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Phylogenetic overview of the Boletineae

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ABSTRACT

The generic and sub-generic relationships in the Boletineae (Boletales) were studied using nuclear large subunit (nuc-lsu), translation elongation factor 1- α (tef1), and DNA directed RNA polymerase largest subunit (RPB1). The Boletineae, with the exclusion of *Hydnomerulius pinastri*, was strongly supported and the status of the families Boletaceae and Paxillaceae is discussed. Members of the genus *Boletus* are found throughout the phylogeny, with the majority not closely related to the type species, *Boletus edulis*. Many of the traditional, morphologically defined genera are not supported as monophyletic and additional sampling and taxonomic revisions are needed. The majority of the Boletineae are confirmed or putatively ectomycorrhizal (ECM), but two putatively mycoparasitic lineages (one lineage of *Buchwaldoboletus lignicola* and *Chalciporus piperatus* and the second *Pseudoboletus parasiticus*) are strongly supported.

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Introduction

The suborder Boletineae (originally considered Agaricales) as a taxonomic rank was first used by Gilbert (1931), and included both poroid and gilled species. This was a step forward in bolete taxonomy, the first time gilled species were included in a concept of a 'bolete'. Since then, the generic and species concepts in the Boletineae have been dominated by those proposed by Singer (1986), which follows the inclusion of gilled species proposed by Gilbert (1931) and Smith & Thiers (1971). The broad outlines of the 'modern' Boletineae, based on multi-locus phylogenetic analyses, were presented by Binder &

Hibbett (2006). The generic-level classifications of Smith & Thiers (1971) and Singer (1986) along with current genera of Boletineae are presented in Table 1. The systems of Smith & Thiers (1971) and Singer (1986) mainly used morphological characters and chemical staining reactions, e.g. the colours produced by placing KOH on the pileipellis, to define genera. Singer (1986) also incorporated the results of chemotaxonomic studies, which identified pigments responsible for colouration and staining reactions. Chemotaxonomic data (Besl et al. 1974, 1986; Besl & Bresinsky 1977, 1979, 1997; Steglich et al. 1977; Bresinsky & Besl 1978) were not available at the time of Smith and Thiers's (1971) work.

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Table 1 – Generic concepts of the Boletineae sensu Smith & Thiers, Singer, and Current.

Boletaceae of Smith & Thiers (1971)	Boletineae of Singer (1986)	Current
Agaricales	Agaricales	Boletales
Boletaceae	Agaricaceae	Boletineae
Boletellus	Incertae sedis	Boletaceae
Boletus ^a	Notholepiota	Afroboletus
Fuscoboletinus	Boletineae	Aureoboletus
Gastroboletus	Boletaceae	Austroboletus
Gyroporus ^b	Boletoidae	Australopilus
Leccinum ^c	Austroboletus	Boletellus
Pulveroboletus	Boletellus	Boletochaete ^f
Tylopilus ^{c,d}	Boletochaete	Boletus
Strobilomyces	Boletus ^a	Borofutus
Suillus ^e	Chalciporus ^a	Bothia
Paxillaceae	Fistulinella	Buchwaldoboletus
Gyrodon	Gastroboletus	Chalciporus
Phylloporus	Leccinum ^c	Chamonixia
	Phylloboletellus	Fistulinella
	Porphyrellus ^d	Gastroboletus
	Pulveroboletus	Gastroleccinum ^f
	Tylopilus ^d	Harrya
	Veloporphyrillus	Heimioporus ^f
	Xanthoconium ^a	Heliogaster
	Gyrodontoideae	Hemileccinum
	Gyrodon	Leccinellum
	Paragyrodon	Leccinum
	Meiogramum	Mycoamaranthus ^f
	Strobilomyceloideae	Notholepiota
	Strobilomyces	Paxillogaster ^f
	Xerocomoideae	Phylloboletellus
	Phylloporus	Phyllobolites
	Tubosaeta	Phylloporus
	Xerocomus ^a	Porphyrellus
	Paxillaceae	Pseudoboletus
	Paxillus	Pulveroboletus
		Retiboletus
		Rhodactina
		Rosbeevera
		Royoungia
		Rubinoboletus
		Sinoboletus ^f
		Spongiforma
		Strobilomyces
		Sutorius
		Tubosaeta
		Tylopilus
		Xanthoconium
		Xerocomellus
		Xerocomus
		Zangia
		Paxillaceae
		Alpova
		Austrogaster ^f
		Gyrodon
		Hoehnelogaster ^f
		Meiogramum ^f
		Melanogaster
		Paragyrodon
		Paxillus

Table 1 – (continued)

Boletaceae of Smith & Thiers (1971)	Boletineae of Singer (1986)	Current
		<i>Incertae sedis</i> <i>Hydnomerulius</i>
a Species of <i>Boletus</i> sensu Smith & Thiers (1971) are distributed across four genera of Singer (1986).		
b Currently classified in <i>Sclerodermatineae</i> .		
c Species of <i>Leccinum</i> sensu Singer are distributed across <i>Leccinum</i> and <i>Tylopilus</i> (pro parte) by Smith & Thiers (1971).		
d Species of <i>Tylopilus</i> sensu Smith & Thiers (1971) are distributed across <i>Tylopilus</i> and <i>Porphyrellus</i> sensu Singer (1986). Single Smith and Thiers.		
e Currently classified in <i>Suillineae</i> .		
f No molecular data available.		

Morphological characters used to delimit genera and species in Boletineae include, but are not limited to: stipe ornamentation, pileipellis and stipitipellis structures, pore surface colour, pore depth, pore mouth diameter, staining reactions of bruised tissues, and staining reactions of different tissues (such as pileus context, stipe context, pileipellis, stipitipellis) to chemicals, typically KOH, 5 % ammonia solution, and FeSO₄. An overview of the presence, absence, and states of key morphological characters of the genera of Boletineae is presented in Table 2. Chemical analysis of pigment production in the boletes has also been used as a taxonomic character and allowed the placement of species not previously thought to be closely related to the boletes, e.g. *Chamonixia* (Boletineae) and *Coniophora* (Coniophorineae), and further strengthened the separation of *Suillus* (Suillineae) from *Boletus* (Steglich et al. 1977; Besl et al. 1986; Besl & Bresinsky 1997).

Smith & Thiers (1971) placed only poroid fungi in the Boletaceae and included members of the modern Suillineae and Sclerodermatineae (Besl & Bresinsky 1997; Jarosch 2001; Binder & Bresinsky 2002a). The modern members of the Boletineae (Table 1) that are gilled, Paxillus and Phylloporus, were placed in the Paxillaceae (Smith & Thiers 1971). Singer's (1986) concept of the Boletineae is almost identical to the modern Boletales (Singer 1986; Binder & Hibbett 2006). The modern Boletineae members are distributed in the Paxillaceae and Boletaceae in Singer's (1986) classification. However, only one genus of Singer's Paxillaceae, Paxillus s.str. (not including Tapinella or Austropaxillus [Tapinellineae]), is included in the modern Boletineae (Binder & Hibbett 2006).

Smith and Thiers were more conservative than Singer when considering whether differences between morphological features warranted a separate genus (Smith & Thiers 1971; Singer 1986). This led Smith and Thiers to 'lump' species into larger genera than those recognized by Singer, except for the genus *Leccinum* (Smith & Thiers 1971; Singer 1986). Overall, Singer recognized 22 genera (not including families that have no modern representatives) of Boletineae and Smith and Thiers recognized 12 genera (including genera in the modern Boletineae that Smith and Thiers placed outside the Boletaceae), including *Suillus* (Table 1). However, Smith and Thiers placed *Paragyrodon* as a section of *Suillus* and stated that *Gyrodon*

lividus was most closely related to *Suillus*; in fact, both *Paragyrododon* and *G. lividus* are members of the modern Boletineae (Paxillineae in Binder & Hibbett 2006) (see Table 1; Smith & Thiers 1971; Singer 1986; Binder & Hibbett 2006).

Some aspects of generic limits and inter-generic relationships in Boletineae have remained unclear. This is due, in part, to alternative interpretations of relatedness based on morphology (as well as lack of resolution in molecular studies). An example is provided by *Boletus*, which Smith and Thiers regarded as 'the most distinctive genus in the family'. However, Smith and Thiers's concept of *Boletus* encompasses multiple genera recognized by Singer, e.g. *Aureoboletus*, *Xerocomus*, *Xanthoconium* (Smith & Thiers 1971; Singer 1986).

Analyses of chemical characters (pigments and colourless compounds that are produced via secondary metabolisms) have been useful for separating large groups, or genera, but overall have been unable to resolve intergeneric relationships among the Boletineae (Besl & Bresinsky 1977, 1979, 1997; Besl et al. 1986; Besl et al. 1974; Binder & Hibbett 2006; Bresinsky & Besl 1978; Steglich et al. 1977). Analyses of DNA sequences have also been informative, but the molecular studies of the last 15 y have failed to resolve generic relationships in the Boletineae and have often focused on only one or a few genera at a time (Binder & Bresinsky 2002b; Binder & Hibbett 2002; Desjardin et al. 2008, 2009; Dentinger et al. 2010; Vizzini et al. 2010; Lebel et al. 2011; Halling et al. 2012a). As more taxa have been sampled in molecular studies, it has become clear that some morphology-based generic concepts do not correspond to monophyletic groups, especially in the larger genera, such as *Boletellus*, *Boletus*, *Tylopilus*, *Xerocomellus*, and *Xerocomus*, but there has been no comprehensive phylogenetic analysis of all the genera of Boletineae. Instead, many studies have focused on finding limits of individual genera or placing species into genera (Binder & Besl 2000; Binder & Bresinsky 2002b; Desjardin et al. 2008, 2009, 2011; Dentinger et al. 2010; Li et al. 2011; Halling et al. 2012a, b; Hosen et al. 2012).

Most previous molecular studies used the nuclear large subunit (nuc-lsu), which has been shown to have little resolving power in the Boletineae when used in isolation (Binder & Hibbett 2006). Continued use of nuc-lsu maintains compatibility between studies and allows placement of species into genera or cryptic forms into a family (e.g. truffle-like species), but additional loci are needed to resolve higher-level relationships in Boletineae (Binder & Besl 2000; Binder & Bresinsky 2002b; Peintner et al. 2003; Binder & Hibbett 2006; Desjardin et al. 2008, 2009; Drehm et al. 2008; Binder et al. 2010; Dentinger et al. 2010; Vizzini et al. 2010; Lebel et al. 2011; Li et al. 2011; Halling et al. 2012a, b). Here, we present a broad phylogenetic analysis of all the major genera of the Boletineae, using three genes: nuclear large subunit ribosomal RNA (nuc-lsu), translation elongation factor 1- α (*tef1*), and DNA directed RNA polymerase II largest subunit (RPB1). *Tef1* and RPB1 were chosen based on the potential they have been shown to have to resolve genera in the Boletineae in previous studies (Binder et al. 2010; Dentinger et al. 2010; Li et al. 2011; Halling et al. 2012a, b; Hosen et al. 2012). The goal of the present study was to develop a phylogenetic framework to guide further exemplar-based sampling and an eventual generic-level reclassification of the Boletineae.

Materials and methods

Taxon sampling—A taxon sampling scheme was designed based on a preliminary analysis of 457 nuc-lsu sequences representing 40 genera and 247 species (Binder & Hibbett 2006), including 42 unnamed species. The nuc-lsu sequences were aligned manually using the PAUP editor and analyzed in the RAXML Blackbox server using the default settings with maximum likelihood (ML) optimization (<http://phylobench.vital-it.ch/raxml-bb/>; Stamatakis et al. 2008; Swofford 2002). Based on the resulting phylogenetic tree (not shown) a sampling scheme was designed, including 93 individuals for which genomic DNA was available, that represents, as broadly as possible, the phylogenetic diversity of the Boletineae as represented in that tree. Amplification of *tef1* and RPB1 genes was attempted using the 93 selected gDNAs. Forty-seven new *tef1*, forty new RPB1 sequences, and seven new nuc-lsu sequences were generated. An additional 28 previously unpublished nuc-lsu sequences were used.

