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Clarifying the butter Boletes: a new genus, *Butyriboletus*, is established to accommodate *Boletus* sect. *Appendiculati*, and six new species are described

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Abstract: The butter boletes (*Boletus* s.l. sect. *Appendiculati*) are an economically important group of ectomycorrhizal fungi whose basidiocarps have a yellow tube layer that often bruises blue, yellow reticulate stipe, mild flavor and firm yellow-tinged flesh that may or may not turn blue when exposed. Morphological characters and molecular data (ITS and LSU) place this group in a separate phylogenetic clade from *Boletus* sensu stricto. Here we establish a new genus, *Butyriboletus*, to accommodate 14 species of butter boletes that range from Asia to Europe, north Africa and North America. We recombine eight previously described butter bolete species and we describe six new species: four from western USA (*Bu. persolidus*, *Bu. primiregius*, *Bu. autumnigius*, *Bu. querciregius*) and two from Yunnan, China (*Bu. yicibus*, *Bu. sanicibus*).

Key words: *Appendiculati*, Boletaceae, butter boletes, *Butyriboletus*, molecular phylogenetics, new genus, new species, taxonomy

Introduction

The genus *Boletus* L. (Boletales, Boletaceae) has been shown to be polyphyletic (Binder and Hibbett 2006, Nuhn et al. 2013), with the porcini (*B. edulis* and close relatives) retained in *Boletus* s. str. and the remaining species in a state of flux (Dentinger et al. 2010). *Boletus* L. sect. Appendiculati Konrad & Maubl. ex. Lannoy & Estadès is a well defined group of edible boletes distinguished by firm flesh, yellow reticulate stipe, yellow pore layer that in most cases turns blue when bruised, and mild-flavored, yellow-tinged flesh that may or may not turn blue when exposed. In North America this group is popularly referred to as the ''butter boletes'' because of the butter-yellow pore surface, stalk and flesh. As far as is known the butter boletes are restricted to the northern hemisphere and appear to be more diverse in temperate climes than in tropical, subtropical or boreal ones. Several species are sold in markets in Europe, the western United States, China, Japan and Mexico and one species is harvested on a massive scale in Yunnan, China. Butter boletes have indirect economic importance in that they form ectomycorrhizal mutualisms with commercially valuable timber (*Pseudotsuga*, *Pinus*, *Abies*, *Quercus*).

Five European butter bolete species have been documented in detail (Marques and Muñoz 2006, Assyov 2012), but great confusion surrounds taxa on other continents. As often is the case with higher fungi, the names of European species (especially *Boletus regius* Krombh. and *B. appendiculatus* Schaeff.) have been liberally applied to similar North American butter boletes (Thiers 1975, Arora 1986, Breitenbach and Kranzlin 1991, Bessette et al. 2000) and to Mexican boletes (Guzmán 1997) as well as Asian members of the group (Hongo 1960). In addition, the name of a northeastern North American species, *B. speciosus* Frost, has been applied by Chinese mycologists to an economically important butter bolete in Yunnan province (Chiu 1948, Wang et al. 2004) by Japanese mycologists to a Japanese species (Imazeki et al. 1988) and by European mycologists (Singer 1967, Moser 1983) to a European butter bolete now recognized as *B. fuscoroseus* Smotlacha or *B. pseudoregius* Hubert ex Estadès.

Taxonomic confusion is not uncommon in that mycologists regularly have encountered new species in poorly studied regions and struggled to settle on distinguishing criteria. The butter bolete group has been difficult to clarify to species on the basis of morphology. Indeed, in this study we found several butter bolete collections in herbaria that were misidentified. DNA sequence data is an additional and often critically useful character to support morphological observations and to elucidate which morphological or ecological characters are useful in distinguishing species. In this paper we include ITS and LSU rDNA gene sequences and set out to compare butter boletes in western USA and Yunnan, China, (two areas where the authors have collected extensively) with butter bolete species originally described from Europe and eastern North America. Butter boletes from other areas such as Japan and Mesoamerica were not examined.

Recent studies have used ITS and/or LSU nrDNA data to determined new species and genera in the Boletaceae (Binder and Bresinsky 2002, Gelardi et al.

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2013, Li et al. 2013). Both regions present difficulties for phylogenetic analyses and require visual examination of alignments. However, in combination with other studies with congruent phylogenetic arrangements (Gelardi et al. 2013, Li et al. 2013, Nuhn et al. 2013), these regions provide sufficient support for our morphological observations. Our goal was to see whether the European names *B. regius* and *B. appendiculatus* have been applied correctly and the species are as widely distributed as the literature indicates or whether western USA and Chinese species are distinct taxa, as morphological observations by DA and others suggest.

MATERIALS AND METHODS

Field and herbarium collections.—Whenever possible, fresh material was 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 we recorded ecological information, such as potential ectomycorrhizal tree hosts and time of fruiting.

DA visited sites in California and other parts of USA over the past 30 y and intensively collected butter boletes in California 2009–2012; JLF compiled collections in California and Oregon 2011–2012. DA has collected and visited markets regularly in Yunnan, China, since 1992. Several of the Yunnan collections were bought from markets, a useful means of obtaining specimens when the specimens are in good condition and the vendor can provide data on habitat and locality. Chiu (1948) described more than a dozen species of boletes from Yunnan on the basis of purchased specimens. Additional vouchered specimens for this study were provided by various other collectors, and we examined herbarium specimens at San Francisco State University (SFSU) and Oregon State University (OSC).

Spores were examined and measured from the most mature specimen in each collection at 1000× magnification with the aid of an Olympus compound microscope with bright field and phase contrast and a Leica DMLB compound microscope. Thirty spores from each type collection were measured and averaged to determine the length-to-width (Q) ratio, which proved to be useful in some cases. However, size and shape of basidia and cystidia and other micromorphological features were not helpful in distinguishing species. Vouchered specimens have been deposited in the herbaria at SFSU and OSC. GPS coordinates (latitude and longitude in decimal degree coordinates) are provided for the designated holotypes; in most cases the coordinates were not recorded on site but extrapolated after collection with Google Earth and Google Maps.

Molecular methods.—DNA was extracted from 75 (12 fresh and 63 dried) basidiocarps. 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 and Bruns 1993); 20 μL PCR were performed with 0.6 units GoTaq and 4 μL 5× green buffer (Promega), 200 μM each dNTP, 0.3 μM each primer, 2.5 mM MgCl2 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. When necessary shorter fragments from older herbarium specimens, such as the type collection of *Boletus abieticola* Thiers, were amplified with following primer pairs ITS1F and ITS4 (5′–tcctccgcttattgatatgc–3′), ITS1 and ITS2, and ITS3 and ITS4; 20 μL PCR were amplified as above with the annealing temperature reduced to 51 C and the extension time reduced to 2 min. PCR products were electrophoresced on 1.5% agarose gels, stained with GelRed nucleic acid stain (1 mg/mL) (Biotium) and visualized with a Kodak EDAS 290 UV transilluminator.

PCR products were purified with QIAquick PCR purification kits (QIAGEN, Valencia, California), prepared with BigDye Terminator Ready Reaction Mix 3.1 and sequenced with an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, California) 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 4.7 (Gene Codes Corp., Ann Arbor, Michigan) and compared to other fungal ITS and LSU sequences in GenBank with BLAST (Altschul et al. 1990). *Boletus rubripes* was used as outgroup for the ITS dataset. *Paxillus involutus* was used as outgroup for the LSU dataset. MAFFT and Clustal X were used to generate multiple sequence alignment (Thompson et al. 1997, Katoh et al. 2002). Alignments were edited manually and visually assessed for regions of homology with BioEdit and Mesquite (Hall 1999, Maddison and Maddison 2011).

Phylogenetic trees, using maximum likelihood, were generated with fastDNAml (Felsenstein 1981, Olsen et al. 1994) through the Mobyle portal of the Pasteur Institute (http:/mobile.pasteur.fr) and MEGA 5 (Tamura et al. 2011). Phylogenetic trees, using parsimony with 1000 bootstrap replicates and 1000 jack-knife replicates, were generated with PAUP* 4.10b10 (Swofford 2002). Consensus trees with 50% majority rule were generated with a tree-bisection-reconnection branch-swapping algorithm. All characters were given equal weight; gaps were treated as missing. For the LSU tree, evolutionary history was inferred with the minimum evolution method (Rzhetsky and Nei 1992). When the number of common sites was less than one-fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. All positions containing gaps and missing data were eliminated. Consensus trees were

examined to confirm branch positions. Trees and aligments were deposited at www/TreeBASE.org

RESULTS

DNA sequences were generated from 75 bolete collections and deposited in GenBank, accession numbers KC184411–KC184489 and KC584784–KC584791 (TABLE I). We generated ITS data from 61 collections and aligned these with an additional nine ITS sequences from GenBank and two from Unite; a total of 776 characters were aligned, with 466 remaining constant, 100 variable and 210 parsimony informative. LSU data from 19 collections were aligned with an additional 21 LSU sequences from GenBank; a total of 501 characters were aligned, with 362 remaining constant, 32 variable and 107 parsimony informative. The optimal LSU tree with the sum of branch length = 0.78036770 is illustrated. Alignments have been deposited at http://purl.org/phylo/treebase/phylows/study/TB2:S13879.

