, whereas spore length for *B. edulis* from both Europe and North America is widely reported as 13-18 µm long. For example, Thiers (1975) reports the spore length as 13-18 µm, Moser (1983) reports it as 13-15.5 (17) µm, Galli (1998) gives the length as 14.5-17.5 µm, and Arora (2008) gives a length of 13-15.5 (17) µm for *B. edulis* var. g*randedulis* . Slightly longer spores are reported by Korhonen et al. (2009) for Scandinavian *B. edulis* (16-19 µm, with a Q of 3). The spores of the southwestern bolete, in contrast, are often longer than 20 µm with a Q of 3.5.

To supplement the morphological data noted above, DNA sequences were generated from 19 *Boletus* collections and deposited in GenBank, accession numbers KC900403- KC900424 (Table 1). We generated LSU data from 8 collections (7 *B. rubriceps* and 1 *B. edulis* var. *grandedulis*) and ITS data from 18 of the collections (15 *B. rubriceps*, 2 *B. edulis* var. *grandedulis* and 1 *B. edulis* from Massachussetts). LSU data shared >99% similarity with European *edulis* species in GenBank; and examination of LSU alignments and pairwise comparisons in GenBank using BLAST support the placement of *B. rubriceps* in the *edulis* branch of *Boletus* s. s. (rather than *B. pinophilus*, which it superficially resembles in some respects).

Pairwise comparison of ITS sequences show that *Boletus rubriceps* differs from the European *B. edulis* by > 1.5 % in the ITS2 region and a portion of the ITS1 region near the 5.8s gene that shows informative variability. Our data also show that *B. rubriceps* differs from both *B. edulis* var. *grandedulis* from the western USA and *B. edulis* from the eastern USA by >1.5 %. This separation, in addition to morphological differences described above, leads us to describe it as a new species below.

Boletus rubriceps D. Arora & J. L. Frank, sp. nov. FIGS. 1, 2A, 2B, 3A, 4

Medium-sized to large basidiocarps with dark red to red-brown pileus, white tubes stuffed with hyphae when young becoming yellow to greenish in age, white stalk with reticulate apex and firm, mild-tasting white flesh. Spores fusoid, (17) 19-23 (24) x 4.5-6µm. Fruiting in summer with spruce and pine. Holotype: USA, New Mexico, Santa Fe County, Santa Fe Ski Basin, 35.79836, -105.8021; under Engelmann spruce, 28 August 2011, *Arora11340* (SFSU).
Mycobank # MB804254

Additional Photos: Arora (1986): Color Plate 143 (as *Boletus edulis*).
Etymology: *rubr-* (from *ruber*, L.) = reddish; *ceps* (L.) = heads (caps), and also evocative of the French name for porcini, *cepes*.

Pileus 8-20 (30) cm broad, convex becoming broadly convex at maturity or sometimes undulating; surface dry or sightly tacky to the touch but typically not viscid unless old or very wet, sometimes with patches of a fine white bloom when very young but otherwise glabrous and occasionally breaking up to form some small scales; color typically dark red to reddish-brown but often developing brown or yellow-brown tones where exposed to sunlight. Context thick, firm, white, typically unchanging when cut but in old or wet specimens sometimes blueing slightly just above the tube layer; taste mild or slightly nutty.

Hymenophore (tube layer) at first white with the individual tubes stuffed with white hyphae such that the pores are not visible, the tube layer becoming yellow as it matures and the pores become discernible, and finally greenish-yellow when fully mature (rarely with some brownish tones); pore surface and tubes usually concolorous and typically not blueing when bruised (but may show slight blueing if old or wet); individual pores < 1 mm broad when mature; tubes at first short in relation to the cap but often 2 cm or more deep when mature.

Stipe 5-15 (20) cm long, 2-6 cm thick at the apex, usually clavate or slightly bulbous when young but less so in age; surface white in most cases, occasionally flushed with brown when older; covered with a fine white reticulum at least over the upper part which may or may not turn brown with age. Context firm, white, unchanging when cut (or showing slight blueing near the tube layer in old or wet specimens).