Single-gene analyses were conducted to assess variability of the intron regions in *tef1* and RPB1, and conflict among individual genes using bootstrapped RAXML searches (results not shown). The intron regions of *tef1* and RPB1 were analyzed by eye and deemed conserved enough to warrant inclusion in the analyses, and no well-supported conflict was detected between topologies of the three genes. The nuc-lsu, *tef1*, and RPB1 genes were then combined into two multi-gene datasets: a 'core' dataset containing 48 species, each of which is represented by all three genes, and an 'extended core' dataset, containing all of the sequences from the core dataset, plus 31 species represented by nuc-lsu and *tef1* sequences. Finally, a constrained analysis of 370 nuc-lsu sequences (a subset of those used in the preliminary analysis), containing 249 sequences publicly available from GenBank and 96 unpublished sequences, was conducted using the ML topology from the extended core dataset as the backbone constraint because the extended core dataset contains more species and provided better resolution.

PCR amplification and sequencing

PCR primer pair EF1-983F—EF1-2218R (Rehner & Buckley 2005) was used for amplification of the *tef1* fragment (approx 900–1200 bp). A new Boletineae specific (with the exception of *Chalciporus* species, which have a divergent sequence at this region) external, forward RPB1 primer, RPB1-Bf 5'-GAATGYA-TATGYGTWAACTGTGG-3' was designed for this study. This primer was designed to bind upstream of intron A, as an alternative to RPB1-Af. RPB1-Bf was paired with RPB1-Cr (Matheny et al. 2002) for amplification of the RPB1 fragment (approx 900–1200 bp).

A touchdown PCR protocol was used to amplify *tef1* and RPB1. The following protocols are for *tef1*: (1) initial denaturation 94 °C for 2 min, (2) denaturation 94 °C for 40 s, (3) 60 °C for 40 s, minus 1 °C per cycle, (4) 72 °C for 1 min, (5) repeat from step 2 for nine cycles, (6) 94 °C for 45 s, (7) 50 °C for 1 min 10 s, (8) 72 °C for 1 min 30 s, (9) repeat from step 6 for 36 cycles, (10) 72 °C for 10 min. For RPB1 PCR protocol see Justo & Hibbett (2011). For sequencing,

Table 2 – Morphological features of selected Boletineae genera.

Genera	Spore ornamentation				Stipe ornamentation					Veil		
	Smooth	Bacillate	Longitudinally ridged	Other	Smooth	Longitudinally striated	Reticulate	Scabrous	Other	None	Partial	Double
Boletaceae												
<i>Afroboletus</i>	○	○	●	○	○	○	○	○	●	●	●**	○
<i>Aureoboletus</i>	●	○	○	○	●	○	○	○	○	●	○	○
<i>Austroboletus</i>	○	○	●	○	●	●	○	○	○	●	○	○
<i>Boletellus</i>	●	○	●	●	●	○	●	○	○	●	●	○
<i>Boletus</i>	●	○	○	○	●	●	●	○	○	●	○	○
<i>Bothia</i>	●	○	○	○	●	○	●	○	○	●	○	○
<i>Buchwaldoboletus</i>	●	○	○	○	●	○	○	○	○	●	○	○
<i>Chalciporus</i>	●	○	○	○	●	○	○	○	○	●	○	○
<i>Gastroboletus</i>	●	○	○	○	●	●	●*	○	○	●	○	○
<i>Hemileccinum</i>	●	○	○	○	○	○	○	○	●	●	○	○
<i>Leccinellum</i>	●	○	○	○	○	○	○	●	○	●	○	○
<i>Leccinum</i>	●	○	○	○	○	○	○	●	○	●	○	○
<i>Notholepiota</i>	●	○	○	○	●	○	○	○	○	●	○	○
<i>Phylloporus</i>	●	●	○	○	●	●	○	○	○	●	○	○
<i>Porphyrellus</i>	●	○	○	○	●	●	○	○	○	●	○	○
<i>Pseudoboletus</i>	●	○	○	○	●	○	○	○	●	●	○	○
<i>Retiboletus</i>	●	○	○	○	○	○	●	○	○	●	○	○
<i>Royoungia</i>	●	○	○	○	○	○	○	○	○	●	○	○
<i>Spongiforma</i>	●	○	○	○	○	○	○	○	○	●	○	○
<i>Strobilomyces</i>	○	○	○	●	○	○	○	○	●	○	●	○
<i>Tylopilus</i>	●	○	○	○	●	○	●	○	○	●	○	○
<i>Xanthoconium</i>	●	○	○	○	●	○	●	○	○	●	○	○
<i>Xerocomellus</i>	●	○	○	○	●	●	○	○	○	●	○	○
<i>Xerocomus</i>	●	●	○	○	●	●	○	○	○	●	○	○
Paxillaceae												
<i>Paragyrodon</i>	●	○	○	○	○	○	○	○	○	○	●	●
<i>Paxillus</i>	●	○	○	○	○	○	○	○	○	●	○	○
<i>Gyrodon</i>	●	○	○	○	○	○	○	○	○	●	○	○
Incertae sedis												
<i>Hydnomerulius</i>	●	○	○	○	○	○	○	○	○	○	○	○

● – Feature present in at least one species in genus.
 ○ – Feature absent in all species.
 ? – unknown.

two additional internal *tef1* primers; EF1-1577F and EF1-1567F, and a different terminal reverse primer, EF1-2212R were used (Rehner & Buckley 2005). An additional four internal primers were used for RPB1: RBP1-2F, RBP1-2.1F, RPB1-2.2F, and RPB1-2.1R (Frøslev et al. 2005; Binder et al. 2010).

Using primer pair LR0R–LR7, (Vilgalys & Hester 1990), seven nuc-*lsu* sequences were generated. The following PCR protocol was used: (1) initial denaturation 95 °C for 2 min, (2) denaturation at 94 °C for 45 s, (3) annealing at 50 °C for 1 min 10 s, (4) extension at 72 °C for 2 min, (5) repeat from step 2 for 34 cycles, (6) 72 °C for 10 min (Binder et al. 2010). Sequencing was done using the same PCR primers and two additional internal primers, LR3R and LR5 (Vilgalys & Hester 1990).

All sequencing was done using the BigDye 3.1 terminator sequencing chemistry (Applied Biosystems, Foster City, California) on an Applied Biosystems 3130 Genetic Analyzer. The

raw sequences were processed using Sequencher™ 4.7 (GeneCodes, Ann Arbor, Michigan). Expected fragment length and alternative primers for all genes can be found at <http://www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.htm>.

Sequence alignment and phylogenetic analysis

Sequences were separately aligned by gene using MAFFT (<http://mafft.cbrc.jp/alignment/server/>; Katoh et al. 2002). All genes were aligned using the Q-INS-i strategy, and were then manually optimized using MacClade 4.08 (<http://macclade.org/>; Maddison & Maddison 1992). The core and extended core datasets were created by concatenating the single gene datasets using MacClade.

All alignments were analyzed using RAxML (Stamatakis et al. 2008) for ML analyses, PAUP*4.0.b10 (Swofford 2002) for maximum parsimony (MP), and PhyloBayes (Lartillot 2004;

	Hymeno- phore		Fruiting body morphology				Hypo- geal	Clamp connec- tions	Cauloba- sidium	Caulo- hymenium	Cystidia				
	Lamel- late	Poroid		Pileate- stipitate	Seco- tioid	Gas- teroid					Resu- pinate	Caulo- cystida	Cheilo- cystida	Chryso- cystidia	Pleuro- cystida
		Regularly poroid	Irregularly poroid												
521	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
522	○	●	○	●	○	○	○	○	○	○	○	○	●	●	●
523	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
524	○	●	○	●	○	○	○	○	●	○	○	○	●	○	●
525	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
526	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
527	●	○	○	●	○	○	○	○	○	○	○	○	●	○	●
528	○	●	●	○	●	○	○	○	○	○	○	○	●	○	●
529	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
530	○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
531	○	○	○	●	○	○	○	○	○	○	○	○	○	○	○
532	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
533	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
534	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
535	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
536	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
537	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
538	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
539	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
540	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
541	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
542	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
543	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
544	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
545	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
546	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
547	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
548	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
549	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
550	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
551	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
552	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
553	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
554	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
555	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
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Lartillot & Philippe 2006; Lartillot et al. 2007) for Bayesian analysis (BY). Alignments have been deposited in TreeBase (# 14219).

ML analyses were run using ML optimization, 1000 bootstrap (BS) replicates, and the GTRCAT and gamma model. MP analyses were performed using parameters published by Justo & Hibbett (2011). BY analysis was done with four MCMC, chains using the CAT-GTR settings. BY analyses was run until convergence, indicated by a max difference output of the 'bpcomp' command of less than 0.1. Nodes were considered well supported if the BS value was greater than 70 % for ML and MP, and posterior probability (PP) for BY was greater than 0.95.

For the constrained analysis, 380 nuc-lsu sequences were aligned using the G-INS-i strategy on MAFFT server (<http://mafft.cbrc.jp/alignment/server/>). Ten duplicate sequences were removed, resulting in a 370 taxon matrix. The alignment was analyzed using the extended dataset ML tree topology as the backbone. Constrained analysis was performed using RAXML with ML optimization, 500 BS replicates, and the GTRCAT and gamma model.

Results

DNA extractions, PCR and sequencing

Tef1, RPB1, and nuc-lsu had 68 %, 42 %, and 100 % success rate for sequencing respectively. The low success rate for RPB1 amplification is probably due to primer mismatches.

Alignment and phylogenetic analysis

See Table 3 for technical output of the nuc-lsu, tef1, and RPB1 ML core and extended analyses, the core and extended MP analyses, and the constrained analysis. Following single-gene analyses to assess conflict, the core nuc-lsu, tef1, and RPB1 alignments were merged, as were the extended nuc-lsu, tef1, and RPB1 datasets. The majority of nuc-lsu sequences were generated with the primer set LR0R—LR5; however, some nuc-lsu sequences used were generated using LR0R—LR7 resulting in longer sequences, by approximately 800 bp. These longer sequences were left untrimmed. Thus,

Table 3 – GenBank accession numbers—Missing.