These data support the recognition of four North American and two Chinese butter boletes as new taxa distinct from each other and from the European ones. Analysis of pairwise comparisons among all species in the new genus reveal base pair differences of 2–5% among species concepts. The closest grouping of species is the western USA red-capped butters, *Bu. primiregius*, *Bu. autumniregius* and *Bu. querciregius*, which differ from each other by > 2% in the ITS regions. All other species in the new genus differ by > 5% in the ITS regions. ITS analysis suggests an affinity between *Bu. fechtneri* and *Bu. sanicibus*. However, other than the western USA red-capped butters and the *Bu. fechtneri-sanicibus* branch, there is low support for clustering species within the genus.

The butter boletes as a whole (including European species) merit generic recognition now that *Boletus* s. str. has been reserved for *B. edulis* and its cognates (Dentinger et al. 2010). In their phylogenetic review of the Boletinae, Nuhn et al. (2013) recognized a ''*B. regius*'' clade that encompasses the butter boletes, clearly distinct from the ''porcini'' clade. While some branches within the new genus are not strongly supported, our ITS and LSU phylogenetic analyses demonstrated that the butter boletes form a monyphyletic clade separate from the core porcini group (FIGS. 1, 2). Support for this clade also was found by Gelardi et al. (2013). Although the LSU tree did not resolve all branches among other related species, the new genus is strongly supported with a 90% bootstrap value. We therefore propose the genus *Butyriboletus* to accommodate the butter boletes. Supported by ITS and LSU data and morphological observations, we transfer eight existing taxa to this genus and describe six new species: four from California, USA, and two from Yunnan, China.

TAXONOMY

Butyriboletus D. Arora & J. L. Frank, gen. nov.
MycoBank MB803207

Basidiocarps epigeous and stipitate. Pileus mostly brown to reddish; tube layer yellow, often turning blue when bruised, stipe yellow or reddish tinged and reticulate over the upper portion; context of pileus pale yellow, turning blue erratically if at all when cut; context of stipe often vinaceous-tinged at the base. Spores fusoid, smooth, brown (olive brown in mass); pileipellis a trichodermium.

MycoBank MB803319
Type: *Butyriboletus appendiculatus* (Schaeffer) D. Arora & J.L. Frank, comb. nov.
Etymology: butyrum (L.) = butter
≡ *Boletus appendiculatus* Schaeffer, Fung. Bavar. Palat. 2: tab. 130. 1763.

These published species are transferred to *Butyriboletus*:

Butyriboletus abieticola (Thiers) D. Arora & J. L. Frank, comb. nov.
MycoBank MB803317
≡ *Boletus abieticola* Thiers, California Mushrooms: a field guide to the Boletes: 39. 1975.

Butyriboletus brunneus (Peck) D. Arora & J. L. Frank, comb. et stat. nov.
MycoBank MB803325
≡ *Boletus speciosus* var. *brunneus* Peck, Ann. Rep. N.Y. St. Mus. 43:85. 1890.

Butyriboletus fechtneri (Velenovsky) D. Arora & J. L. Frank, comb. nov.
MycoBank MB803320
≡ *Boletus fechtneri* Velenovsky, České Houby 4–5: 704. 1922.

Butyriboletus pseudoregius (Huber) D. Arora & J.L.Frank, comb. et stat. nov.
MycoBank MB804787
≡ *Boletus appendiculatus* subsp. *pseudoregius* Huber, *Z. Pilzk.* 17:87. 1938.

Butyriboletus regius (Krombholz) D. Arora & J. L. Frank, comb. nov.
MycoBank MB803323
≡ *Boletus regius* Krombholz, Naturgetr. Abbild. Beschr. Schwämme (Prague) 2:3. 1832.

TABLE I. List of collections sequenced with GenBank numbers, herbarium and collection information

Species	Collection no.	Herbarium code		Collector	Date	Location	Tree associates	ITS	LSU
Butyriboletus									
abieticola	Arora11086	SFSU	OSC148260	D. Arora	25 Jul 2011	Lassen National Forest, CA	*Abies*	KC184411	
abieticola	Arora11087	SFSU		D. Arora	25 Jul 2011	Lassen National Forest, CA	*Abies & Pinus*	KC184412	KC184413
abieticola	Cooke38653*	SFSU		W. B. Cooke	8 Aug 1967	Mt. Shasta, CA	*Abies concolor*	KC184414	
abieticola	HDT44741	SFSU		H. Thiers	26 Aug 1982	Sierra Co., CA	conifers	KC184415	
abieticola	HDT46430	SFSU		H. Thiers	2 Oct 1983	Siskiyou Co., CA	*Abies*	KC184416	
abieticola	HDT52625	SFSU		H. Thiers	31 Aug 1989	Sierra Co., CA	conifers	KC184417	
abieticola	JLF2564	SFSU		N. Siegel	9 Nov 2011	Jedediah Smith SP, CA	conifers including *Abies*	KC184418	KC184418
abieticola	ODELL6742		OSC67698	B. Frondrick/ M. Ellestad	24 Aug 1999	Grass Mtn, Lincoln Co., OR	*Abies, Tsuga, Pseudotsuga*	KC184419	
autumniregius	ARI063		OSC148259	A. Mujic	Sep 2011	W. of Corvallis, OR	mixed conifers		KC184420
autumniregius	Arora11095	SFSU		D. Arora	29 Oct 2011	Plumas Co., CA	mixed conifers	KC184421	
autumniregius	Arora11096	SFSU	OSC148261	D. Arora	29 Oct 2011	Plumas Co., CA	mixed conifers	KC184422	
autumniregius	Arora11108	SFSU	OSC148262	W. So	14 Nov 2011	San Mateo Co., CA	mixed woods	KC184423	KC184424
autumniregius	Arora11112*	SFSU	OSC148243	D. Arora	15 Nov 2011	Mendocino Co., CA	*Pseudotsuga*	KC184425	KC184425
autumniregius	Arora11113	SFSU	OSC148263	D. Arora	15 Nov 2011	Mendocino Co., CA	*Pseudotsuga*	KC184426	
autumniregius	HDT43782	SFSU	OSC148264	H. Thiers	Oct 1981	Mendocino Co., CA	mostly *Notholithocarpus*	KC184427	
autumniregius	JDS289		OSC148265	J. Scelza	14 Oct 2010	East of Ashland, OR	mostly *Picea engelmannii*	KC184428	KC184428
autumniregius	JLF2271	SFSU	OSC148266	J.L. Frank	30 Oct 2011	Williams, OR	mixed conifers	KC184429	KC184429
autumniregius	JLF2275		OSC148267	J.L. Frank	30 Oct 2011	Williams, OR	mixed conifers	KC184430	
autumniregius	ODELL4653		OSC66191	M. Widmer	20 Oct 1997	Dorena Lake, Lane Co., OR	*Pseudotsuga*	KC184431	
brunneus	Arora11221	SFSU		D. Arora	11 Sep 2011	Dublin, NH	*Tsuga*	KC184471	KC184471
brunneus	DW75591	SFSU		D. Wasilewksi	4 Sep 2011	Rickett's Glen SP, PA	mixed woods	KC184472	
brunneus	HDT50456	SFSU		H. Thiers	15 Aug 1986	North Adams, MA	*Tsuga*	KC184473	
fechtneri	AT2003097	SFSU		A. Taylor	12 Jul 2003	Uppsala, Sweden	*Fagus sylvatica*	KC584784	
persolidus	Arora027	SFSU		F. Menge	6 Dec 2002	Santa Cruz Co., CA	mixed hardwoods		KC184440
persolidus	Arora11102	SFSU	OSC148269	P. Carpenter	30 Oct 2011	Santa Cruz Co., CA	*Quercus agrifolia*	KC184441	
persolidus	Arora11103*	SFSU	OSC148270	D. Arora	1 Nov 2011	Henry Cowell SP, CA	*Quercus agrifolia*	KC184442	KC184442
persolidus	Arora11109	SFSU	OSC148271	D. Arora	16 Nov 2011	Amador Co., CA	*Quercus, Arctostaphylos, Pinus*	KC184443	
persolidus	Arora11110	SFSU	OSC148272	D. Arora	16 Nov 2011	Amador Co., CA	*Quercus, Arctostaphylos, Pinus*	KC184444	KC184444
persolidus	Halling15	SFSU		R. Halling	21 Nov 1973	Amador Co., CA	*Quercus*	KC184445	
persolidus	HDT10943	SFSU		H. Thiers	4 Dec 1963	San Mateo Co., CA	mixed woods	KC184446	
primiregius	AHS69419	SFSU		K.A. Harrison	17 Aug 1964	Valley Co., ID	conifers	KC184447	