Spores dark olive-brown in mass, fusoid, thin-walled, smooth, pale brown or yellowish in KOH, (17) 19-23 (24) x 4.5-6 (6.25) µm (Q=3.5). Basidia clavate, mostly 4-spored; hymenial cystidia inconspicuous, fusoid-ventricose; caulocystidia likewise and scarcely protruding. Pileus cuticle an interwoven trichodermium, with very few if any erect elements.

Specimens examined: USA, NEW MEXICO, Santa Fe County, Santa Fe Ski Basin, 35.79836, -105.8021, under Engelmann spruce at 10,000 ft. elevation, 28 August 2011, *Arora11340* (Holotype: SFSU; Isotype: OSC148289); ARIZONA: Apache County, Hwy 180 1 mi. W of New Mexico border, *Arora11338* (SFSU); along Forest Rd. 281 10 mi. SE of Alpine, *Arora11339* (SFSU). COLORADO: Conejos County, 1 mi. up Red Lake Rd. near Las Cumbres Pass, *Arora12006* (SFSU); Dolores County, Dunton Meadows parking area, *Arora11331* & *Arora11332* (SFSU); Mesa County, near Grand Junction, *Arora11336* (SFSU); near Mesa, *Arora12007* (SFSU); San Miguel County, Lizard Head Pass trailhead, *Arora11333* , Arora 11334 & Arora 11335 (SFSU); Telluride ski lift, *Arora11337* (SFSU). NEW MEXICO: Rio Arriba County, Forest Rd. 42B, 1 mi. S. of Hopewell Lake, *Arora12003* (SFSU); along Hwy 64 2 mi. W of Hopewell Lake, *Arora12004* (SFSU); Taos County, Forest Access Road 34 above Cabresta Lake, *Arora12005* (SFSU).

Occurrence: Solitary or in groups on ground in montane conifer forests, associated primarily with spruce (*Picea*) but also with pine (*Pinus*) and possibly fir (*Abies*). Abundant in the southern Rocky Mountains and southwestern United States following summer thunderstorms (July-September), especially at the edges of meadows, trails, ski runs, etc. The exact northern and southern limits of its range have not been established but we suspect it occurs in northern Mexico; it has not been found on the West Coast.

Discussion:
Often called "Rocky Mountain Red-capped King Bolete" or "Rocky Mountain Red" for short, *B. rubriceps* is the common *Boletus* s.s. of the high elevation forests of Colorado, New Mexico and Arizona. The most notable fieldmark is its dark red pileus (FIGS. 1A, 1B, 2A). It differs from *B. edulis* var. *grandedulis* Arora & Simonini by having a yellowish to green mature pore surface (FIG. 2B) rather than brown or cinnamon, and by its pileus color. The pileus of *B. edulis* var. *grandedulis* is brown or yellow-brown, sometimes developing reddish tones in sunlight but then typically having a yellowish margin (FIG. 2C); it is not known to occur in the Southwest. Spruce-associated *B. edulis* in the Pacific Northwest has a much paler (pale tan to brown) pileus; *B. rex-veris* Arora & Simonini fruits in the spring and has not been found in the Southwest; *B. fibrillosus* Thiers has a fibrillose brown pileus and brownish stipe; and *B. regineus* Arora & Simonini has a dark brown pileus (at least when young) overlaid with a whitish bloom. All of the taxa mentioned above have shorter spores than those of *B. rubriceps*. The difference in spore length and Q ratio between *B. rubriceps* and other members of the *B. edulis* complex in North America and Europe is noticeable even without measuring the individual spores (FIGS. 3A, 3B).

B. rubriceps is considered by many to be the most delicious of western USA porcini and it is often seen in summer farmers' markets in the Southwest (FIG. 4A, B). It is worth noting that Arora12007, collected by Tony Yancher in Colorado, is a rare, bright yellow morph of *B. rubriceps* (see FIG. 4C) with no red or brown pigments in the pileus. A bright yellow morph has also been observed for *B. fibrillosus,* normally a brown-capped species, in the Sierra Nevada of California.