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Afroboletus luteolus</i>	00-436	Africa	—	D. Arora	KF030238	KF030397	KF030392
<i>Aureoboletus gentilis</i>	Pug1	Maindreieck, Germany	3-Oct-95	J. Schreiner	DQ534635	KF030399	—
<i>Aureoboletus innixus</i>	MB 03-104	Lincoln, MA, USA	Aug-03	M. Binder	KF030239	KF030400	—
<i>Aureoboletus moravicus</i>	Xle1	Maindreieck, Germany	19-Spt-98	J. Schreiner	DQ534641	KF030403	—
<i>Aureoboletus roxanae</i>	DS626-07	Chestnut Ridge Park, NY, USA	25-Oct-06	E. Both	KF030311	KF030402	KF030381
<i>Aureoboletus thibetanus</i>	AFTOL-450	Kunming, Yunnan, China	—	Z.-L. Yang	AY700189	DQ029199	DQ435800
<i>Austroboletus gracilis</i>	112/96	MA, USA	30-Aug-96	N. Arnold	DQ534624	KF030425	KF030358
<i>Boletellus chrysenteroides</i>	3838	Towy Park, North Collins, NY	10-Oct-95	E. Both	KF030312	KF030432	KF030383
<i>Boletellus projectellus</i>	AFTOL-713	Cape Cod, MA, USA	14-Sep-03	M. Binder	AY684158	AY879116	AY662660
<i>Boletellus shichianus</i>	AFTOL-532	Yunnan, China	2003	L. Wang	AY647211	DQ408145	—
<i>Boletinellus merulioideus</i>	AFTOL-575	Rock House, MA, USA	29-Aug-02	M. binder	AY684153	DQ056287	DQ435803
<i>Boletus abruptibulbus</i>	4588	Cape San Blas, FL, USA	1-Apr-05	E. Both	KF030302	KF030401	KF030388
<i>Boletus aereus</i>	REH8721	Redwood National Park, Humbolt Co., CA, USA	17-Nov-05	R.E. Halling	KF030339	KF030426	KF030377
<i>Boletus amygdalinus</i>	112605ba	Mendocino Co., CA, USA	26-Nov-05	B. Neill	JQ326996	JQ327024	KF030360
<i>Boletus appendiculatus</i>	Bap1	Bavaria, Germany	10-Aug-95	J. Schreiner	AF456837	JQ327025	KF030359
<i>Boletus bicolor</i> var <i>bicolor</i>	MB 07-001	Chestnut Ridge Park, NY, USA	28-Jul-95	E. Both	KF030370	KF030405	KF030370
<i>Boletus bicolor</i> var <i>borealis</i>	2858	Erie Co., NY, USA	—	E. Both	JQ326998	JQ327021	—
<i>Boletus calopus</i>	Bc1	Bavaria, Germany	7-Sep-94	N. Arnold	AF456833	JQ327019	—
<i>Boletus carminipes</i>	MB 06-061	Erie Co., NY, USA	4-Aug-06	M. Binder, E. Both	JQ327001	JQ327022	KF030363
<i>Boletus dupainii</i>	JAM 0607	Butner, NY, USA	8-Mar-00	E. Both	KF030413	KF030413	KF030361
<i>Boletus edulis</i>	Be3	Bavaria, Germany	14-Sep-94	M. Binder	KF030282	GU187682	GU187444
<i>Boletus firmus</i>	MB 06-060	Chestnut Ridge Park, NY, USA	3-Aug-06	M. Binder	KF030368	KF030408	KF030368
<i>Boletus inedulis</i>	MB 06-044	Erie Co., NY, USA	3-Aug-06	M. Binder, E. Both	JQ327013	JQ327020	KF030362
<i>Boletus luridiformis</i>	AT2001087	Berkshire, England, UK	—	A. F. S. Taylor	JQ326995	JQ327023	—
<i>Boletus morrisii</i>	8206	Concord, MA, USA	2-Aug-06	B. Neill	KF030433	KF030433	—
<i>Boletus pallidus</i>	179/97	Bavaria, Germany	—	—	AF457409	KF030424	KF030396
<i>Boletus peckii</i>	3959	Erie Co., NY, USA	4-Aug-95	A. R. Clark, E. Both	JQ326999	JQ327026	—
<i>Boletus pseudosensibilis</i>	DS615-07	Chestnut Ridge Park, NY, USA	7-Jul-95	E. Both	KF030257	KF030407	—
<i>Boletus pulchriceps</i>	DS 4514	Chiricahua Mnts, AZ, USA	1-Aug-91	—	KF030261	KF030409	KF030376
<i>Boletus pulverulentus</i>	9606	West Newton, MA, USA	9-Jun-06	Bill Neill	KF030313	KF030418	KF030364
<i>Boletus regius</i>	11 265	Mendocino Co., CA, USA	26-Nov-05	Bill Neill	KF030411	KF030411	—
<i>Boletus rhodosanguineus</i>	4252	Chestnut Ridge Park, NY, USA	12-Jul-98	E. Both	KF030252	KF030412	—
<i>Boletus roseopurpureus</i>	MB 06-059	Chestnut Ridge Park, NY, USA	30-Jul-06	A. Taylor, M. Binder	KF030262	KF030410	KF030372
<i>Boletus rufomaculatus</i>	4414	Chestnut Ridge Park, NY, USA	6-Aug-97	E. Both	KF030248	KF030406	KF030369
<i>Boletus semigastroideus</i>	PBM 3076	Arataki Visitor Center, Auckland, New Zealand	6-May-09	P.B. Matheny	KF030352	KF030430	KF030384
<i>Boletus separans</i>	DPL 2704	Texas	2000	D. Lewis	KF030329	KF030431	KF030385
<i>Boletus subalpinus</i>	27 882	—	—	J. Trappe	KF030340	KF030427	KF030379

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Boletus</i>	72 206	Jefferson, NH	22-Jul-06	B. Neill	KF030303	KF030404	KF030374
<i>subglabripes</i>							
<i>Boletus tenax</i>	REH6871	New York Botanical Garden, NY, USA	25-Jul-95	R.E. Halling	KF030320	KF030437	–
<i>Boletus variipes</i> var <i>fagicola</i>	4249	Cheboygan Co., MI, USA	10-Aug-68	A. H. Smith	JQ327014	JQ327017	KF030378
<i>Bothia castanella</i>	MB 03-053	Massachussetts	25-Jun-05	M. Binder	DQ867117	KF030421	KF030382
<i>Buchwaldoboletus</i>	Pul1	Maindreieck, Germany	9-Sep-95	J. Schreiner	JQ326997	JQ327040	–
<i>lignicola</i>							
<i>Chalciporus rubinus</i>	DS4640-3	Germany	–	J. Schreiner	KF030283	KF030440	–
<i>Chalciporus</i>	MB 04-001	Rutland, MA, USA	28-Sep-04	M. Binder	DQ534648	GU187690	GU187453
<i>piperatus</i>							
<i>Chalciporus</i>	4302	Deer Meadows, Tuleet, NY	14-Sep-98	E. Both	KF030284	KF030441	–
<i>pseudorubnellus</i>							
<i>Gyrodon lividus</i>	GL1	Bavaria, Germany	11-Aug-95	H. Besl, W. Helfer	AF098378	GU187701	GU187461
<i>Hemileccinum</i>	Bim1	Bavaria, Germany	21-Sep-95	J. Schreiner	AF139715	JQ327034	KF030375
<i>impolitum</i>							
<i>Hydnomerulius</i>	CFMR:MD312	USDA Forest Products Laboratory	13-Nov-19	–	GU187580	GU187708	GU187462
<i>pinastri</i>							
<i>Leccinellum corsicum</i>	Buf 4507				KF030347	KF030435	KF030389
<i>Leccinellum</i>	930809/1	France	9-Aug-93	G. Lannoy	AF139694	KF030434	–
<i>crocipodium</i>							
<i>Leccinum albellum</i>	MB 06-040	Erie Co., NY, USA	30-Jul-06	M. Binder	JQ327007	JQ327038	–
<i>Leccinum scabrum</i>	Ls1	Austria	14-Sep-95	M. Binder	AF139705	JQ327039	–
<i>Paragyrodon</i>	MB 06-066	Iowa City, IA, USA	22-Spt-06	M. Binder	GU187593	GU187737	–
<i>sphaerosporus</i>							
<i>Paxillus</i>	Pf1	Bavaria, Germany	21-Aug-95	L. Kriegelsteiner	AF167680	GU187736	–
<i>filamentosus</i>							
<i>Paxillus</i>	Po1	Bavaria, Germany	21-Aug-98	Ch. Hahn	AY177256	KF030442	–
<i>obscurisporus</i>							
<i>Paxillus vernalis</i>	Pv2	Canada	31-Aug-97	T. Lohmeyer	AY645059	DQ457629	–
<i>Phylloporus pelletieri</i>	Pp1	Bavaria, Germany	9-Sep-95	M. Kronfeldner	AF456818	JQ327036	KF030390
<i>Porphyrellus</i>	MB 97-023	Bavaria, Germany	9-Sep-96	M. Beisenherz	DQ534643	GU187734	GU187475
<i>porphyrosporus</i>							
<i>Pseudoboletus</i>	Xpa1	Bavaria, Germany	9-Jul-95	A. Bresinsky	AF050646	KF030443	KF030394
<i>parasiticus</i>							
<i>Retiboletus griseus</i>	202/97	MA, USA	30-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456834	KF030414	KF030373
<i>Royoungia boletoides</i>	27 546	New South Wales, Australia	25-Oct-99	J. Trappe	JX889655	JX889696	–
<i>Royoungia boletoides</i>	AWC4137	Victoria, Australia	28-May-01	T. Lebel et al.	DQ534663	JX889700	–
<i>Spongiforma</i>	DED 7873	Thailand	7-Jul-05	D.E. Desjardin	EU685108	KF030436	KF030387
<i>thailandica</i>							
<i>Strobilomyces</i>	Sf1	Bavaria, Germany	12-Aug-95	J. Enzmann	DQ534626	JQ327037	AY858963
<i>floccopus</i>							
<i>Strobilomyces</i> sp	REH8514	Cayo District, Belize	3-Oct-03	R.E. Halling	EU685109	KF030398	–
<i>Tylopilus badiceps</i>	78 206	Holliston, MA, USA	28-Jul-06		KF030335	KF030429	–
<i>Tylopilus felleus</i>	AT2001011	Stadsskogen, Uppsala, Sweden	17-Sep-01	A. F. S. Taylor	JQ326993	JQ327015	KF030380
<i>Tylopilus</i>	MB 06-053	Erie Co., NY, USA	3-Aug-06	E. Both	JQ326994	JQ327016	–
<i>ferrugineus</i>							
<i>Tylopilus</i>	MB 06-056	Chestnut Ridge Park, NY, USA	4-Aug-06	E. Both	KF030350	KF030439	KF030395
<i>plumbeoviolaceus</i>							
<i>Xanthoconium</i>	3518	Lake Mize, Gainesville, FL, USA	10-Aug-92	E. Both	KF030353	KF030428	KF030386
<i>stramineum</i>							
<i>Xerocomellus</i>	Xch1	Bavaria, Germany	8-Aug-95	M. Binder	AF050647	KF030415	KF030365
<i>chrysenteron</i>							
<i>Xerocomellus</i>	PBM 1331	West Coast, USA	–	P.B. Matheny	KF030297	KF030420	–
<i>rubellus</i> cf <i>West</i> <i>Coast</i>							

(continued on next page)