TABLE I. Continued

Species	Collection no.	Herbarium code		Collector	Date	Location	Tree associates	ITS	LSU
primiregius	Arora11081	SFSU	OSC148273	D. Arora	2 Jul 2011	Stanislaus National Forest, CA	conifers including *Abies*	KC184448	
primiregius	Arora11084	SFSU	OSC148274	D. Arora	1 Jul 2011	El Dorado National Forest, CA	*Abies*	KC184449	
primiregius	Arora11085	SFSU	OSC148275	D. Arora	2 Jul 2011	El Dorado National Forest, CA	conifers including *Abies*	KC184450	
primiregius	DBB00606		OSC148276	D. Bojantchev	31 May 2008	Dunsmuir, CA	*Quercus, Pseudotsuga, Abies*		KC184451
primiregius	HDT47714	SFSU		H. Thiers	11 Jun 1984	Sierra Co., CA	*Abies*	KC184452	
primiregius	JLF1973		OSC148277	J.L. Frank	28 May 2011	Dunsmuir, CA	*Abies, Pseudotsuga, Quercus*	KC184453	KC184453
primiregius	JLF2029		OSC148278	J.L. Frank	26 Jun 2011	Dunsmuir, CA	*Abies, Pseudotsuga, Quercus*	KC184454	
primiregius	JLF2030*	SFSU	OSC148279	J.L. Frank	26 Jun 2011	McCloud, CA	*Abies*	KC184455	KC184455
primiregius	JLF2097		OSC148280	L. Jeandin	11 Jul 2011	near Lost Creek Lake, OR	*Abies*	KC184456	
primiregius	JLF2139		OSC148281	J.L. Frank	9 Aug 2011	Rogue River NF, OR	conifers including *Abies*	KC184457	
pseudoregius	MG383a	SFSU		M. Gelardi	29 Oct 2010	Lazio, Italy	*Quercus, Fraxinus*	KC184458	KC184458
pseudoregius	SP613117	SFSU		S. Kelly	30 Aug 2004	Buckinghamshire, UK	*Quercus*	KC584785	
querciregius	Arora11098	SFSU	OSC148282	D. Arora	3 Nov 2011	Santa Cruz Co., CA	*Quercus agrifolia*	KC184459	
querciregius	Arora11099	SFSU	OSC148283	F. Menge	3 Nov 2011	Santa Cruz Co., CA	*Quercus agrifolia*	KC184460	
querciregius	Arora11100*	SFSU	OSC148284	F. Menge	31 Oct 2011	Santa Cruz Co., CA	*Quercus agrifolia*	KC184461	KC184461
querciregius	Arora12012	SFSU	OSC148285	N. Janson	18 Nov 2012	Santa Rosa, CA	*Quercus agrifolia, Q. lobata*	KC584788	
querciregius	01MWB052012		OSC148286	M. Beug	20 May 2012	Klickitat Co., WA	*Quercus garryana*	KC584786	
querciregius	01MWB120512		OSC148287	M. Beug	5 Dec 2012	Klickitat Co., WA	*Quercus garryana*	KC584787	
regius	MG407a	SFSU		M. Gelardi	16 Jun 2011	Lazio, Italy	*Quercus cerris*	KC184462	KC184462
regius	MG408a	SFSU		M. Gelardi	18 Jun 2011	Lazio, Italy	*Quercus, Fagus, Castanea*	KC584789	KC584790
regius	PN40600	SFSU		P. Nedelev	16 Jun 2011	Kostinbrod, Bulgaria	*Quercus*	KC184463	
roseoflavus	Arora977	SFSU		D. Arora	20 Jul 1997	Lu Feng, Yunnan	mixed woods with pine	KC184432	
roseoflavus	Arora11053	SFSU	OSC148268	D. Arora	23 May 2011	Lu Feng, Yunnan	mixed woods	KC184433	KC184433
roseoflavus	Arora11054	SFSU		D. Arora	23 May 2011	Lu Feng, Yunnan	mixed woods	KC184434	KC184435
roseoflavus	Arora11058	SFSU		D. Arora	23 May 2011	Lu Feng, Yunnan	mixed woods	KC184436	KC184436
roseoflavus	Arora11076	SFSU		D. Arora	13 Jun 2011	Dali, Yunnan	*Pinus yunnanensis*	KC184437	
roseoflavus	Arora11077	SFSU		D. Arora	13 Jun 2011	Dali, Yunnan	mixed woods	KC184438	
roseoflavus	Trappe19504		OSC59355	D. Arora	19 Jul 1996	Yunnan	market	KC184439	
sanicibus	Arora99211*	SFSU		D. Arora	4 Jul 1999	Shilin, Yunnan	Mixed woods	KC184469	KC184470
subappendiculatus	AT2010197	SFSU		A. Taylor	14 Aug 2010	Scotland	*Quercus*	KC584791	KC584791
yicibus	Arora9727*	SFSU		D. Arora	3 Aug 1997	Lijiang, Yunnan	*Pinus yunnanensis*	KC184474	KC184475

TABLE I. Continued

Species	Collection no.	Herbarium code	Collector	Date	Location	Tree associates	ITS	LSU
Other spp. sequenced								
Boletus auripes	Arora11224	SFSU	D. Arora	20 Sep 2011	East Haddam, CT	mixed woods with oaks	KC184476	KC184477
Boletus auripes	DW73672	SFSU	D. Wasilewski	15 Aug 2011	Luzerne Co., PA	*Quercus*	KC184478	
Boletus barrowsii	Arora12009	SFSU	W. So	10 Aug 2012	New Mexico	conifers	KC184479	KC184480
Boletus edulis	Arora11223	SFSU	D. Arora	15 Sep 2011	Athol, MA	*Tsuga*	KC184481	
Boletus frostii	JLF2548	SFSU	J.L. Frank	16 Aug 2012	Holderness, NH	mixed woods	KC812303	
B.edulis var. *grandedulis*	Arora12011	SFSU	D. Arora	20 Nov 2006	Mendocino Co., CA	*Pinus*		KC184482
Boletus mirabilis	JLF2235	SFSU	J.L. Frank	21 Oct 2011	Marion Co., OR	conifers	KC184483	
Boletus regineus	JLF2273	SFSU	J.L. Frank	30 Oct 2011	Williams, OR	mixed conifers with *Arbutus*	KC184484	KC184485
Boletus rex-veris	JLF2012	SFSU	J.L. Frank	19 Jun 2011	McCloud, CA	*Abies*	KC184486	
Boletus roseopurpureus	JLF2565	SFSU	N. Siegel	9 Aug 2012	near Bonden, WV	*Quercus, Fagus, Betula*	KC184464	KC184465
Boletus roseopurpureus	JLF2566	SFSU	N. Siegel	10 Aug 2012	Cooper Rock SF, WV	mixed woods	KC184466	KC184467
Boletus roseopurpureus	JLF2567	SFSU	N. Siegel	16 Aug 2012	Devil's Hopyard SF, CT	mixed woods		KC184468
Boletus rubripes	JLF2078	SFSU	J.L. Frank	12 Jul 2011	Klamath Co., OR	conifers	KC184487	
Boletus smithii	JLF2240	SFSU	J.L. Frank	20 Oct 2011	Marion Co., OR	conifers	KC184488	KC184489

* Holotype.