ITS sequence data separate *B. rubriceps* by more than 1% from all other described species in *Boletus* s. s. There is more variation in the ITS2 region, where the difference is more than 1.5%. Korhonen et al. (2009) considered the IGS1 region, but found it to be less variable than the ITS in the *B. edulis* complex. They recognized two species of porcini (*B. pinetorum* and *B. betulicola*) that appear phylogenetically conspecific with Eurasian *B. edulis*. Dentinger et al. (2010) corroborated ITS with LSU, ATP6 and RPB1, and included at least two species from Europe (*B. persoonii* and *B. venturii*) that also appear to be conspecific with the Eurasian *B. edulis*. Feng et al. (2012) noted that the porcini clade shows less variability in the ITS region than many other groups, and designated 0.7% as a threshold for species identification. According to their data, however, even this fine a threshold did not differentiate among four described porcini species with clearcut morphological differences. Several sequences in GenBank contain single nucleotide polymorphisms (SNPs) that would require corroborating sequence data to establish confidence in these small apparent variations. These SNPs erode bootstrap values for branches in this tightly-knit clade and may contribute to the concept of *B. edulis* as a widespread and variable species.

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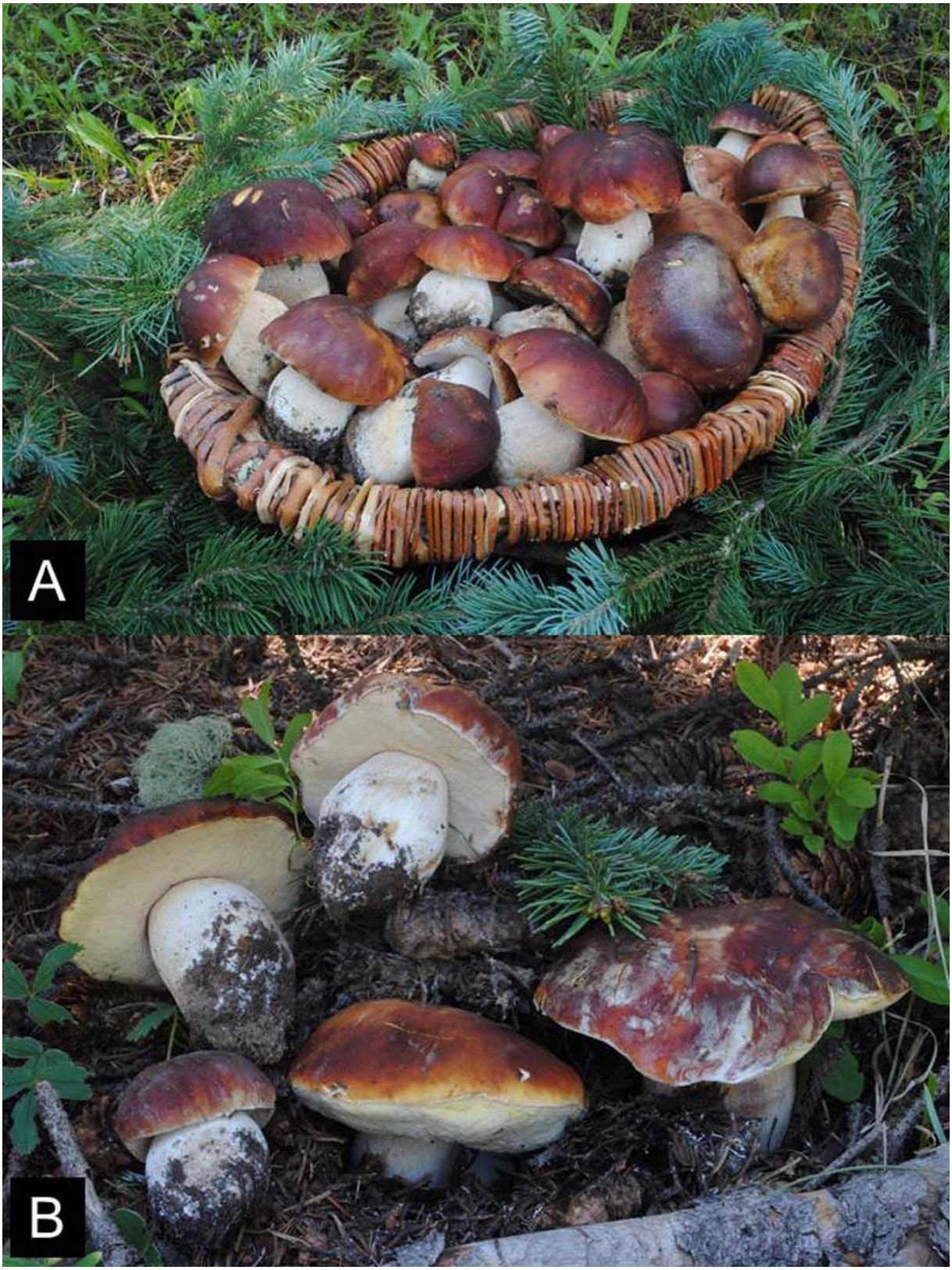