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Xerocomellus rubellus</i> cf East Coast	MB 03-033	Worcester, MA, USA	23-Jul-03	M. Binder	KF030294	KF030419	KF030371
<i>Xerocomellus zelleri</i>	REH8724	Redwood National Park, Humboldt Co., CA, USA	18-Nov-05	R.E. Halling	KF030271	KF030416	KF030366
<i>Xerocomus badius</i>	MB 03-098a	Rutland, MA, USA	19-Aug-03	M. Binder	KF030355	KF030423	KF030393
<i>Xerocomus badius</i>	Xb2	Bavaria, Germany	Sep-95	A. Bresinsky	KF030357	KF030422	—
<i>Xerocomus cisalpinus</i>	AT2005034	Upsala, Uppland, Finland	30-Jul-05	A.F. S. Taylor	KF030354	KF030417	KF030367
<i>Xerocomus perplexus</i>	MB 00-005	Rutland, MA, USA	5-Aug-00	M. Binder	JQ003702	KF030438	—
<i>Xerocomus subtomentosus</i>	Xs1	Bavaria, Germany	10-Aug-95	J. Enzmann, A. Bresinsky	AF139716	JQ327035	KF030391
Constrained data set taxa							
<i>Alpova diplophloeus</i>	17685	—	—	J. Trappe	AF071454		
<i>Alpova diplophloeus</i>	Adi1	Canada	Sep-87	W. Steglich, B. Steffan	AF352035		
<i>Alpova trappei</i>	28 042	—	—	J. Trappe	KF030307		
<i>Alpova trappei</i>	16 394	—	—	J. Trappe	AF071456		
<i>Aureoboletus auriporus</i>	35/94	MA, USA	28-Aug-97	H. Besl	DQ534636		
<i>Aureoboletus auriporus</i>	DD971	Orange Co, NC, USA	—	—	AY612819		
<i>Aureoboletus citrinoporus</i>	REH8719	Humboldt Co, CA, USA	16-Nov-05	R.E. Halling	KF030298		
<i>Auroboletus innixus</i>	136	—	—	—	KF030240		
<i>Aureoboletus viridiflavus</i>	DD972	Orange Co, NC, USA	—	—	AY612805		
<i>Austroboletus flavidus</i>	DPL7541	—	—	—	KF030351		
<i>Austroboletus mucosus</i>	TH6300	Pakaraima Mnts, Guyana	—	—	AY612798		
<i>Austroboletus niveus</i>	M312	New Zealand	10-May-95	A. Bresinsky	DQ534622		
<i>Austroboletus novaezealandicae</i>	M50	New Zealand	17-Mar-95	A. Bresinsky	DQ534623		
<i>Boletellus ananas</i>	TH8819	Guyana	—	T.W. Henkel	HQ161853		
<i>Boletellus ananas</i>	TH6264	Pakaraima Mnts, Guyana	—	T.W. Henkel	AY612799		
<i>Boletellus cf ananiceps</i>	00-335	Africa	2000	D. Arora	KF030330		
<i>Boletellus betula</i>	134/96	MA, USA	31-Aug-96	N. Arnold	AF050642		
<i>Boletellus betula</i>	DD9852	Orange Co, NC, USA	—	—	AY612797		
<i>Boletellus chrysenteroides</i>	54/97	MA, USA	21-Aug-97	N. Arnold, H. Besl	DQ534634		
<i>Boletellus dicymbophilus</i>	TH8840	Guyana	—	T.W. Henkel	HQ161852		
<i>Boletellus mirabilis</i>	CBS 136.60	—	—	—	AF050652		
<i>Boletellus mirabilis</i>	REH8717	Big Lagoon Park, Humboldt Co, CA, USA	15-Nov-05	R.E. Halling	KF030299		
<i>Boletellus piakaii</i>	TH8077	Guyana	—	T.W. Henkel	HQ161861		
<i>Boletellus projectellus</i>	sn2 Hor	—	—	—	KF030300		
<i>Boletellus russellii</i>	12/96	MA, USA	6-Aug-96	N. Arnold	AF050651		
<i>Boletellus russellii</i>	DPL6698	Texas	2000	D. Lewis	KF030325		
<i>Boletellus sp</i>	TAA195080	Valle de Mai, Praslin, Seychelles	—	—	AM412293		
<i>Boletineae sp EcM</i>	L2481_Bol5	L'Abondance, Mahe, Seychelles	—	—	AM412263		
<i>Vateriopsis</i>	26 763	—	—	J. Trappe	KF030268		
<i>Boletus abieticola</i>	REH8720	Shasta/Trinity National Forest, Trinity Co, CA, USA	15-Nov-05	R.E. Halling	KF030269		
<i>Boletus atkinsonianus</i>	4471	Bigelow Hollow State Park, Conn, USA	18-Aug-00	B. Neill, E. Both	KF030241		

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Boletus atkinsonianus</i>	4557	Tucker Co, WV, USA	28-Aug-00	W.C. Woody, E. Both	KF030242		
<i>Boletus aurantioruber</i>	MEN11-0 MB	Cape Cod, MA, USA		M. Binder	KF030342		
<i>Boletus bicolor</i>	TH6933	Watuaga Co., NC, USA	—	—	AY612800		
<i>Boletus bicolor</i>	snOH	Ohio, USA	—	D. Lewis	KF030258		
<i>Boletus bicolor</i>	MB 06-037	Chestnut Ridge Park, NY, USA	3-Aug-06	M. Binder	KF030250		
<i>Boletus bicolor</i> var <i>subreticulatus</i>	3818	North Collins Town Park, NY, USA	10-Aug-95	E. Both	KF030247		
<i>Boletus billieae</i>	4558	Well Fleet, Cape Cod, MA, USA	25-Aug-01	B. Neill, E. Both	KF030265		
<i>Boletus calopus</i>	112606	Mendocino Co., CA, USA	26-Nov-05	B. Neill	KF030279		
<i>Boletus campestris</i>	5/96	Eastabrook Woods, MA, USA	9-Aug-96	N. Arnold	DQ534640		
<i>Boletus carminipes</i>	4591	Berea, Cuyalioga Co, OH, USA	22-Jul-01	P. Monk, E. Both	KF030259		
<i>Boletus cf edulis</i>	—	Turkey	—	—	AF462355		
<i>Boletus cf edulis</i>	197/84	China	—	—	AF462357		
<i>Boletus cf luridus</i>	MAN 011	Costa Rica	1-Jun-04	M.A. Neves	KF030288		
<i>Boletus coniferarum</i>	7/94	CA, USA	Oct-94	W. Steglich	AF456827		
<i>Boletus eastwoodiae</i>	AT2003096	—	—	A.F. S. Taylor	KF030253		
<i>Boletus eastwoodiae</i>	TDB-1000C	Marin Co., CA, USA	28-Nov-87	T. D. Bruns	AF071528		
<i>Boletus edulis</i>	OSC FAC 1615	—	—	—	AF071457		
<i>Boletus edulis</i>	No. 15	Yugoslavia	—	—	AF462356		
<i>Boletus edulis</i>	HN141	Watuaga Co., NC, USA	—	—	AY612802		
<i>Boletus edulis</i>	FO 46874	Germany	—	—	AF291300		
<i>Boletus erythropus</i>	3131	Raquette Lake, NY, USA	21-Jul-98	E. Both	KF030291		
<i>Boletus erythropus</i>	Ber1	Bavaria, Germany	1-Oct-95	J. Enzmann, H. Besl	AF139683		
<i>Boletus fechtneri</i>	AT2003097	—	—	A.F. S. Taylor	KF030270		
<i>Boletus fechtneri</i>	Bf1	Bavaria, Germany	1-Sep-94	M. Beisenherz	AF456821		
<i>Boletus fibrillosus</i>	PBM1342	WA, USA	—	P.B. Matheny	KF030343		
<i>Boletus fibrillosus</i>	Thiers 6995	Jackson Forest, Mendocino Co, CA, USA	21-Nov-92	R.E. Halling, E. Both	KF030344		
<i>Boletus floridanus</i>	BD368	Parque Nacional de Guanacaste, Area de Conservacion Guanacaste, Costa Rica	—	B. Dentinger	HQ161859		
<i>Boletus gertrudiae</i>	86/97	MA, USA	23-Aug-97	N. Arnold	AF457407		
<i>Boletus glabellus</i>	00-194	Boston Cemetary, Boston, MA, USA	—	B. Neill	KF030318		
<i>Boletus gyrodontoides</i>	MS5	Malaysia	8-Apr-91	N. Arnold, H. Besl	DQ534651		
<i>Boletus hypocarcinus</i>	DPL6899	USA	—	D. Lewis	KF030260		
<i>Boletus inedulius</i>	NCJ14	Orange Co, NC, USA	—	D. Dremel	AY612803		
<i>Boletus inedulius</i>	sn2002	NY, USA	—	E. Both	KF030281		
<i>Boletus junquilleus</i>	Bju1	Keilberg-Waldmichbach, Salzbuckel, Germany	9-Nov-96	J. Schreiner	DQ534645		
<i>Boletus leptospermi</i>	M23	New Zealand	15-Mar-95	A. Bresinsky	DQ534632		
<i>Boletus longicurvipes</i>	8/97	USA	14-Aug-97	N. Arnold	AF139688		
<i>Boletus longicurvipes</i>	TH6944	Watuaga Co., NC, USA	—	—	AY612812		
<i>Boletus luridus</i>	Bl2	Bavaria, Germany	14-Aug-95	M. Beisenherz	AF139686		
<i>Boletus luteocupreus</i>	Blu1	Karlbürg, Hagwald, Germany	8-Aug-95	J. Schreiner	DQ534657		
<i>Boletus minato- olivaceus</i>	4091	Chestnut Ridge Park, NY, USA	26-Jun-97	E. Both	KF030243		
<i>Boletus modestus</i>	229/97	MA, USA	1-Sep-97	N. Arnold	DQ534659		
<i>Boletus nobilis</i>	BD239 (MIN)	Duke Forest, Durham, NC, USA	—	—	EU232002		
<i>Boletus oliveisporus</i>	DPL6823	Texas	2000	D. Lewis	KF030254		
<i>Boletus pallidoroseus</i>	BD396	Pine Bend Bluff Scientific and Natural Area, MS, USA	—	B. Dentinger	HQ161860		
<i>Boletus pallidoroseus</i>	sn Hor	Suffolk Co, NY, USA	2000	J. Horman	KF030305		
<i>Boletus pinophilus</i>	No. 4	Bavaria, Germany	20-Jul-92	H. Besl	AF462358		
<i>Boletus pinophilus</i>	42/93	—	—	—	AF462359		
<i>Boletus pulcherrimus</i>	00-291	Mendocino Co., CA, USA	—	A. Mohr, E. Both	KF030256		
<i>Boletus quercophilus</i>	BDCR0417 (MIN)	along Rio Savegre, San Gerardo de Dota, San Jose, Costa Rica	—	—	EU232001		
<i>Boletus radicans</i>	Brad1	—	—	—	AF336241		
<i>Boletus regius</i>	Bre1	Bavaria, Germany	24-Aug-95	L. Kriegelsteiner	DQ534653		

(continued on next page)