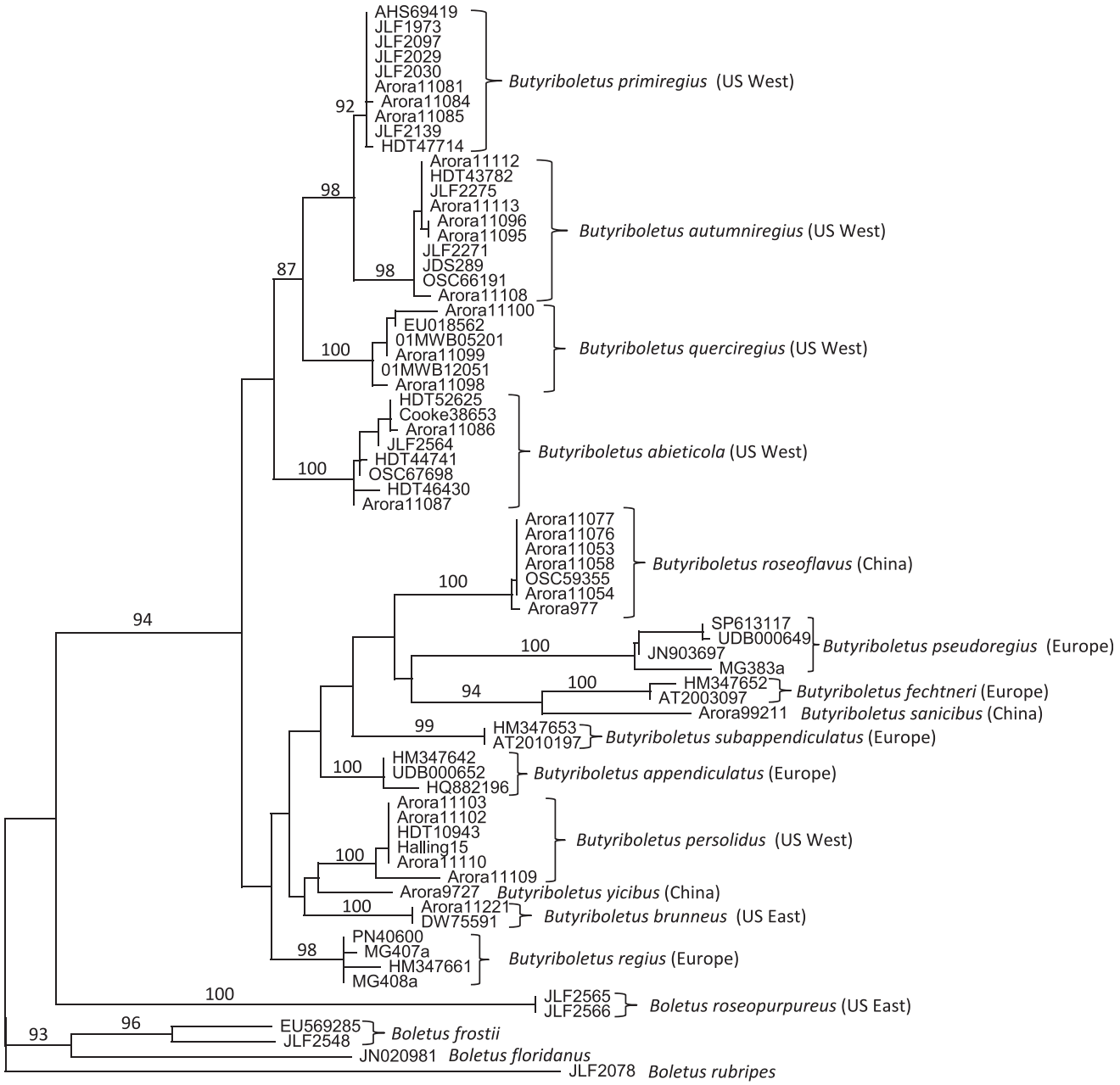

FIG. 1. ML tree illustrating phylogenetic relationships of species in the new genus *Butyriboletus* inferred from ITS nrDNA using fastDNAml. Tree topology corresponds to the optimal ML tree. Bootstrap values (> 50%) from the parsimony analysis (1000 replicates) using PAUP* are above branches.

Butyriboletus roseoflavus (Hai B. Li & Hai L.Wei) D. Arora & J.L. Frank., comb. nov.
MycoBank MB804788
≡ *Boletus roseoflavus Hai B. Li & Hai L. Wei*, Mycol Prog 2013.01.24

Butyriboletus subappendiculatus (Dermek, Lazebnicek & Veselsky) D. Arora & J. L. Frank, comb. nov.
MycoBank MB803326
≡ *Boletus subappendiculatus* Dermek, Lazebn. & J. Veselský, Fungorum Rariorum Icones Coloratae 9:13. 1979.

Although Assyov (2012) published *Boletus fuscoroseus* Smotlacha as an earlier synonym for *B. pseudoregius* Hubert ex Estadès, we recombined the latter name because it is better understood. It is likely that other *Boletus* species (e.g. *Boletus pulchriceps* E.E. Both, A.E. Bessette & R. Chapman) will be transferred to *Butyriboletus* (see Nuhn et al. 2013).

The following six species are newly described; regional identification keys are provided after the descriptions.

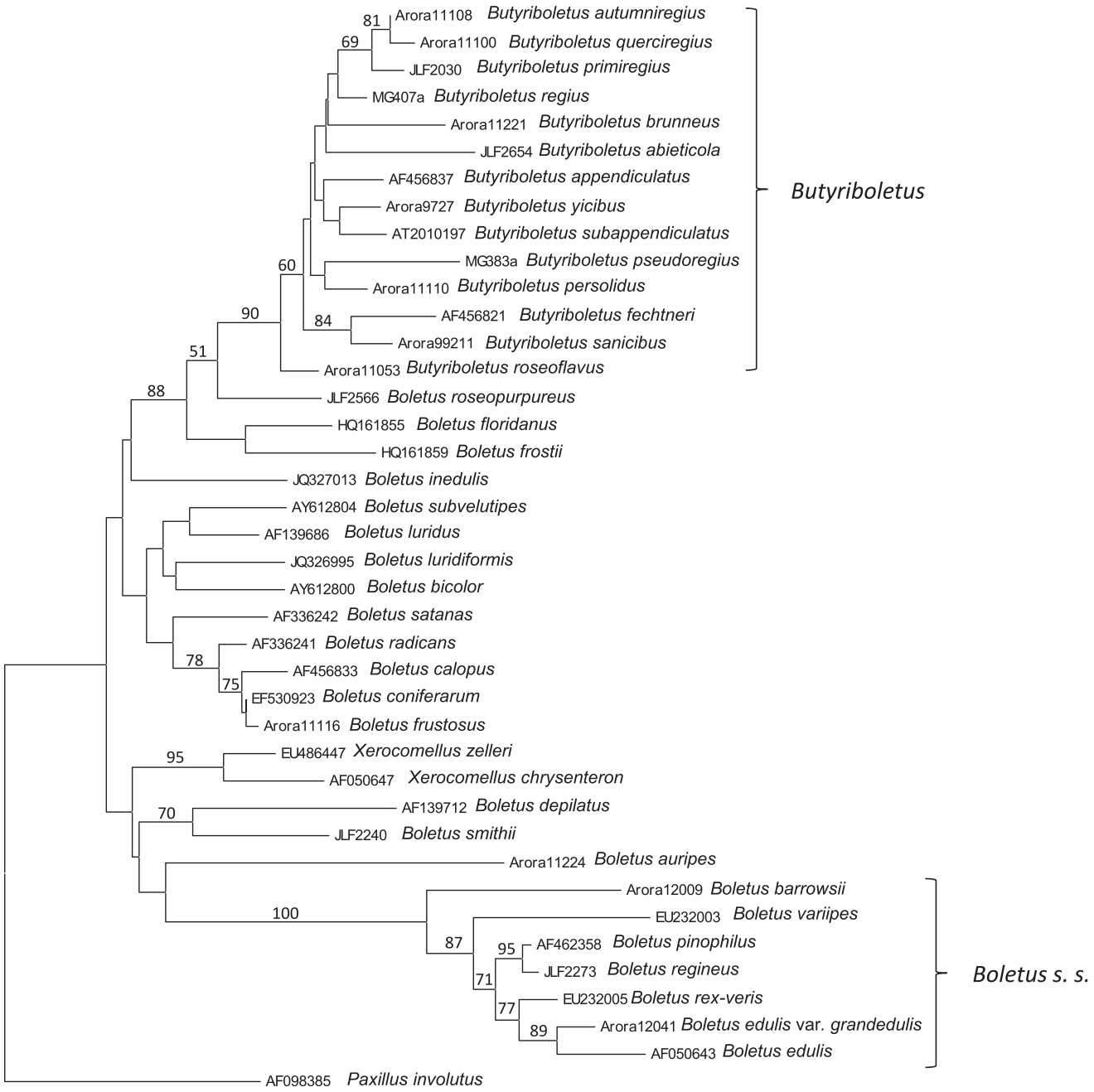

FIG. 2. Maximum likelihood tree generated in MEGA 5 with LSU nrDNA, showing the new genus *Butyriboletus* separate from the core group of *Boletus* s. str. The tree with the highest log likelihood is shown. Bootstrap values (> 50%) from the parsimony analysis (1000 replicates) using PAUP* are above branches.

Butyriboletus persolidus D.Arora & J.L. Frank, sp. nov. FIG. 3A

Medium-sized to large basidiocarps with brown pileus, yellow tube layer that usually turns blue when bruised, yellow reticulate stalk and firm, mild-tasting flesh that may turn blue erratically when exposed. Spores fusoid. Fruiting with oaks, typically in the fall. MycoBank MB803208

Holotype: USA, California, Santa Cruz County, Henry Cowell Redwoods State Park, 37.02618, −122.03884, in a nearly pure stand of *Quercus agrifolia*, 1 Nov 2011, *Arora11103* (SFSU).

= *Boletus appendiculatus* sensu Thiers, California Mushrooms: A Field Guide to the Boletes: 41 (1975)

FIG. 3. A. *Butyriboletus persolidus* (Arora11103). B. *Bu. primiregius* (Arora11084). C. *Bu. autumniregius* (Arora11095). D. *Bu. querciregius* (Arora11100). E. *Bu. querciregius* (Arora11098). F. *Bu. sanicibus* (Arora99211). G. *Bu. yicibus* (Arora9727).

FIG. 4. A. *Butyriboletus primiregius* in California market. B. *Bu. abieticola* with distinctive fibrillose scales (JLF2564, photo by Noah Siegel). C. Yi women with *Bu. yicibus* and *Lactarius* sp. D. *Bu. roseoflavus* on Kunming street, Yunnan, China.

Additional photos: Arora (1986): color plate 134; Arora (1991):163.

Etymology: per (L.) = very; *solidus* (L.) = solid or dense.