FIGURE 1. *Boletus rubriceps*. A. Typical young basidiocarps. B. Basidiocarps showing dark red pileus and patches of whitish bloom sometimes evident especially where the pileus is creased.

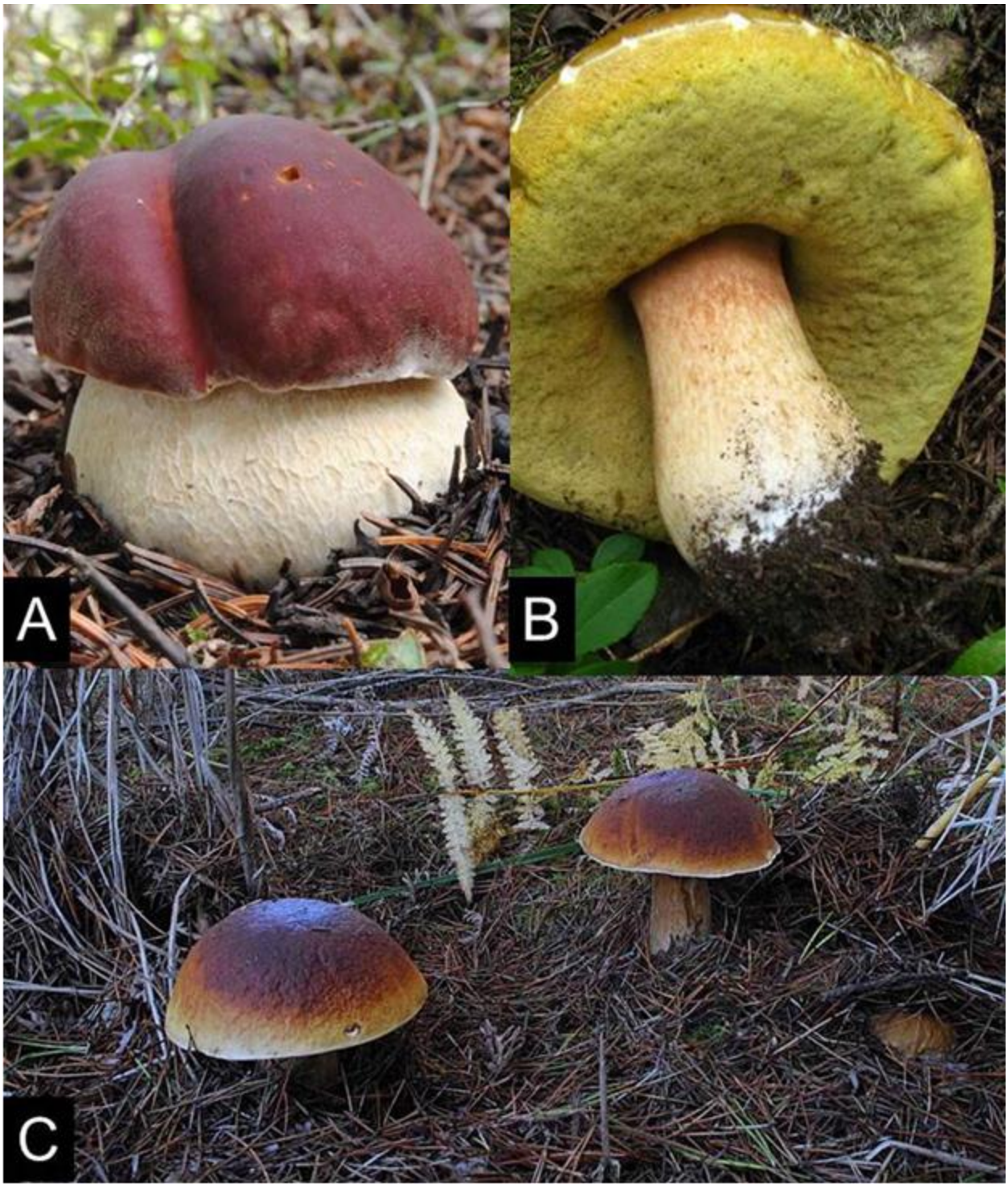

FIGURE 2. *Boletus rubriceps*. A. Button showing dark red pileus and reticulate stipe. B. Mature basidiocarp showing yellow or greenish-yellow pore surface. C. B. *edulis* var. *grandedulis* in coastal Oregon pine forest, showing reddish-brown pileus with yellowish margin.