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Boletus rex-veris</i>	JFA13101	Chinook Pass, WA, USA	—	—	EU232005		
<i>Boletus rhodopus</i>	4590	Letchworth State Park, Wyoming Co, NY, USA	30-Jul-05	E. Both	KF030289		
<i>Boletus rhodoxanthus</i>	Brh1	USA	Jul-94	W. Steglich	DQ534647		
<i>Boletus roseipes</i>	4515	Essex B. John's Brook Lodge, NY, USA	8-Aug-93	E. Both	KF030301		
<i>Boletus roseopurpureus</i>	3765	Chestnut Ridge Park, NY, USA	26-Jul-96	E. Both	KF030264		
<i>Boletus</i>	4497	Storrs, Uconn Campus, Conn, USA	10-Aug-96	E. Both	KF030263		
<i>Boletus rubripes</i>	8722	Redwood National Park, Humbolt Co., CA, USA	17-Nov-05	R.E. Halling, E. Both	KF030280		
<i>Boletus</i>	3094	Auburn, Worcester Co, MA, USA	7-Aug-84	B. Both	KF030266		
<i>Boletus rubrofibrillosus</i>	MB 05-003	MA, USA	—	M. Binder	KF030304		
<i>Boletus satanas</i>	Bs2	Germany	—	M. Binder	AF336242		
<i>Boletus sensibilis</i> var <i>subviscidus</i>	3929	NTCP—	8-Sep-91	E. Both	KF030310		
<i>Boletus separans</i>	183/86	USA	1986	W. Steglich	AF457404		
<i>Boletus smithii</i>	8727	Humbolt Co, CA, USA	19-Nov-05	R.E. Halling	KF030244		
<i>Boletus</i> sp	4689	—	—	R.E. Halling	KF030319		
<i>Boletus</i> sp	4696	—	—	R.E. Halling	KF030331		
<i>Boletus</i> sp	4698	—	—	R.E. Halling	KF030324		
<i>Boletus</i> sp cf <i>bicolor</i>	3921	Buffalo, NY, USA	29-Aug-95	E. Both	KF030290		
<i>Boletus speciosus</i>	13/96	Flints Pond, MA, USA	9-Aug-95	N. Arnold	DQ534654		
<i>Boletus speciosus</i> var <i>brunneus</i>	4258	Chestnut Ridge Park, NY, USA	15-Jul-98	E. Both	KF030317		
<i>Boletus</i>	4070	NCES, NY, USA	23-Sep-96	E. Both	KF030341		
<i>subcaerulescens</i>							
<i>Boletus subluridellus</i>	3737	NCES, NY, USA	2-Jul-95	E. Both	KF030249		
<i>Boletus subvelutipes</i>	RV98.102	Giles Co, VA, USA	—	—	AY612804		
<i>Boletus tenax</i>	6641	New York Botanical Garden, NY, USA	Aug-91	R.E. Halling	KF030321		
<i>Boletus torosus</i>	Btor1	Garmisch, Bavaria, Germany	31-Jul-95	M. Beisenherz, L. Kieglsteiner, J. Schreiner	DQ534466		
<i>Boletus variipes</i>	BD378	USA	—	B. Dentinger	HQ161846		
<i>Boletus variipes</i>	BD245 (MIN)	Duke Forest, Durham, NC, USA	—	B. Dentinger	EU232003		
<i>Boletus vermiculosus</i>	222/97	Flints Pond, MA, USA	1-Sep-97	N. Arnold	DQ534646		
<i>Boletus violaceofuscus</i>	GMB501	—	—	—	AF457403		
<i>Chalciporus</i>	8434	Germany	—	J. Schreiner	KF030285		
<i>amarellus</i>							
<i>Chalciporus</i>	Cam1	Bavaria, Germany	18-Sep-94	A. Bresinsky	AF456835		
<i>amarellus</i>							
<i>Chalciporus</i>	27 620	Australia	—	J. Trappe	DQ534652		
<i>ovalisporus</i>							
<i>Chalciporus</i>	Cp1	Bavaria, Germany	17-Sep-94	M. Binder	AF336244		
<i>piperatus</i>							
<i>Chalciporus</i>	NSL 15	New Zealand	20-Mar-95	A. Bresinsky	DQ534464		
<i>piperatus</i>							
<i>Chalciporus</i>	BN07	NH, USA	2005	B. Neill	KF030286		
<i>pseudorubinelus</i>							
<i>Chalciporus</i>	DS612 07	Rocky Pt. National Resources Mgmt. Area, Suffolk Co, NY, USA	30-Sep-02	J. Herman, M. Herman, E. Both	KF030287		
<i>pseudorubinelus</i>							
<i>Chalciporus</i>	191/81	ME, USA	Aug-81	W. Steglich	EU685106		
<i>rubinelus</i>							
<i>Chamonixia</i>	92/83	Bavaria, Germany	4-Oct-83	H. Besl	AF336245		
<i>caespitosa</i>							
<i>Chamonixia</i> sp	Muroi361	—	—	J. Trappe	DQ218598		
<i>Durianella</i>	AWW240	Selangor Provence, Malaysia	—	A.W. Wilson	EU293062		
<i>echinulata</i>							

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Fistulinella viscida</i>	238	New Zealand	26-Apr-95	A. Bresinsky	AF456826		
<i>Gastroboletus turbinatus</i>	19/95	WA, USA	18-Aug-95	N. Arnold, J. Ammirati	AF336248		
<i>Gastroboletus vividus</i>	27 480	—	—	J. Trappe	KF030245		
<i>Gyrodon lividus</i>	GLM1	Bavaria, Germany	16-Sep-98	A. Bresinsky	AF167677		
<i>Gyrodon monticola</i>	30/96	Colombia	27-Apr-96	Ch. Hahn	AF352040		
<i>Harrya chromapes</i>	BD377	USA	—	B. Dentinger	HQ161856		
<i>Harrya chromapes</i>	sn1	—	—	—	AF139709		
<i>Harrya chromapes</i>	sn2	—	—	—	AY612834		
<i>Heimioporus retispora</i>	MS6	Malaysia	8-Apr-91	H. Besl	AF050650		
<i>Heliogaster columellifera</i>	TNS-F-11696	Mt. Kuram, Kyoto Pref., Kyoto-shi, Sakyo-ku, Japan	—	T. Orihara	EF183541		
<i>Heliogaster columellifera</i>	TNS-F-11700	Aichi Pref., Okazaki-shi	—	T. Orihara	EF183544		
<i>Hemileccinum depilatum</i>	Bd1	Bavaria, Germany	11-Sep-94	A. Bresinsky	AF139712		
<i>Hydnomerulius pinastri</i>	412	Canada	10-Oct-73	J.H. Ginns	AF352044		
<i>Hydnomerulius pinastri</i>	Z. Wang sn	CA, USA	22-Aug-04	Z. Wang	DQ534667		
<i>Leccinellum carpini</i>	930808	France	8-Aug-93	G. Lannoy	AF139691		
<i>Leccinellum corsicum</i>	931101/1 GL	France	1-Nov-93	G. Lannoy	AF139693		
<i>Leccinellum lepidum</i>	110684	Italy	11-Jun-84	H. Besl	AF139698		
<i>Leccinum aerugineum</i>	8909241AE	Belledonne 38, Seiglieres, France	24-Sep-84	A. Estades	DQ534618		
<i>Leccinum albellum</i>	TH6968	Watuaga Co., NC, USA	—	—	AY612811		
<i>Leccinum aurantiacum</i>	La1	Bavaria, Germany	11-Sep-94	M. Beisenherz	AF139689		
<i>Leccinum aurantiacum</i>	HN1573	Giles Co, VA, USA	—	—	AY612810		
<i>Leccinum callitrichum</i>	GR92103	—	—	—	AF139690		
<i>Leccinum cerinum</i>	MK11800	—	—	—	AF139692		
<i>Leccinum duriusculum</i>	880904/4 GL	France	4-Sep-88	Wolfer	AF139695		
<i>Leccinum flavostipitatum</i>	24/98	USA	23-Aug-98	N. Arnold	AF139696		
<i>Leccinum manzanitae</i>	TDB-969	CA, USA	21-Nov-94	T. D. Bruns	DQ534613		
<i>Leccinum monticola</i>	BDCR14	Cerro de la Muerta summit, Costa Rica	—	B. Dentinger	HQ161869		
<i>Leccinum nigellum</i>	GPL4676	France	11-Sep-79	G. Redeuilh	AF139699		
<i>Leccinum palustre</i>	MK11107	Finland	2-Sep-92	M. Korhonen	AF139701		
<i>Leccinum percandidum</i>	9210040	France	10-Apr-92	A. Estades	AF139702		
<i>Leccinum piceinum</i>	Lp1	Pruz in Tyrol, Austria	14-Sep-94	M. Binder, H. Besl	DQ534614		
<i>Leccinum quercinum</i>	196695	NGS Ludwigshain, Kelheim, Germany	5-Sep-94	A. Reisinger	DQ534612		
<i>Leccinum rigidipes</i>	196696	Massif Belledonne 38, Prabert, France	11-Oct-89	A. Estades	DQ534617		
<i>Leccinum rotundifoliae</i>	MK7676:251	Finland	—	M. Korhonen	AF139704		
<i>Leccinum rugosiceps</i>	TH6967	Watuaga Co., NC, USA	—	—	AY612813		
<i>Leccinum scabrum</i>	NCJ26	Watuaga Co., NC, USA	—	—	AY612814		
<i>Leccinum schistophilum</i>	921024/1 GL	59 Waredin, Rost, France	24-Oct-92	G. Lannoy	DQ534615		
<i>Leccinum varicolor</i>	Lvar1	Bavaria, Germany	19-Sep-95	J. Enzmann	AF139706		
<i>Leccinum versipelle</i>	Lv2	Bavaria, Germany	6-Sep-94	H. Besl	AF139707		

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Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Melanogaster ambiguus</i>	Ma2	USA	13-Aug-89	R. Watling	AF352046		
<i>Melanogaster broomeianus</i>	Mbr1	Bavaria, Germany	28-Jun-95	M. Binder	AF098383		
<i>Melanogaster tuberiformis</i>	628	USA	11-Oct-83	J. Trappe	AF167679		
<i>Melanogaster tuberiformis</i>	9666	—	—	J. Trappe	AF074919		
<i>Melanogaster variegatus</i>	REG 384	Germany	21-Jul-80	H. Besl	DQ534668		
<i>Octaviania asterosperma</i>	Octa1	Bavaria, Germany	3-Sep-97	L. Kriegelsteiner	DQ534619		
<i>Octaviania tasmanica</i>	TL2329	Australia	—	—	HQ647147		
<i>Paragyrodon sphaerosporus</i>	TDB-420	Becker Co, MN, USA	22-Aug-80	T. D. Bruns	AF071531		
<i>Paxillus filamentosus</i>	PfM1	Bavaria, Germany	12-Sep-95	H. Besl	AF167681		
<i>Paxillus involutus</i>	Pi3	Bavaria, Germany	10-Sep-95	M. Binder	AF167682		
<i>Paxillus involutus</i>	Pi2	Bavaria, Germany	3-Sep-95	M. Binder	AF167683		
<i>Paxillus involutus</i>	Pi5	Bavaria, Germany	8-Oct-95	M. Binder	AF167684		
<i>Paxillus involutus</i>	PiM2	Bavaria, Germany	20-Sep-98	A. Bresinsky	AF167686		
<i>Paxillus involutus</i>	PiM1	Bavaria, Germany	22-Sep-98	M. Jarosch	AF167701		
<i>Paxillus involutus</i>	RV98.135	Watuaga Co., NC, USA	—	—	AY612815		
<i>Phylloboletellus chloephorus</i>	XAL 3388	Municipio Coatepec, El Grande, Vercruz, Mexico	5-Aug-01	V. Bandala	DQ534658		
<i>Phylloporus arenicola</i>	27 954	USA	—	J. Trappe	JQ003704		
<i>Phylloporus bellus</i>	MCA559	—	—	—	AY612817		
<i>Phylloporus centroamericanus</i>	MAN 037	Costa Rica	—	M. A. Neves	JQ003664		
<i>Phylloporus alborufus</i>	MAN 022	Costa Rica	—	M. A. Neves	JQ003678		
<i>Phylloporus leucomycelinus</i>	MB 05-007	MA, USA	—	M. Binder	KF030322		
<i>Phylloporus phaeoxanthus</i>	MAN 017	Costa Rica	—	M.A. Neves	JQ003669		
<i>Phylloporus purpureus</i>	MAN 050	Costa Rica	—	M.A. Neves	JQ003672		
<i>Phylloporus rhodoxanthus</i>	161/96	MA, USA	1-Sep-96	N. Arnold	DQ534631		
<i>Phylloporus rhodoxanthus</i>	SAR 89/457	Durham, Co, NC, USA	—	—	U11925		
<i>Porphyrellus porphyrosporus</i>	00-348	Africa	—	D. Arora	KF030348		
<i>Porphyrellus brunneus</i>	M225	New Zealand	24-Apr-95	A. Bresinsky	DQ534630		
<i>Porphyrellus porphyrosporus</i>	Pop4	Bavaria, Germany	28-Aug-97	T. Wagner, M. Binder	KF030346		
<i>Porphyrellus porphyrosporus</i>	Pop1	Bavaria, Germany	9-Sep-96	M. Beisenherz	DQ534642		
<i>Porphyrellus pseudoscaber</i>	REH8716	Big Lagoon Park, Humbolt Co, CA, USA	15-Nov-05	R.E. Halling	EU685107		
<i>Porphyrellus sordidus</i>	148/98	Estabrook Woods, MA, USA	28-Aug-98	N. Arnold	DQ534644		
<i>Porphyrellus sp</i>	00-356	Africa	—	D. Arora	KF030349		
<i>Pseudoboletus parasiticus</i>	151/97	Blue Valley, Macon, NC, USA	25-Aug-97	N. Arnold	DQ534655		
<i>Pulveroboletus auriflammeus</i>	DD973	Orange Co, NC, USA	—	—	AY612818		
<i>Pulveroboletus curtisii</i>	TH6943	Watuaga Co., NC, USA	—	—	AY612820		
<i>Pulveroboletus ravenelii</i>	64/96	MA, USA	25-Aug-98	N. Arnold	KF030306		
<i>Retiboletus retipes</i>	RV98.127	Giles Co, VA, USA	—	—	AY612821		
<i>Pulveroboletus sp</i>	00-317	Africa	2000	D. Arora	KF030332		
<i>Pulveroboletus sp</i>	00-428	Africa	2000	D. Arora	KF030333		
<i>Pulveroboletus sp</i>	00-507	Africa	2000	D. Arora	KF030334		