Pileus 8–25(30) cm broad, convex becoming broadly so at maturity or sometimes plane; surface dry or sightly tacky to the touch but not viscid, glabrous but typically with a fine, pallid bloom when young; mostly tan to brown, but at times cinnamon-brown and at other times yellowish brown to buff, sometimes with darker rusty brown blushes. Context firm and dense, thick, pale yellow, typically unchanging in the button stage but with age usually turning blue at least somewhat when cut (often slowly and erratically but more dramatically in older specimens); flavor mild.

Hymenophore (tube layer) pale yellow becoming bright yellow and eventually olive yellow; pore surface usually concolorous with the tubes (rarely slightly

reddish), typically turning blue quickly when bruised except in the button stage; tubes at first short in relation to the pileus context, but 1–2 cm or longer when mature; individual pores typically less than 1 mm broad.

Stipe 5–15(25) cm long, 3–7 cm thick at the apex, usually clavate or bulbous when young but often equal or with a slightly tapered base when mature; surface butter-yellow to bright yellow throughout, although sometimes also with brownish or reddish stains especially downward; finely reticulate over at least the upper portion, the reticulation yellow but often becoming brownish after spores are shed. Context firm, yellow except for the base, which usually has a distinct vinaceous tinge, turning blue only slightly or not at all when cut.

Spores dark olive brown in mass, 12–15 × 4–5 µm (Q = 3.2, n = 30), subfusoid, thin-walled, smooth, pale brown or yellowish in KOH. Basidia clavate, four-spored; hymenial cystidia fusoid-ventricose. Pileus cuticle an interwoven trichodermium with few if any erect hyphal tips.

Specimens examined: USA, CALIFORNIA: Santa Cruz County, Henry Cowell Redwoods State Park, in a nearly pure stand of *Quercus agrifolia*, 1 Nov 2011, *Arora11103* (Holotype: SFSU, Isotype: OSC148241). Santa Cruz County: *Arora027* (SFSU, OSC), *Arora11101* (SFSU), *Arora11102* (SFSU, OSC); Amador County, *Arora11109* (SFSU, OSC), *Arora11110* (SFSU, OSC); San Mateo County, *Halling15* (SFSU), *HDT10943* (SFSU).

Occurence: Solitary or in groups in soil under hardwoods or in mixed forests, usually associated with live oak (e.g. *Quercus agrifolia*), probably also with other fagaceous trees (e.g. *Notholithocarpus densiflorus*, *Chrysolepis chrysophylla*); typically fruiting Oct–Dec and sometimes prolific after the first fall rains. So far verified only from California, occurring in both the coastal region and in the Sierra Nevada foothills. Reports of *Boletus appendiculatus* sensu Thiers under conifers in the Pacific Northwest (e.g. Tylutki 1987) appear to be misidentifications based on *Butyriboletus abieticola*, *Bu. primiregius* and/or *Bu. autumniregius*. However, reports of *Boletus appendiculatus* with *Q. garryana* on Vancouver Island, British Columbia, are likely based on either this species and/or *Bu. querciregius*.

Comments: This species is remarkable even among butter boletes for its thick, dense flesh. The cap sometimes has rusty brown areas but lacks the rose, pink or vinaceous tints of other California butter boletes. It is most likely to be mistaken for *Bu. querciregius*, which also is associated with oaks. The latter has a distinctly reddish pileus when fresh but faded specimens can approach color of *Bu. persolidus*. *Bu. querciregius*, however, tends to be softer than *Bu. persolidus* and is much more likely to have blushes of red on the stipe (although not consistently). Also, its pileus is often blotched with yellow or pink while that of *Bu. persolidus* tends to remain evenly brown.

Butyriboletus primiregius D. Arora & J.L. Frank, sp. nov. FIGS. 3B, 4A

Medium-sized to large basidiocarps with reddish or vinaceous pileus fading to brown or olive, yellow tube layer that usually turns blue when bruised, yellow reticulate stalk and firm, mild-flavored flesh that may turn blue erratically when exposed. Spores fusoid. Fruiting under mountain conifers, typically in late spring.

MycoBank MB803209

Holotype: USA, California, Siskiyou County: 5 mi E. of McCloud, 41.28788, −122.07308, under *Abies*, 26 Jun 2011, *JLF2030* (SFSU).

Etymology: primi (L.) = first, because it is the first of the butter boletes to appear each year in western USA mountains; *regius* (L.) = kingly, a reflection of its market name (king butter bolete) but also referring to its European relative, *B. regius*.

Pileus 8–25(30) cm broad, convex, becoming broadly convex at maturity or at times plane or undulating; surface dry or sightly tacky to the touch but not viscid; glabrous or breaking up to form many scales (but these much smaller than in *Bu. abieticola*); color at first wholly or mostly dark red or vinaceous red (but color often obscured by a layer of brown dust), often developing dingy brown, tan, ocher-brown or dingy olive brown tones as it ages or is exposed to light; the vinaceous red tints sometimes disappearing entirely or more often remaining visible at least near the margin. Context firm and dense, thick, pale yellow, typically unchanging in the button stage but at maturity usually turning blue at least somewhat when cut (often slowly and erratically and most pronounced just above the tube layer); flavor mild.

Hymenophore (tube layer) pale yellow becoming bright yellow and eventually olive yellow or greenish; pore surface usually concolorous with the tubes, typically turning blue quickly when bruised except in the button stage; tubes at first short in relation to the pileus context, but 1–2 cm or longer when mature; individual pores typically less than 1 mm broad.

Stipe 5–15(25) cm long, 2–7 cm thick at the apex, usually clavate or bulbous when young but often becoming more or less equal with age; surface butter yellow to bright yellow throughout or commonly with dark red (rhubarb red) stains toward the base, often bruising blue when touched or rubbed; finely reticulate over at least the upper portion, the reticulation yellow but often becoming brownish after spores are shed, rarely reddish; base often terminating in one or more rootlike processes originating deep in the soil. Context firm, yellow except for the

base, which usually has a distinct vinaceous tinge, not turning blue or turning blue only slightly when cut but larval tunnels often rhubarb red.

Spores dark olive brown in mass, (12)13–17(18) × 3.5–5 μm (Q = 3.5, n = 30); fusoid, thin-walled, smooth, pale brown or yellowish in KOH. Basidia clavate, four-spored; hymenial cystidia fusoid-ventricose. Pileus cuticle an interwoven trichodermium with few if any erect hyphal tips.

Specimens examined: USA, CALIFORNIA: Siskiyou County, 5 mi E. of McCloud, under *Abies*, 26 Jun 2011, *JLF2030* (Holotype: SFSU, Isotype: OSC148242). El Dorado County, *Arora11081* (SFSU, OSC), *Arora11084* (SFSU, OSC), *Arora11085* (SFSU, OSC); Sierra County, *HDT47714* (SFSU); Siskiyou County, *DBB00606* (OSC), *JLF1973* (OSC), *JLF2029* (OSC); IDAHO: Pearl Creek, Upper Payette Lake, Valley County, *AHS69419* (SFSU); OREGON: Jackson County, *JLF2097* (OSC), *JLF2139* (OSC).

Occurence: Solitary or in groups in soil under conifers, fruiting in the late spring and early summer (May–Jul). It is associated primarily with *Abies* spp. at 4000–7000 feet, but DBB00606 was found at 2800 feet in an oak forest with scattered conifers. It is the first of the butter boletes to appear each year, typically fruiting close on the heels of *Boletus rex-veris* D. Arora & Simonini and at least 2 wk before *Butyriboletus abieticola* (Thiers) D. Arora & J.L. Frank. It is common in the Sierra Nevada of California and the Mount Shasta region and also occurs in the mountains of Oregon and Idaho. It is absent from the coastal region as far as is known.

Comments: This species can be distinguished from close relatives by its late spring fruiting season and the pronounced tendency of the cap to fade to dingy olive brown or paler when it is exposed to sunlight. The scales on the cap, if present, are smaller than in *Bu. abieticola* and not obviously fibrillose. In the Mount Shasta area of northern California, where the type collection was made, this species is harvested commercially for sale to select markets and restaurants in USA, usually under the name King Butter Bolete (FIG. 4A).

Butyriboletus autumniregius D. Arora & J.L. Frank, sp. nov. FIG. 3C

Medium-sized to large basidiocarps with reddish to vinaceous pileus, yellow tube layer that usually turns blue when bruised, yellow reticulate stalk and firm, mild-flavored flesh that may turn blue erratically when exposed. Spores fusoid. Fruiting in the fall in mixed woods and under conifers.

MycoBank MB803210

Holotype: USA, California: Mendocino County, Anchor Bay, 38.80983, −123.5693, in a forest of Douglas-fir (*Pseudotsuga menziesii*) and redwood, 15 Nov 2011, *Arora11112* (SFSU).

Etymology: autumni (L.) = autumn, for its autumn fruiting habit; *regius* (L.) = kingly, and also refers to its European relative, *B. regius*.

Pileus 8–30 cm broad, convex becoming broadly convex at maturity or sometimes plane; surface dry or sightly tacky to the touch but not viscid; glabrous or breaking up to form many small scales (but these typically smaller than in *Bu. abieticola*); color wholly or mostly rose red to vinaceous red or at times mottled with tan or yellow. Context firm and dense, thick, pale yellow, typically unchanging in the button stage but in age usually turning blue at least somewhat when cut (often slowly and erratically); flavor mild.