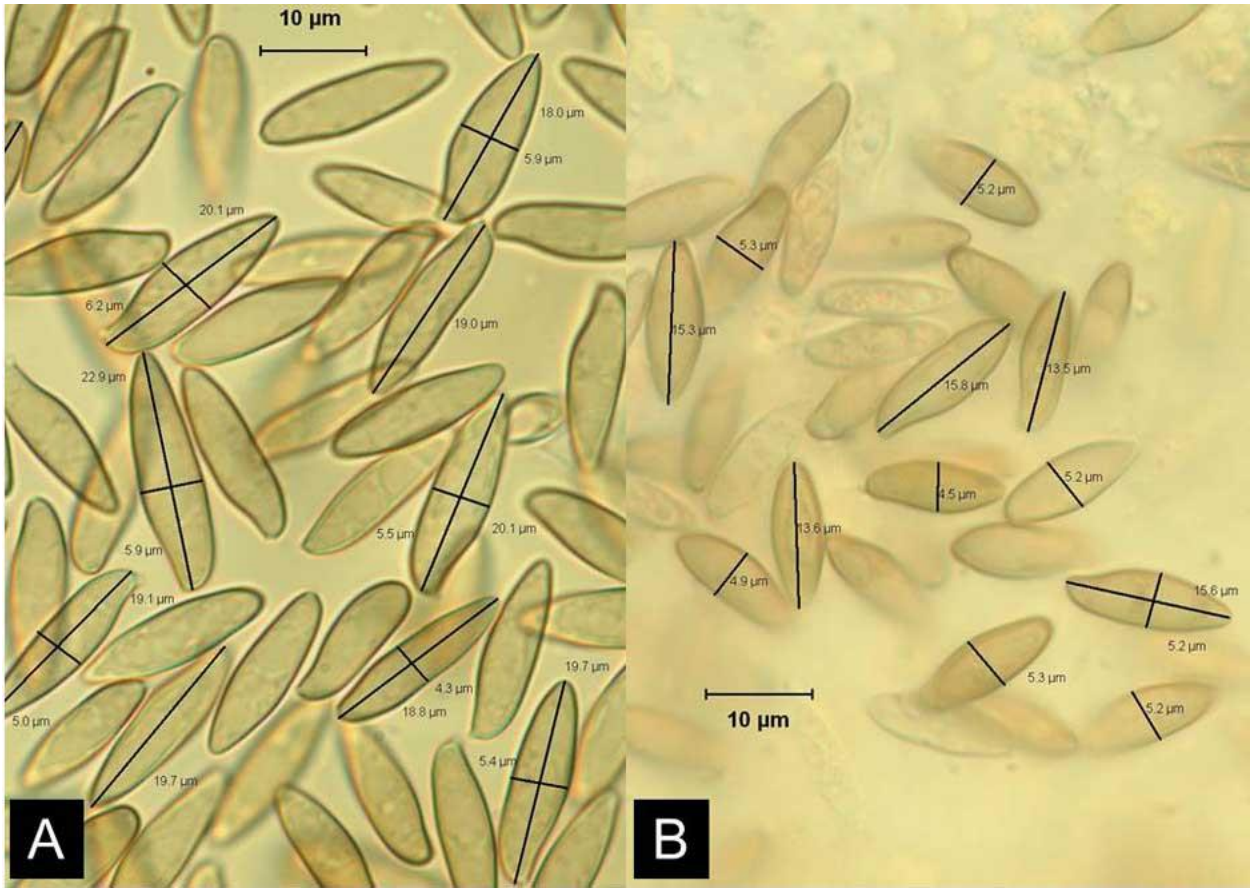

FIGURE 3. A. Spores, many of them longer than 20 µm, from *B. rubriceps* (Arora 11331). B. Spores of *B. edulis* var. *grandedulis* (JLF 2965).