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Retiboletus flavoniger</i>	REH7247	San José, Costa Rica	8-Jun-98	R.E. Halling	AF456828		
<i>Retiboletus flavoniger</i>	REH7189	San José, Costa Rica	24-Nov-93	R.E. Halling	AF456829		
<i>Retiboletus griseus</i>	Both sn	NY, USA	—	E. Both	KF030308		
<i>Retiboletus nigerrimus</i>	Tyni1	Japan	15-Jul-96	R. Marumoto	AF456832		
<i>Retiboletus ornatipes</i>	93/97	USA	23-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456825		
<i>Retiboletus ornatipes</i>	186/97	USA	30-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456819		
<i>Retiboletus retipes</i>	96/97	USA	23-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456830		
<i>Retiboletus retipes</i>	Japan1	Japan	10-Jul-97	H. Shibata	AF456807		
<i>Retiboletus retipes</i>	REH7567	Cartago, Costa Rica	28-May-96	R.E. Halling	AF456808		
<i>Retiboletus retipes</i>	55/97	USA	21-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456811		
<i>Retiboletus</i> sp	MAN 053	Costa Rica	12-Jun-04	M.A. Neves	KF030327		
<i>Rossbeevera pachyderma</i>	MEL2079350	New Zealand	—	—	HQ647157		
<i>Rossbeevera pachyderma</i>	M42	New Zealand	15-Mar-95	A. Bresinsky	DQ534620		
<i>Rossbeevera vittatispora</i>	MEL2293673	Australia	—	—	HQ647163		
<i>Rossbeevera westraliensis</i>	MEL2233755	Australia	—	—	HQ647164		
<i>Rubinoletus</i> sp	01-590	Africa	2001	—	KF030314		
<i>Tylophilus ballouii</i>	TH6385	Pakaraima Mnts, Guyana	—	—	AY612823		
<i>Spongiforma squarepantsii</i>	LHFB14	Malaysia	—	T.D. Bruns, D. E. Desjardin	HQ724509		
<i>Spongiforma squarepantsii</i>	LHFB01	Malaysia	—	T.D. Bruns, D. E. Desjardin	HQ724510		
<i>Strobilomyces dryophilus</i>	72 106	—	—	—	KF030345		
<i>Strobilomyces floccopus</i>	HN0027	Orange Co, NC, USA	—	—	AY612824		
<i>Strobilomyces</i> sp	177/97	MA, USA	26-Aug-97	N. Arnold	DQ534627		
<i>Sutorius eximius</i>	40/97	MA, USA	20-Aug-97	N. Arnold	AF139684		
<i>Tylophilus aff chromapes</i>	01-513	Africa	2001	D. Arora	JX889672		
<i>Tylophilus aff conicus</i>	01-533	Africa	2001	D. Arora	KF030328		
<i>Tylophilus aff eximius</i>	01-528	Africa	2001	D. Arora	JQ327002		
<i>Tylophilus aff virens</i>	01-541	Africa	2001	D. Arora	JX889677		
<i>Tylophilus alboater</i>	TH6941	Durham, Co, NC, USA	—	—	AY612832		
<i>Tylophilus alboater</i>	TDB-1207	Duncombe, Co., NC, USA	3-Sep-88	T. D. Bruns	AF139708		
<i>Tylophilus atronicotianus</i>	WV	Camp Creek State Forest, WV, USA	14-Aug-02	W.C. Woody, E. Both	KF030293		
<i>Tylophilus atronicotianus</i>	—	Clarion Public Hunting Area, Clarion Co, PA, USA	18-Sep-04	W. Sturgeon, E. Both	EU685110		
<i>Tylophilus badiceps</i>	NCJ20	Watuaga Co., NC, USA	—	—	AY612833		
<i>Tylophilus badiceps</i>	MB 03-052	MA, USA	Aug-03	M. Binder	KF030336		
<i>Tylophilus cf chromapes</i>	01-549	Africa	2001	D. Arora	JX889671		
<i>Tylophilus felleus</i>	—	—	—	—	AF071466		
<i>Tylophilus felleus</i>	HKAS54926	Marburg, Germany	—	—	HQ326933		
<i>Tylophilus indecicus</i>	98/98	USA	26-Aug-98	N. Arnold, W. Helfer	AF456820		
<i>Tylophilus intermedius</i>	BD277	Nerstrand-Big Woods State Park, MN, USA	—	B. Dentinger	HQ161875		
<i>Tylophilus rhoadsiae</i>	RV98.261	Giles Co, VA, USA	—	—	AY612836		

(continued on next page)

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Tylopilus</i>	152/98	MA, USA	28-Aug-98	N. Arnold	DQ534629		
<i>rubrobrunneus</i>							
<i>Tylopilus</i> sp	00-311	Africa	2000	D. Arora	KF030309		
<i>Tylopilus</i> sp	190/83	USA	18-Sep-83	W. Steglich	AF457405		
<i>Tylopilus</i> sp	00-366	Africa	2000	D. Arora	KF030337		
<i>Tylopilus</i> sp	00-388	Africa	2000	D. Arora	KF030338		
<i>Tylopilus</i> sp	204/97	MA, USA	30-Aug-97	N. Arnold	AF456813		
<i>Tylopilus</i>	9306tv	Plymouth, MA, USA	3-Sep-06	B. Neill	KF030315		
<i>variobrunneus</i>							
<i>Tylopilus</i>	sn Hor	—	—	—	KF030316		
<i>variobrunneus</i>							
<i>Tylopilus virens</i>	—	Japan	1997	R. Marumoto	DQ534621		
<i>Xanthoconium affine</i>	78/94	USA	31-Aug-94	W. Steglich	AF457406		
<i>Xanthoconium affine</i>	RV98.112	Giles Co, VA, USA	—	—	AY612838		
<i>Xanthoconium affine</i>	BD217	Mnt. Lake Biological Station, Mountain Lake, VA, USA	—	B. Dentinger	HQ161854		
<i>var maculosum</i>							
<i>Xanthoconium</i> sp	MB 06-049	Dutch Grove, Erie Co, NY, USA	4-Aug-06	M. Binder	KF030292		
<i>Xerocomus</i>	TH6304	Pakaraima Mnts, Guyana	—	—	AY612839		
<i>amazonicus</i>							
<i>Xerocomellus</i>	Xar1	Spessart, Germany	6-Sep-95	L. Krieglsteiner	KF030295		
<i>armeniacus</i>							
<i>Xerocomus badius</i>	Xb1	Bavaria, Germany	Sep-95	M. Binder	AF050648		
<i>Xerocomus badius</i>	MB 03-098(a b)	Rutland, MA, USA	19-Aug-03	M. Binder	KF030355, KF030356		
<i>Xerocomus bubalinus</i>	MB 05-008	Beaver St., Worcester, Worcester Co, MA, USA	5-Oct-05	M. Binder	KF030296		
<i>Xerocomellus</i> cf <i>chrysenteron</i>	PBM925	USA	—	—	KF030272		
<i>Xerocomellus</i> cf <i>porosporus</i>	MB 00-017	Woodland St., Worcester, Worcester Co, MA, USA	2-Aug-00	M. Binder	KF030277		
<i>Xerocomellus</i>	IB20000405	—	—	—	AF514809		
<i>chrysenteron</i>							
<i>Xerocomellus</i>	REH8723	Redwood National Park, Humbolt Co., CA, USA	17-Nov-05	R.E. Halling	KF030276		
<i>chrysenteron</i>							
<i>Xerocomellus</i>	TDB-635	Crawford, Co., MI, USA	8-Aug-83	T. D. Bruns	AF071537		
<i>chrysenteron</i>							
<i>Xerocomellus</i>	Xpor1	Bavaria, Germany	17-Sep-95	M. Binder	AF050645		
<i>porosporus</i>							
<i>Xerocomellus</i>	IB19880304	—	—	—	AF514810		
<i>porosporus</i>							
<i>Xerocomellus</i>	IB19990957	—	—	—	AF514811		
<i>porosporus</i>							
<i>Xerocomellus</i>	Xpru1	Bavaria, Germany	21, Aug-98	L. Krieglsteiner	AF050644		
<i>pruinatus</i>							
<i>Xerocomellus</i>	IB19980368	—	—	—	AF514827		
<i>pruinatus</i>							
<i>Xerocomellus</i>	GR22465	—	—	—	AF514818		
<i>ripariellus</i>							
<i>Xerocomellus</i>	IB19980360	—	—	—	AF514816		
<i>ripariellus</i>							
<i>Xerocomellus rubellus</i>	GS961	—	—	—	AF514829		
<i>Xerocomellus rubellus</i>	IB19990917	—	—	—	AF514828		
<i>Xerocomellus zelleri</i>	27 888	—	—	J. Trappe	KF030275		
<i>Xerocomus</i>	JAM0539	Cantabria, Las Rozas, Spain	21-Sep-04	J.A. Munoz	KF040544		
<i>chrysonemus</i>							
<i>Xerocomus cisalpinus</i>	5823-130998	Germany	13-Sep-98	J. Schreiner	KF030274		
<i>Xerocomus cisalpinus</i>	IB20000701	—	—	—	AF514812		
<i>Xerocomus dryophilus</i>	IB19990901	—	—	—	AF514823		
<i>Xerocomus dryophilus</i>	IB19991057	—	—	—	AF514822		
<i>Xerocomus fennicus</i>	H126	—	—	—	AF514820		
<i>Xerocomus fennicus</i>	RJ126	—	—	—	AF514821		
<i>Xerocomus hortonii</i>	84/94	MA, USA	23-Aug-94	W. Steglich	AF139713		

Table 3 – (continued)

Species	Isolate ID	Location	Date	Collector/ Identifier	nuc-lsu	tef1	rpb1
<i>Xerocomus illudens</i>	64/98	USA	24-Aug-98	N. Arnold, W. Helfer	AF139714		
<i>Xerocomus illudens</i>	DD9854	Orange Co, NC, USA	—	—	AY612840		
<i>Xerocomus lanatus</i>	MB 95-074	Bavaria, Germany	13-Sep-95	M. Binder	DQ534633		
<i>Xerocomus silwoodensis</i>	JAM0612	Araba, Albania	9-Aug-05	J.A. Munoz	KF030323		
<i>Xerocomus</i> sp	RV98.123	Giles Co, VA, USA	—	—	AY612841		
<i>Xerocomus spadiceus</i>	OKM25919	Clallam Co, WA, USA	—	—	AY612842		
<i>Xerocomus subtomentosus</i>	IB19980452	—	—	—	AF514831		
<i>Xerocomus subtomentosus</i>	IB19991000	—	—	—	AF514832		
<i>Xerocomus truncatus</i>	63/97	USA	21-Aug-97	N. Arnold, H. Besl	DQ534625		
<i>Xerocomus truncatus</i>	REH8718	CA, USA	—	R.E. Halling	KF030273		

the proportion of gaps and undetermined characters in the nuc-lsu dataset is artificially inflated due to the use of different primer pairs.

For the BY analyses, maximum differences in split frequency between runs dropped to zero after approximately 51 000 cycles for the core dataset and 27 000 for the extended dataset, and the chains were then stopped. The chains for each dataset were analyzed with the readpb program, removing 10 % of the samples as burn-in. A total of 91 789 trees were used to estimate PPs for the core dataset, and 49 367 trees for the extended dataset (Table 4).

Taxonomy

Boletus separans (Peck), Bull. Buffalo Soc. Nat. Sci. 1: 59.(1873).