Hymenophore (tube layer) pale yellow becoming bright yellow and eventually olive yellow or greenish; pore surface usually concolorous with the tubes, typically turning blue quickly when bruised except in the button stage; tubes at first short in relation to the pileus context but 1–2.5 cm or longer when mature; individual pores typically less than 1 mm broad.

Stipe 5–15(25) cm long, 3–7 cm thick at the apex, usually clavate and sometimes markedly bulbous when young but often less so (at times equal) with age; surface butter yellow to bright yellow throughout or commonly with dark red (rhubarb red) stains toward the base, often bruising blue when touched or rubbed; finely reticulate over at least the upper portion, the reticulation yellow or occasionally reddish but often becoming brown after spores are shed. Context firm, pale yellow except for the base, which usually has a distinct vinaceous tinge, turning blue slightly or not at all when cut.

Spores dark olive brown in mass, fusoid, thin-walled, smooth, pale brown or yellowish in KOH, (12)13–16.5(18) × 4–5 μm (Q = 3.5, n = 30). Basidia clavate, four-spored; hymenial cystidia fusoid-ventricose. Pileus cuticle an interwoven trichodermium with few erect hyphal tips.

Specimens examined: USA, CALIFORNIA: Mendocino County, near Anchor Bay in a forest of Douglas-fir (*Pseudotsuga menziesii*) and redwood, 15 Nov 2011, *Arora11112* (Holotype: SFSU, Isotype: OSC148243). Humboldt County, *Arora11114* (SFSU); Mendocino County, *Arora11113* (SFSU, OSC), *HDT43782* (SFSU, OSC); Plumas County, *Arora11095* (SFSU, OSC), *Arora11096* (SFSU, OSC); San Mateo County, *Arora11108* (SFSU, OSC). OREGON: Benton County: *ARI063* (OSC); Jackson County, *JDS289* (OSC); Josephine County, *JLF2271* (SFSU, OSC), *JLF2275* (OSC); Lane County, *OSC66191* (OSC).

Occurence: Solitary or in groups on ground in coastal forests as well as mid-elevation forests of the Sierra Nevada and Cascades; associated with conifers and possibly also with hardwoods. Most of our

collections were made in mixed woods, making identification of the mycorrhizal host speculative, but the type collection likely was associated with Douglas-fir (*Pseudotsuga menziesii*) in that there were no other ectomycorrhizal tree hosts in the area; also, this species has been observed in pure stands of second-growth Douglas-fir. Other possible but unverified tree hosts for this species (based on other collections) include spruce (*Picea engelmannii*), tanoak (*Notholithocarpus densiflorus*), madrone (*Arbutus menziesii*), fir (*Abies* spp.) and pine (*Pinus* spp.). Fruiting generally in the fall (Sep–Dec). This species is fairly common from the central California coastal region northward and also at low to mid-elevations in the Sierra Nevada, Siskiyous and Cascades; its northern limit has not been determined but at least one of its hosts, Douglas-fir, extends into British Columbia, Canada.

Comments: This species is best distinguished from *Bu. primiregius* by its fall fruiting season. Its cap does not tend to fade as much as that species but this may be a function of growing in more humid conditions than any inherent difference in pigment stability. From *Bu. querciregius* it differs in habitat (it has not been found to form mycorrhizal associations with *Quercus*) and more vivid pileus color (tending toward deep rose red or vinaceous red when fresh rather than pinkish red, pinkish brown or yellowish) and in its longer spores with larger Q ratio.

Butyriboletus querciregius D. Arora & J.L. Frank, sp. nov. FIG. 3D, E

Medium-sized to large basidiocarps with reddish or yellow-tinged pileus, yellow tube layer that usually turns blue when bruised, yellow reticulate stalk and firm, mild-flavored flesh that may turn blue erratically when exposed. Spores short-fusoid. Fruiting in fall with oaks.

MycoBank MB803211

Holotype: USA, California, Santa Cruz County: near Aptos, 36.96860, −121.84540, under coast live oak (*Quercus agrifolia*), 31 Oct 2011, *Arora11100* (SFSU).

Additional photos: Arora (1986): color plate 133 (as *Boletus regius*).

Etymology: querci (L.) = pertaining to oak; *regius* (L.) = kingly, and also refers to its European relative, *B. regius*.

Pileus 6–20(25) cm broad, convex becoming broadly convex at maturity or sometimes plane; surface dry or sightly tacky to the touch but not viscid; glabrous or occasionally breaking up to form some small scales; reddish or dull red when young and fresh or yellow overlain with red (i.e. reddish orange overall with more yellow at the margin) or red blotched with yellow but often fading to pale reddish brown, pinkish tan, yellow or yellowish buff with age or where exposed to light. Context firm, thick, pale yellow, typically unchanging in the button stage but with age usually turning blue at least somewhat when cut; flavor mild.

Hymenophore (tube layer) pale yellow becoming bright yellow and eventually olive yellow or greenish; pore surface usually concolorous with the tubes, typically turning blue quickly when bruised except in the button stage; tubes at first short in relation to the pileus context but 1–2 cm or longer when mature; individual pores typically less than 1 mm broad.

Stipe 5−15(20) cm long, 2–6 cm thick at the apex, usually clavate or slightly bulbous when young but often more or less equal in age; surface butter yellow to bright yellow throughout or with beautiful red to dark red blushes toward the base, sometimes bruising blue when pressed but variable in this respect; finely reticulate over at least the upper portion, the reticulation yellow or occasionally reddish but often becoming brown after spores are shed. Context firm, pale yellow except for the base, which usually has a distinct vinaceous tinge, turning blue little if at all when cut.

Spores dark olive brown in mass, fusoid, thin-walled, smooth, pale brown or yellowish in KOH, (10)11–15(16) × 3.5–5 μm (Q = 3.0, n = 30). Basidia clavate, four–spored; hymenial cystidia mostly fusoid-ventricose. Pileus cuticle an interwoven trichodermium with few erect hyphal tips.

Specimens examined: USA, CALIFORNIA: Santa Cruz County, near Aptos under coast live oak (*Quercus agrifolia*), coll. by F. Menge, 31 Oct 2011, *Arora11100* (Holotype: SFSU, Isotype: OSC148244). Santa Cruz County, *Arora11097* (SFSU), *Arora11098* (SFSU, OSC), *Arora11099* (SFSU, OSC), *Arora11106* (SFSU). Sonoma County, Santa Rosa, *Arora12012* (SFSU). WASHINGTON: Klickitat County, *01MWB052012* (OSC), *01MWB120512* (OSC).

Occurence: Solitary or in groups in leaf litter under oaks (mainly *Q. agrifolia* and *Q. garryana*, less commonly with *Q. lobata* and other *Quercus* spp.). It fruits mostly in the fall (Sep–Dec), but the first of the Washington collections listed above was made in May and DA has found it in coastal California in June. It is possible that this species also occurs with tanoak, but such a relationship has not been verified. It is common after early fall rains in the oak woodlands of California, infrequent northward to Washington and possibly British Columbia. We were able to identify an ITS DNA sequence in GenBank (EU018562) of an ectomycorrhizal root tip from *Q. garryana* in Oregon as *Bu. querciregius* (FIG. 1); its basic ectomycorrhizal morphology is tan, monopodial and tortuous (Moser et al. 2009). The distribution and tree host preferences of this species appear to

closely parallel those of the oak-associated population of *Boletus barrowsii* Thiers & Smith (Arora 2008).

Comments: This is the third species, along with *Bu. primiregius* and *Bu. autumniregius*, encompassed by Thiers' (1975) concept of *Boletus regius*. The latter species, however, turns blue only rarely when cut and has not been verified to occur in North America. *Bu. querciregius* is most likely to be confused with *Bu. autumniregius* in that both species fruit in the fall and their ranges overlap. *Bu. querciregius* differs in its mycorrhizal host, shorter spores with smaller Q ratio, and also by its different pileus color: red or dull red or even orange-red, sometimes blotched with yellow before fading to pinkish brown, tan or yellowish. *Bu. querciregius* also can be mistaken for *Bu. persolidus* (see comments under that species for a comparison). Some collections of *Bu. querciregius* feature a beautiful blush of red over the lower portion of the stipe as shown in color plate 133 of Arora (1986), but this is not a consistent character. It is worth noting that Arora11097 grew under the same tree as the specimens Arora (1986) photographed 30 y earlier.

Butyriboletus yicibus D. Arora & J.L. Frank, sp. nov. FIGS. 3F, 4C

Medium-sized basidiocarps with brown pileus, yellow tube layer that usually stains blue-gray when bruised, slender yellow reticulate stalk and firm, mild-flavored flesh that may stain blue-gray erratically when exposed. Spores fusoid or subfusoid. Fruiting in summer in pine forests and mixed woods.