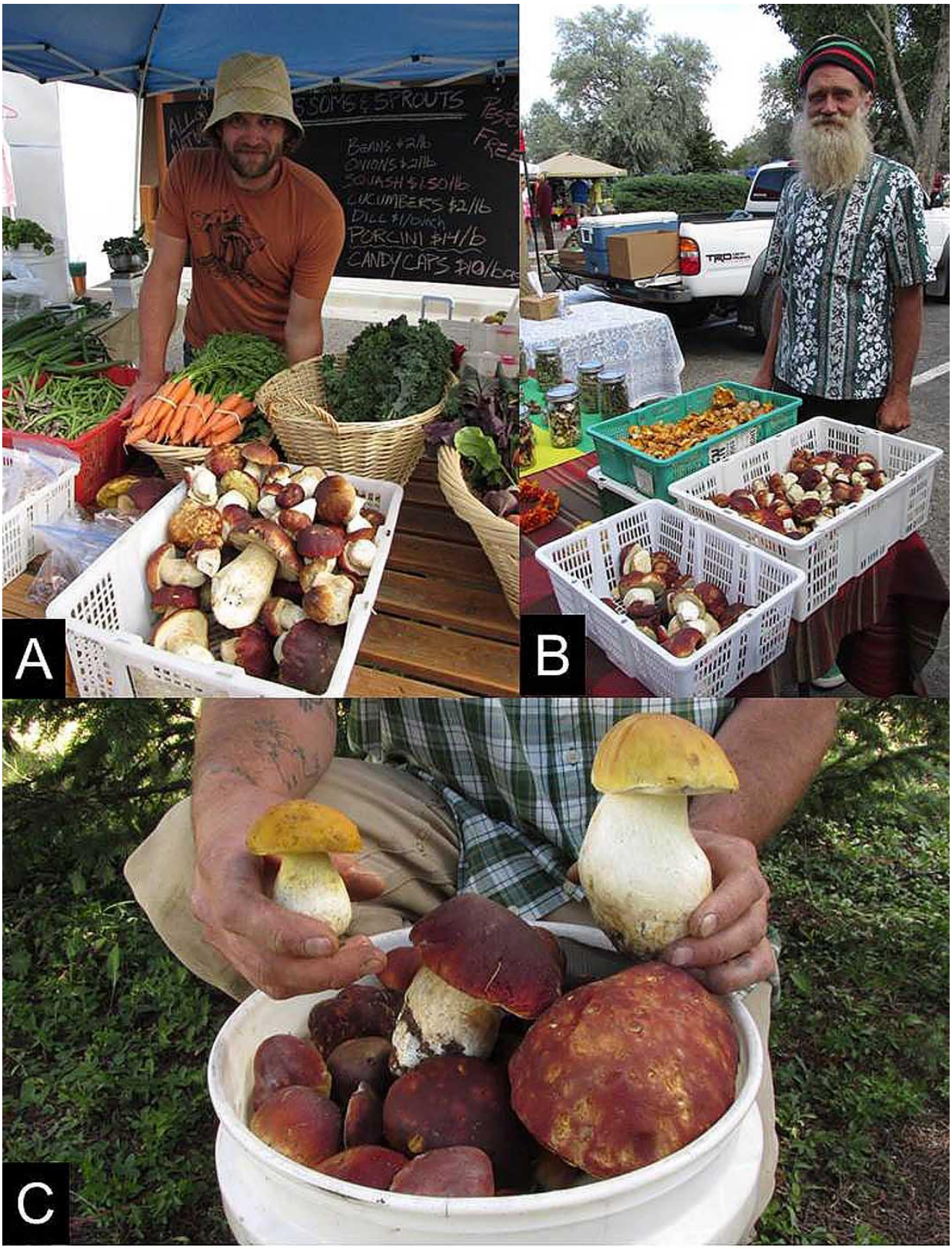

FIGURE 4. A, B. *B. rubriceps* for sale in New Mexico farmers' market. C. A rare bright yellow morph of *B. rubriceps* with typical specimens for comparison.

TABLE 1. Collection information (including location, date, habitat, and GenBank accession numbers) for specimens sequenced and/or examined for this study.

Taxon	**Collection #**	**Collector**	**Date**	**Location**	**Habitat**	**ITS**	**LSU**
Boletus rubriceps	Arora11331	D. Arora	08/22/11	USA:Colorado:Dolores Co.	spruce	KC900403	KC900404
Boletus rubriceps	Arora11332	D. Arora	08/23/11	USA:Colorado:Dolores Co.	spruce	KC900405	
Boletus rubriceps	Arora11333	D. Arora	08/22/11	USA:Colorado:San Miguel Co.	spruce		
Boletus rubriceps	Arora11334	D. Arora	08/22/11	USA:Colorado:San Miguel Co.	spruce	KC900406	KC900407
Boletus rubriceps	Arora11335	D. Arora	08/24/11	USA:Colorado:San Miguel Co.	spruce	KC900408	KC900408
Boletus rubriceps	Arora11336	J Sirjesse	08/01/11	USA:Colorado:Mesa Co.	conifers	KC900409	KC900410
Boletus rubriceps	Arora11337	J Sirjesse	08/01/11	USA:Colorado:San Miguel Co.	spruce		