Synonyms: *Boletus edulis* spp. *separans* (Peck) Singer, Amer. Midl. Nat. 37: 26 (1947).

B. edulis f. *separans* (Peck) Vassilkov, BekyiGrib: 15 (1966).

Xanthoconium separans (Peck) Halling & Both, Bull. Buffalo Soc. Nat. Sci. 36: 240 (1998).

TYPE: New York. Greenbush, August 1872, C.H. Peck (NYS).

Mycobank #: 226550.

Boletus subalpinus (Trappe & Thiers) M. Nuhn, Manfr. Binder, A. F. S. Taylor, Halling, Hibbett, *comb. nov.*

Basionym: *Gastroboletus subalpinus* Trappe & Thiers Brittonia 21: 245 (1969).

TYPE: Oregon. Hood River Co., Cloud Gap, Trappe 607 (Holotype: OSC).

Mycobank #: 804290.

Boletus semigastroideus M. Nuhn, Manfr. Binder, A. F. S. Taylor, Halling, Hibbett, *nom. nov.* for *Secotium areolatum* G. Cunn., New Zealand J. Sci. Technol. 23: 172B (1942), non *Boletus areolatus* Berkeley, Hooker's J. Bot. Kew Gard. Misc. 4: 138 (1852).

Synonym: *Notholepiota areolata* (G. Cunn.) E. Horak, New Zealand J. Bot. 9: 479 (1971).

TYPE: New Zealand. Auckland, Swanson, J. Dingley, IV. 1943 (Holotype: PDD 4595).

Mycobank #: 804291.

Discussion

Overview of phylogenetic relationships in Boletineae

In both the three-gene core and extended datasets, the Boletineae and Boletaceae are upheld with maximum supported values in all analyses (BS = 100 %, MP = 100 %, PP = 1.0, reported as [BS/MP/PP] hereafter), but the backbone of the Boletaceae remains poorly resolved. Nonetheless, there are

Table 4 – Alignment Information from ML and MP analyses.

Alignment	ML			MP			
	Positions	Alignment patterns	% Gaps and undetermined characters	Characters	Constant characters	Uninformative characters	Informative characters
nuc-lsu, core	1537	482	35	N/A	N/A	N/A	N/A
tef1, core	1327	702	12	N/A	N/A	N/A	N/A
RPB1, core	1490	1079	27	N/A	N/A	N/A	N/A
nuc-lsu, extended	1952	702	35	N/A	N/A	N/A	N/A
tef1, extended	1345	763	13	N/A	N/A	N/A	N/A
RPB1, extended	Same as core			N/A	N/A	N/A	N/A
nuc-lsu, constrained	2000	858	56	N/A	N/A	N/A	N/A
Three gene core	N/A	N/A	N/A	4349	2434	543	1327
Three gene extended	N/A	N/A	N/A	4759	2732	558	1469

eleven strongly support, named clades shared in both the three-gene core and extended, and an additional seven supported, named clades in the three-gene extended (see Figs 1 and 2 for clades; clades 1–11 are shared, clades 12–18 are only in the three-gene extended analysis). The named, supported clades may provide the basis for an eventual generic level reclassification of the *Boletaceae*. The major groups, which we informally label as anaxoboletus, leccinoid, hypoboletus, bicolor, dupainii, regius, carminipes, chalciporus, and *Paxillaceae* are all upheld by at least two of the three analysis methods (above 70 % for BS and MP, and 0.95 for PP) in the three-gene core and extended phylogenies. However, two groups, chalciporus and *Paxillaceae*, are represented by single taxa in the three-gene core analysis. The overall tree topology

is identical for the core and extended analyses. Eleven strongly supported sub-groups within the anaxoboletus, leccinoid, and hypoboletus groups are discussed below. Except for the *Paxillaceae*, group names do not correspond to formal taxa. Group names were designated based on the most inclusive clade with strong support and are only meant to facilitate discussion.

The anaxoboletus group unites the genera *Afroboletus*, *Boletus*, *Gastroboletus*, *Notholepiota*, *Porphyrellus*, *Tylopilus*, *Strobilomyces*, *Xanthoconium*, *Xerocomellus*, and species referred to as *Xerocomus* but shown to not be closely related to *Xerocomus subtomentosus*, the type species of *Xerocomus*. *Boletus*, *Gastroboletus*, *Notholepiota*, and *Xanthoconium* found in anaxoboletus are members of the ‘porcini’ group as defined by Dentinger

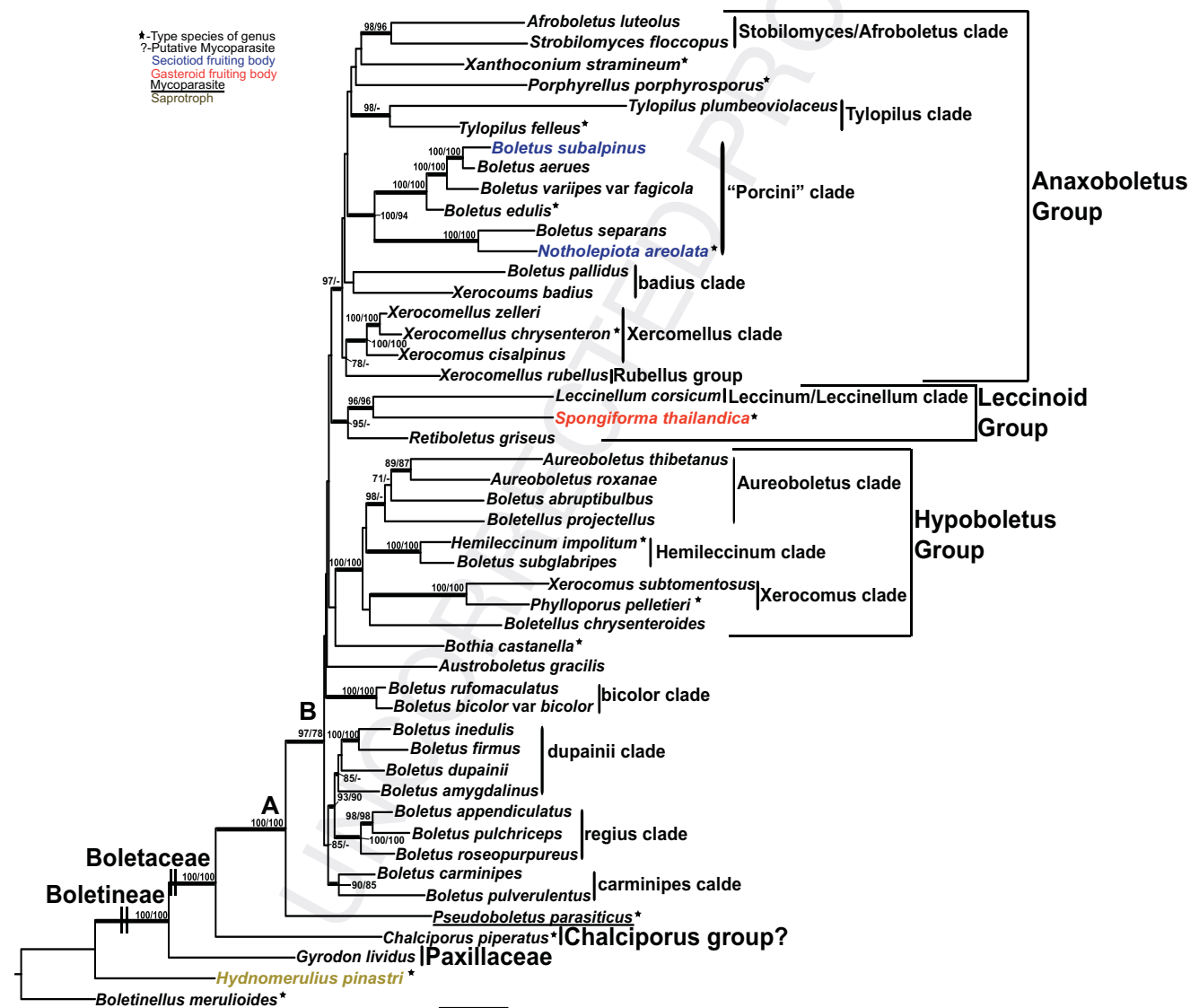


Fig 1 – Phylogenetic relationships of the Boletineae inferred from *nuc-18S*, *tef1*, and *RPB1* using RAxML, PhyloBayes, and PAUP*. This is referred to as the three-gene core analysis in the text. The tree topology corresponds to the optimal ML tree. Support values ≥ 70 % BS for ML and MP are displayed [ML/MP], and PPs ≥ 0.95 % are depicted by bold branches. Groups and clades that were recovered in the analysis are indicated, and three taxonomic ranks are indicated: Boletineae, Boletaceae and Paxillaceae. Nodes indicated by A and B indicate the most inclusive and second most inclusive clades that lack a formal taxonomic rank, respectively. Numbered clades 1–11 are the clades the core analysis shares with the extended analysis.

et al. (2010) and is equivalent to Singer's *Boletus* section *Boletus* (Singer 1986) containing the type species: *Boletus edulis*. The leccinoid group unites the genera *Leccinellum*, *Leccinum*, *Spongiforma*, and *Retiboletus*. The hypoboletus group contains members of *Aureoboletus*, *Boletellus*, *Boletus* (non-porcini), *Hemileccinum*, *Phylloporus*, and *Xerocomus* (the clade with the type species). The relationship between these three lineages is unresolved. *Chalciporus* spp. and *Buchwaldoboletus lignicola* are supported as a clade, designated *chalciporus* group.

Two other nodes receive support, node A and node B (see Figs 1 and 2). Node A supports the sister group relationship between the *chalciporus* group and all other members of the *Boletaceae* (see Figs 1 and 2), and node B supports *Pseudoboletus parasiticus* as the sister group of the non-*chalciporus* group *Boletaceae*. All other nodes with support correspond to groups that could be considered genera.

Diverse morphology-based generic concepts have been proposed in the *Boletineae*, with most genera containing a small or moderate (≤ 50) number of species (Pilát 1969; Smith & Thiers 1971; Singer 1986; Binder & Besl 2000; Binder & Bresinsky 2002b; Binder & Hibbett 2006; Sutara 2008; Halling et al. 2012a, b). Twenty-three genera are included in the core analysis, representing roughly half of the total genera of the *Boletineae* that have molecular data available, and 28 genera are included in the extended analysis. In the extended analysis, the following genera are recovered as monophyletic: *Strobilomyces*, *Leccinum*, *Leccinellum*, *Royoungia*, *Chalciporus*, and *Paxillus*. However, the following genera are not monophyletic using current generic concepts: *Aureoboletus*, *Boletellus*, *Boletus*, *Xanthoconium*, *Xerocomellus*, and *Xerocomus*.

The most difficult taxonomic issue facing boletologists concerns the genus *Boletus*. The type species of *Boletus*, *B. edulis*, is nested in the strongly supported 'porcini' clade, which is part of the *anaxoboletus* group, but many other species of '*Boletus*' are distributed among the distantly-related bicolor, *dupainii*, *regius*, and *carminipes* clades (Fig 2). Members of the '*porcini*,' bicolor, *dupainii*, *regius*, and *carminipes* clades all share smooth spores, a spore print colour reported as some hue of olive brown, tube trama of the boletoid type, and other characters (Smith & Thiers 1971; Singer 1986; Bessette et al. 2000; Ladurner & Simonini 2003; Muñoz 2005; Dentinger et al. 2010). However, the bicolor, *dupainii*, *regius*, and *carminipes* clade species are distinguished from the '*porcini*' clade by the lack of a white pore surface when young and absence of the 'stuffed' pore characteristic (Dentinger et al. 2010). The present study lacks the necessary sampling to attempt to resolve the *Boletus* spp. that are not members of *Boletus* s.str., which may represent additional independent clades.