MycoBank MB803256

Holotype: China, Yunnan, Lijiang County, 26.9947, 100.2524, scattered in pine forest (*Pinus yunnanensis*) on the road from Lijiang to Jade Dragon Snow Mountain, 3 Aug 1997, Arora9727 (SFSU).

Etymology: Yi = the largest ethnic minority in Yunnan; *cibus* (L.) = food.

Pileus 6–15 cm broad, with an incurved margin becoming broadly convex; surface predominantly dull brown, sometimes with reddish brown areas, glabrous, not viscid. Context thick, firm, pale yellow, turning blue slightly and erratically when cut, at least in most specimens; odor and flavor mild.

Hymenophore (tube layer) pale yellow when fresh, becoming greenish yellow with age, the pore surface typically bruising bluish (blue-gray) when rubbed, but the staining may be absent in buttons; tubes relatively short for a long time but eventually 2 cm long or more, typically depressed around the stipe; pores small (< 1 mm).

Stipe relatively long and slender, 2–4 cm thick at apex and 10–20 cm long, equal or swollen at the base, predominantly pale yellow or yellowish buff when fresh but often brownish stained below; surface often staining bluish gray when pressed or rubbed; upper portion finely reticulate. Context yellow, brighter yellow toward the base and with or without a vinaceous tinge at the extreme base; turning blue little if at all when cut.

Spores dark olive brown in mass, fusoid, thin-walled, smooth, pale brown or yellowish in KOH, (11)12–15(16) × 3.5–5 μm (Q = 3.3, n = 30). Basidia clavate, four-spored, hymenial cystidia mostly fusoid-ventricose. Pileus cuticle an interwoven trichodermium.

Specimens examined: CHINA, YUNNAN: Lijiang County, scattered in pine forest on the road from Lijiang to Jade Dragon Snow Mountain, 3 Aug 1997, *Arora9727* (Holotype: SFSU, Isotype: OSC148246).

Occurence: Scattered on ground in pine forest (*Pinus yunnanensis*); fruiting in the summer (Jul–Sep). Verified from Lijiang County, Yunnan, but observed in adjacent areas and probably more widely distributed.

Comments: This species can be distinguished from *Bu. sanicibus* by its leggy, more gracile stature and tendency to stain blue-gray rather than blue when bruised, and both species differ from *Bu. roseoflavus* in lacking red tones in the pileus. *Bu. yicibus* is eaten by the Yi minority of Lijiang and probably by other minorities such as the Naxi and Bai. DA encountered it several times while conducting research in Yunnan, but only the type collection was examined for this study.

Butyriboletus sanicibus D. Arora & J.L. Frank, sp. nov. FIG. 3G

Medium-sized to fairly large basidiocarps with brown pileus, yellow tube layer that usually turns blue when bruised, thick, yellow reticulate stalk and firm, mild-flavored flesh that usually turns blue when exposed. Spores fusoid or subfusoid. Fruiting in summer in pine forests and mixed woods.

MycoBank MB803257

Holotype: China, Yunnan, Shilin County, 24.76972, 103.34972, scattered in pine forest (*Pinus yunnanensis*) in Sani village near the Stone Forest, 4 Jul 1999, *Arora99211* (SFSU).

Etymology: Sani = an ethnic minority especially numerous to the east of Kunming, Yunnan; *cibus* (L.) = food.

Pileus 7−20 cm broad, with an incurved margin becoming broadly convex; surface predominantly dull brown, in young specimens often overlaid with a pallid sheen, not viscid. Context thick, firm, pale yellow, normally turning blue when cut; odor and flavor mild.

Hymenophore (tube layer) pale yellow becoming bright yellow, slightly greenish with age, typically bruising blue when rubbed, but the staining may be absent in buttons; tubes relatively short for a long time but eventually 2 cm long or more, typically depressed around the stipe; pores small (< 1 mm).

Stipe relatively robust, 2.5 cm or more thick, equal or somewhat clavate, typically pale yellow when fresh and turning blue when bruised (but some variation observed according to how wet or dry the mushroom is), finely reticulate at least over the upper portion. Context pale yellow, typically turning blue only slightly if at all when cut (in contrast to the pileus context), typically with a vinaceous tinge in the basal area.

Spores dark olive brown in mass, subfusoid, thin-walled, smooth, pale brown or yellowish in KOH, 11−15 × 4−5 µm (Q = 2.8, n = 30). Basidia clavate, four-spored; hymenial cystidia mostly fusoid-ventricose. Pileus cuticle an interwoven trichodermium.

Specimens examined: CHINA, YUNNAN: Shilin County, scattered in mixed forest with pines present (*Pinus yunnanensis*) outside a Sani village near the Stone Forest, 4 Jul 1999, *Arora99211* (Holotype: SFSU, Isotype: OSC148247). Lijiang County, *Arora9726*.

Occurence: Scattered on ground in pine forests (*Pinus yunnanensis*) or mixed woods; fruiting in summer (Jun–Sep). Known from Shilin and Lijiang counties in Yunnan and likely occurring through much of mid-elevation Yunnan.

Comments: This species is more robust than *Bu. yicibus* and is phenotypically reminiscent of the European species, *Bu. appendiculatus* and *Bu. fechtneri*. Indeed, it is closely related to the latter (FIGS. 1, 2). This species is commonly eaten by Sani people in Shilin County, Yunnan. It is occasionally seen in farmers' markets but not in the abundant quantities typical of the pallid- or reddish capped species, *Bu. roseoflavus*.

To enable identification in the field, separate dichotomous keys are provided for the *Butyriboletus* species of western USA and Yunnan, China. Only one species, *Bu. brunneus*, has been identified from eastern North America. For a key to European species, see Assyov (2012).

KEY TO *BUTYRIBOLETUS* SPECIES OF CALIFORNIA, OREGON, WASHINGTON AND IDAHO, USA

1. Pileus at least partly (often entirely) pink, red, rose or vinaceous when fresh (but may fade with age) 2
1. Pileus without pink, red, rose or vinaceous tones ... 5
   2. Pileus with fibrillose scales at maturity; associated with conifers, especially fir .. *Bu. abieticola*
   2. Pileus not as above (if surface breaks up into scales when mature then the scales not fibrillose); associated with hardwoods or conifers 3
3. Pileus reddish to pinkish; spores mostly 11–15 µm long with a Q ratio of 3; associated primarily if not exclusively with oaks ..... *Bu. querciregius*
3. Pileus typically vinaceous to deep rose when fresh; spores mostly 13–17 um long with a Q-ratio of 3.5; associated primarily with conifers but possibly also with oaks and tanoaks 4
   4. Fruiting in late spring-early summer (May–Jul) in mountains ..... *Bu. primiregius*
   4. Fruiting in fall (Sep–Dec) in mountains as well as lowlands ..... *Bu. autumniregius*
5. Pileus appressed fibrillose or slightly felt-like when young and breaking up into fibrillose scales by maturity; associated with conifers .... *Bu. abieticola*
5. Not as above; associated with hardwoods, especially oak 6
   6. Pileus some shade of brown; stipe typically without red tones ..... *Bu. persolidus*
   6. Pileus yellow or yellowish; stipe sometimes with red tones ..... *Bu. querciregius*

KEY TO *BUTYRIBOLETUS* SPECIES OF YUNNAN, CHINA

1. Pileus whitish to slightly yellowish to pink or dark red but not brown ..... *Bu. roseoflavus*
1. Pileus some shade of brown 2
   2. Stipe typically longer than width of pileus; pore and stipe surfaces typically staining bluish gray when bruised ..... *Bu. yicibus*
   2. Stipe typically as long as or shorter than width of pileus; pore and stipe surfaces typically staining blue or blue-green when bruised .. *Bu. sanicibus*

DISCUSSION

North American, Chinese and European butter boletes form a new monophyletic genus, *Butyriboletus*, containing at least 14 taxa (FIGS. 1, 2). In most cases these taxa can be distinguished by their geographic location, habitat, fruiting season and traditional field characters such as pileus color and staining reactions. Micromorphology is not particularly helpful in *Butyriboletus*: Taxa examined by the authors have similar basidia, hymenial cystidia, an epicuticular trichodermium and smooth, long-ellipsoid to fusoid (''boletoid'') spores.

Five species of *Butyriboletus* occur in western USA: *Bu. abieticola* Thiers plus four newly described species. Three of the newly described species (*Bu. autumniregius*, *Bu. primiregius*, *Bu. querciregius*) have a reddish pileus when fresh. Together they comprise Thiers' (1975) concept of *Boletus regius*, while the fourth new species, *Bu. persolidus*, has a brown pileus and corresponds with Thiers' (1975) concept of *Boletus appendiculatus*. Our ITS data (FIG. 1) show

that *Bu. primiregius* and *Bu. autumniregius* have the closest affinity to each other and to *Bu. querciregius*.

Three of the five western USA species are widely distributed and associated primarily with Pinaceae (*Bu. abieticola*, *Bu. autumniregius*, *Bu. primiregius*), while two associate primarily with Fagaceae (*Bu. persolidus*, *Bu. querciregius*). Of the conifer-dwelling species, *Bu. autumniregius* and *Bu. primiregius* can be distinguished by fruiting season: generally May–Jul for *Bu. primiregius* versus Sep–Dec for *Bu. autumniregius*. *Bu. abieticola* Thiers generally fruits from July until early October and may overlap temporally with either of the previous species but consistently shows fibrillose scales on the mature pileus (FIG. 4B).