Boletus rubriceps	Arora11338	D. Arora	08/26/11	USA:Arizona:Apache Co.	pine, oak	KC900411	KC900411
Boletus rubriceps	Arora11339	D. Arora	08/26/11	USA:Arizona:Apache Co.	pine, oak	KC900412	KC900412
Boletus rubriceps (holotype)	Arora11340	D. Arora	08/28/11	USA:New Mexico: Santa Fe Co.	spruce	KC900413	
Boletus rubriceps	Arora12003	D. Arora	08/02/12	USA:New Mexico: Rio Arriba Co.	spruce	KC900414	
Boletus rubriceps	Arora12004	D. Arora	08/02/12	USA:New Mexico: Rio Arriba Co.	spruce	KC900415	
Boletus rubriceps	Arora12005	D. Arora	08/03/12	USA:New Mexico: Taos Co.	spruce	KC900416	
Boletus rubriceps	Arora12006	D. Arora	08/08/12	USA:Colorado: Conejos Co.	spruce	KC900417	
Boletus rubriceps	Arora12007	T. Yancher	08/09/12	USA:Colorado:Mesa Co.	spruce	KC900418	
Boletus rex-veris	Arora12042	D. Arora	06/15/12	USA:California:El Dorado Co.	fir	KC900419	KC900420
Boletus edulis var. *grandedulis*	Arora12014	D. Arora	11/21/12	USA:California:Mendocino Co.	pine	KC900421	KC900422
Boletus edulis var. *grandedulis*	Arora12041	D. Arora	11/18/06	USA:California:Mendocino Co.	pine	KC900420	
Boletus edulis	Arora11223	D. Arora	09/15/11	USA:Massachusetts: Worcester Co.	hemlock	KC184481	