In addition to growing in pure conifer stands, *Bu. autumniregius* frequently occurs in mixed forests where its ectomycorrhizal host is speculative. The oak-associated *Bu. querciregius* fruits at the same time as *Bu. autumniregius* but differs in pileus color and by shorter spores. The other oak-associated species, *Bu. persolidus*, is easily distinguished by its brown pileus.

The five classic butter boletes of Europe and northern Africa as described by Assyov (2012), *Boletus appendiculatus*, *Bu. fechtneri*, *Bu. fuscoroseus* (= *Bu. pseudoregius*), *B. regius* and *B. subappendiculatus*, were found to nest within *Butyriboletus* (FIGS. 1, 2). Our results show no overlap in the occurrence of butter bolete species among North America and Europe or China. A close affinity between *Bu. sanicibus* (China) and *Bu. fechtneri* (Europe) was supported by both ITS and LSU data (FIGS. 1, 2).

One bolete from eastern USA, *Butyriboletus brunneus* (≡ *Boletus speciosus var. brunneus* Peck), also falls clearly within the butter bolete clade. We were unable to locate any recent collections of the typical variety of *Boletus speciosus* Frost, described as having a ''scarlet lake red'' pileus (Frost 1874); therefore we raised var. *brunneus* to species rank in the new combination. Most of the *Boletus speciosus* collections from Tennessee sequenced by Li et al. (2013) have ITS data identical to that which we obtained from several collections of *Boletus roseopurpureus* Both, A.E.Bessette, & Roody (Bessette et al. 2000). *B. roseopurpureus* is an outlier to *Butyriboletus*, according to our data. Other than *B. roseopurpureus*, the closest relatives to *Butyriboletus* are *Boletus frostii* J.L. Russell and *Boletus floridanus* (Singer) Murrill, two distinctive boletes with raised reticulation on the stalk and reddish pore surface beaded with golden droplets when young. A third species from eastern USA, *Boletus auripes* Peck, was placed in sect. Appendiculati by Snell and Dick (1970) but was found to have different affinities (FIGS. 1, 2). As a result it remains in the residual pool of *Boletus* s.l. species awaiting future reassignment.

As for the two newly described species from Yunnan, both are associated primarily (although perhaps not exclusively) with pine. Both are eaten locally (FIG. 4C) and occasionally sold in farmers' markets but are not nearly as prevalent or as economically valuable as the recently described *Bu. roseoflavus* (FIG. 4D). The pileus of that species is often reddish or pinkish as described by Li et al. (2013), but in our Yunnan collections is frequently pallid as well but not brown.

Our study of the butter boletes was not intended to be exhaustive. Given the geographical breadth and great biological diversity of China it is likely that additional unrecognized species of *Butyriboletus* occur there. We did not include Japanese taxa in this study (see *Boletus regius* sensu Hongo 1960 and *B. speciosus* sensu Imazeki et al. 1988). Butter boletes also have been reported from the highlands of Mexico under the names *Boletus regius* and *B. appendiculatus* (Guzmán 1997). *Boletus pulchriceps* E.E. Both, A.E. Bessette & R. Chapman (Bessette et al. 2000), described as occurring with oak in southern Arizona, has the aspect of other butter boletes and belongs to the ''regius clade'' according to Nuhn et al. (2013). Additional unsequenced taxa from eastern North America also may belong to *Butyriboletus*.

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LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Arora D. 1986. Mushrooms demystified. 2nd ed. Berkeley, California: Ten Speed Press. 959 p.

———. 1991. All that the rain promises, and more: a hip pocket guide to western mushrooms. Berkeley, California: Ten Speed Press. 263 p.

———. 2008. California porcini: three new taxa, observations on their harvest and the tragedy of no commons. Econ Bot 62:356–375, doi:10.1007/s12231-008-9050-7

Assyov B. 2012. Revision of *Boletus* section *Appendiculati* (Boletaceae) in Bulgaria with a key to the Balkan species. Turk J Bot 36:408–419.

Bessette AE, Roody W, Bessette AR. 2000. North American Boletes. Syracuse, New York: Syracuse Univ. Press. 396 p.

Binder M, Bresinsky A. 2002. *Retiboletus*, a new genus for a species complex in the Boletaceae producing retipolides. *Feddes Repertorium* 113:30–40, doi:10.1002/1522-239X(200205)113:1/2<30::AID-FEDR30>3.0.CO;2-D

———, Hibbett DS. 2006. Molecular systematics and biological diversification of Boletales. Mycologia 98: 971–981, doi:10.3852/mycologia.98.6.971

Breitenbach J, Kranzlin F. 1991. Fungi of Switzerland. Vol. 3. Boletes and agarics. Mykologia Lucerne. 361 p.

Chiu WF. 1948. The boletes of Yunnan. Mycologia 40:199–231, doi:10.2307/3755085

Dentinger BT, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau PA, Nagasawa E, Soytong K, Taylor AF, Watling R, Moncalvo JM, McLaughlin DJ. 2010. Molecular phylogenetics of porcini mushrooms (Boletus section Boletus). Mol Phylogenet Evol 57: 1276–1292, doi:10.1016/j.ympev.2010.10.004

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376, doi:10.1007/BF01734359

Frost C. 1874. Catalogue of boleti of New England, with descriptions of new species. Bull Buffalo Soc Nat Sci 2: 100–105.

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and roots. Mol Evol 2:113–118.

Gelardi M, Vizzini A, Ercole E, Voyron S, Sun J-Z, Liu X-Z. 2013. *Boletus sinopulverulentus*, a new species from Shaanxi province (central China) and notes on *Boletus* and *Xerocomus*. Sydowia 65:45–57.

Guzmán G. 1997. Los nombres de los hongos y lo relacionado con ellos en América Latina. Mexico: Instituto de Ecología. 356 p.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Hongo T. 1960. The Agaricales of Japan 1–2: Rhodophyllaceae, Paxillaceae, Gomphidiaceae, Boletaceae and Strobilomycetaceae. Acta Phytotaxon Geobotan 18: 97–112.

Imazeki R, Otani Y, Hongo T. 1988. Fungi of Japan. Tokyo: Yama-Kei Publishers. 624 p.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30: 3059–3066, doi:10.1093/nar/gkf436

Li H, Wei H, Peng H, Ding H, Wang L, He L, Fu L. 2013. *Boletus roseoflavus*, a new species of *Boletus* section Appendiculati from China. Mycol Prog 24:1–11.

Maddison WP, Maddison DR. 2011. Mesquite 2.75: a modular system for evolutionary analysis. http://mesquiteproject.org

Marques G, Muñoz JA. 2006. Révision des espèces européennes du genre Boletus section Appendiculati. Étude sur la base de caracteres morphologique et d'analyse des polymorphismes des fragments de restriction (PCR-RFLP). Bull Trim Soc Mycol Fr 122:353–366.

McCarthy C. 1998. Chromas 1.45. Southport, Queensland, Australia: Griffith Univ.

Moser AM, Frank JL, D'Allura JA, Southworth D. 2009. Ectomycorrhizal communities of *Quercus garryana* are similar on serpentine and nonserpentine soils. Plant Soil 315:185–194, doi:10.1007/s11104-008-9743-9

Moser M. 1983. Keys to Agarics and Boleti [of Europe]. London: Roger Phillips. 535 p.

Nuhn M, Binder M, Taylor A, Halling R, Hibbett D. 2013. Phylogenetic overview of the Boletineae. Fungal Biol 117:479–511, doi:10.1016/j.funbio.2013.04.008

Olsen GJ, Matsuda H, Hagstrom R, Overbeek R. 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput Appl Biosci 10:41–48.

Rzhetsky A, Nei M. 1992. A simple method for estimating and testing minimum evolution trees. Mol Biol Evol 9: 945–967.

Singer R. 1967. Die Röhrlinge II. Die Boletoideae *und* Strobilomycetaceae. In: Die Pilze Mitteleuropas 6:1–151.

Snell W, Dick E. 1970. The Boleti of northeastern North America. Lehre, Germany: J. Cramer. 115 p.

Swofford DL. 2002. PAUP* 4.0: phylogenetic analysis using parsimony and other methods. Sunderland, Massachusetts: Sinauer Associates.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2729, doi:10.1093/molbev/msr121

Thiers HD. 1975. California mushrooms: a field guide to the Boletes. Hafner Press. 261 p.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882, doi:10.1093/nar/25.24.4876

Tylutki E. 1987. Mushrooms of Idaho and the Pacific Northwest. Vol 2. Non-gilled Hymenomycetes. Moscow: Univ. Idaho Press. 232 p.

Wang X, Liu P, Yu F. 2004. Color atlas of wild commercial mushrooms in Yunnan. Kunming, China: Yunnan Science & Technology Press. 136 p.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.