While the need for a reclassification of *Boletus* has been evident for some time (Binder & Hibbett 2006), certain infrageneric taxa within *Boletus* have been shown to be problematical. For example, *Boletus* sect. *Subtomentosus* sensu Smith & Thiers (1971) is represented by members of the *carminipes* clade and *Boletus pallidus*, this latter appears in the distantly related *badius* clade of the *anaxoboletus* group (Fig 2). Similarly, species of *Boletus* sect. *Calopodes* sensu Singer (1986) are distributed across the *carminipes* clade and the *regius* clade (Fig 2). Even some putative species have proven to be polyphyletic. Specifically, *Boletus bicolor* var. *bicolor* and *B. bicolor* var. *borealis* sensu Smith & Thiers (1971) occur in two separate

groups, the bicolor and *carminipes* clades, respectively and are not, based on our results, the same species.

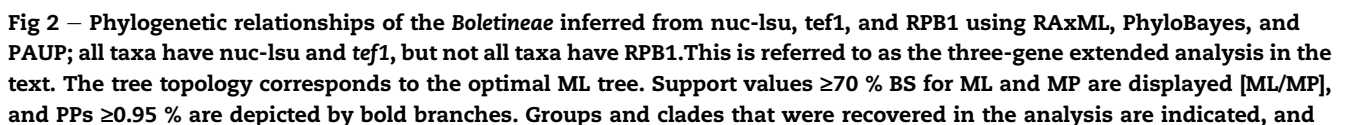
Ecological diversity in Boletineae

The great majority of the species of the *Boletineae* are ectomycorrhizal (ECM) (Binder & Hibbett 2006; Tedersoo et al. 2010). However, *Pseudoboletus parasiticus* and species of the *Chalciporus* group (*Chalciporus piperatus* and *Buchwaldoboletus lignicola*) appear to be mycoparasites (Modess 1941; Godbout & Fortin 1985; Yamada & Katsuya 1995; Kasuya & Igarashi 1996; Högborg et al. 1999; Taylor et al. 2003; Zeller et al. 2007; Tedersoo et al. 2010). *Pseudoboletus parasiticus*, which produces its fruiting bodies on those of its host, *Scleroderma citrinum* (*Sclerodermatineae*), is well characterized as a mycoparasite, but evidence on the nutritional mode of *Chalciporus piperatus* is not as clear-cut.

Chalciporus piperatus was originally considered to be ECM, but several synthesis attempts failed to show formation of ECM with tree species in vitro (Modess 1941; Godbout & Fortin 1985; Yamada & Katsuya 1995; Kasuya & Igarashi 1996; Zeller et al. 2007). Garrido (1988) reported successful ECM synthesis using *C. piperatus*, but Tedersoo et al. (2010) urged caution, stating that the illustrations of Garrido (1988) do not resemble true ECM hyphal formations. Additionally, isotopic analysis suggested that *C. piperatus* may be saprotrophic (Högborg et al. 1999; Taylor et al. 2003). However, isotopic values for saprotrophic species, especially leaf litter decayers, and some mycoparasites can overlap with isotopic values for ECM species (Hobbie et al. 1999; Högborg et al. 1999; Hobbie et al. 2001).

Ecological observations suggest a mycoparasitic nutritional mode for *C. piperatus*, which has been found in association with its putative host, *Amanita muscaria* (*Agaricales*), in New Zealand and Australia (Robinson 2010). *Pinus radiata* is a non-native species in New Zealand that forms ECM with *A. muscaria*, which is thought to have been introduced along with *P. radiata*. *Amanita muscaria* has made a host jump onto the native *Nothofagus*, and *C. piperatus* has been observed fruiting in proximity to *A. muscaria* associated with *Nothofagus*, implying that it followed *A. muscaria*'s mycorrhizal host shift (Tedersoo et al. 2010; Dunk et al. 2012).

Buchwaldoboletus lignicola, which is the sister taxon of *Chalciporus*, also appears to be mycoparasitic. Fig 3 depicts the results of a confrontation experiment in which the hyphae of *B. lignicola* can be seen wrapping around hyphae of *Phaeolus schweinitzii*, a known brown-rot tree pathogen. *Buchwaldoboletus lignicola* is associated with *P. schweinitzii* in the field and appears to be dependent on the latter for growth (Szczechpka & Sokół 1984), which contradicts suggestions that it is saprotrophic (Pantidou 1962; Pilát 1965). Collectively, the confrontation analysis and association with *P. schweinitzii* of *B. lignicola*, and the isotopic and ecological evidence for *C. piperatus* support a mycoparasitic nutritional mode for some members of the *Chalciporus* group (Taylor et al. 2003; Zeller et al. 2007). Because the *Chalciporus* group and *P. parasiticus* are nested within the *Boletineae* (Figs 1 and 2), the genera may represent parallel shifts in nutritional mode from the ECM nutritional mode shared by members of the *Paxillaceae* and all of the species above node B (Figs 1 and 2).



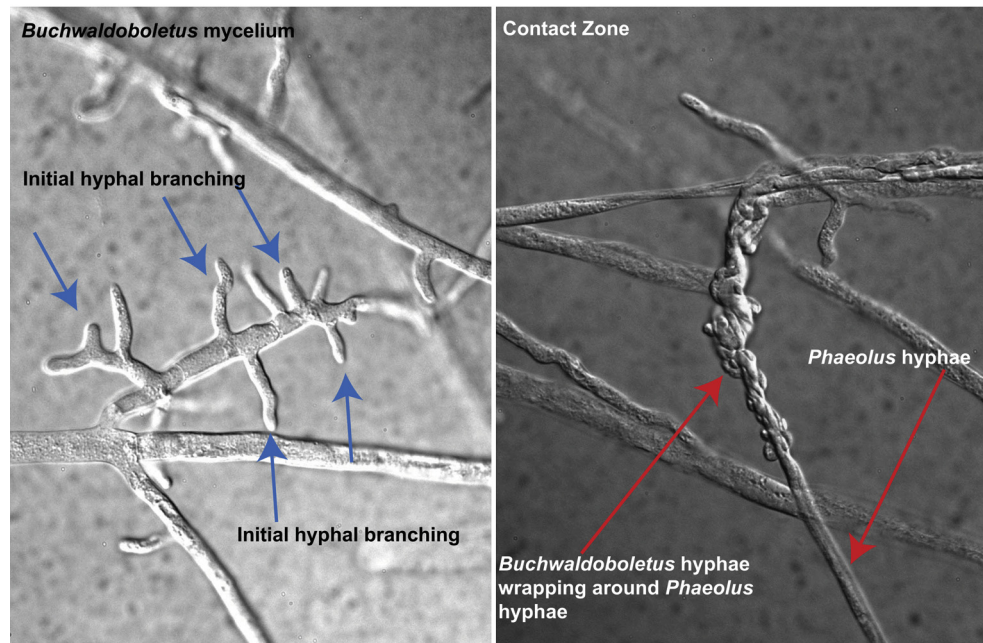
Confrontation test between *Buchwaldoboletus* and *Phaeolus*

Fig 3 – Confrontation analysis between the hyphae of *Buchwaldoboletus lignicola* and *Phaeolus schweinitzii*. Initial hyphal branching of *Buchwaldoboletus lignicola* is seen in the image on the left and indicated by blue arrows. In the image on the right, the black arrow indicates the *P. schweinitzii* hypha and where the hyphae of *B. lignicola* are wrapping around a hyphal strand of *P. schweinitzii*, indicated by a white arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Clade-by-clade discussion of Boletineae

Because the topologies of the three-gene core and extended phylogenies are congruent, the following discussion refers to the more inclusive extended phylogeny. Discussion of the systematic and morphological features of the clades is confined to the taxa presented in Fig 2. The congruent Fig 2 clades in the constrained analysis (Sup. Fig 1) are discussed only when well-supported or otherwise noteworthy, but due to the lack of support for nearly all nodes on the constrained tree (Sup. Fig 1), morphological and systematic features are generally not discussed. For complete contents of the constrained analysis clades see Table 5 and Sup. Fig 1 for tree topology. **anaxoboletus group** [94/94/0.99]—*Anaxoboletus* comprises six clades: *Tylopilus*, *Strobilomyces* (including *Afroboletus luteolus*), *Boletus sensu stricto*, *Xerocomellus*, ‘*rubellus*,’ and ‘*badius*’ and two unresolved genera typified by *Xanthoconium stramineum* and *Porphyrellus porphyrosporus*. The relationships between clades are not resolved.

Tylopilus clade [99/-/1.0]—The *Tylopilus* species in the extended analysis are recovered as monophyletic, with a placement within the tree that is similar to that in Dentinger et al. (2010). Li et al. (2011) reported *Tylopilus* as polyphyletic

considering the placement of *Tylopilus chromapes* outside the clade containing the type species, *Tylopilus felleus*. Recently, two new genera, *Harrya* and *Australopilus*, have been described to accommodate the *T. chromapes* complex (Halling et al. 2012b). Despite these recent works, there still remain species called *Tylopilus* that are not supported as members of *Tylopilus* s.str. or lack enough molecular and morphological data to place in a different genus or erect a new genus; e.g. *Tylopilus alboater*, *Tylopilus atronicotianus*, *Tylopilus badiceps*, *Tylopilus rhoadsiae*, *Tylopilus variobrunneus*, *Tylopilus virens* (see Sup. Fig 1; Binder & Hibbett 2006). The *Tylopilus* clade containing *T. felleus* in Li et al. (2011) corresponds to this study’s *Tylopilus* clade. The least inclusive clade containing all the species from the three-gene analysis receives no support in the constrained analysis; nevertheless it contains only species placed in *Tylopilus*.

The traditional morphological concept of *Tylopilus* is that of smooth spored species, with or without a reticulum on the stipe, a spore print of pinkish to various brown hues, and a pore surface that is frequently white when young and turns pink, yellow, brown, grey or black with age (Bessette et al. 2000; Singer 1986; Smith & Thiers 1971; Watling 2008). The variable pore surface colour with age is one of the characters that

three taxonomic ranks are indicated: Boletineae, Boletaceae and Paxillaceae. Nodes indicated by A and B indicate the most inclusive and second most inclusive clades that lack a formal taxonomic rank, respectively. Clade numbers 1–11 represent the clades the extended analysis shares with the core analysis, numbers 12–18 represent clades in the extended analysis only.

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Table 5 – Contents of Sup. Fig. 1.

Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
Anaxoboletus	Tylopilus	<i>Tylopilus plumbviolaceus</i> MB06-056 <i>Tylopilus felleus</i> AT2001011 ^a	<i>Tylopilus badiceps</i> 72806 <i>Tylopilus ferrugineus</i> MB06-053	<i>Tylopilus badiceps</i> MB06-053xx <i>Tylopilus indecisus</i> AF456820 <i>Tylopilus intermedius</i> HQ161875 <i>Tylopilus rubrobrunneus</i> <i>Tylopilus plumbeoviolaceus</i> MB06-056? <i>Tylopilus variobrunneus</i> 9306 <i>Tylopilus variobrunneus</i> sn Hor <i>Rubinoboletus</i> 1590 28S <i>Tylopilus ballouii</i> AY612823 <i>Tylopilus felleus</i> sn Aurora <i>Tylopilus felleus</i> AF071466 <i>Tylopilus alboater</i> AF139708
	Strobilomyces	<i>Strobilomyces floccopus</i> Sf1 <i>Afroboletus luteolus</i> 00436 ^a	<i>Strobilomyces</i> sp 4515	<i>Strobilomyces floccopus</i> AY612824 <i>Strobilomyces dryophilus</i> 72106 <i>Strobilomyces</i> sp. DQ534627 <i>Boletus</i> sp. 4689
	'Porcini' clade	<i>Boletus subalpinus</i> 27882 <i>Boletus aereus</i> 8721 <i>Boletus variipes</i> var. <i>fagicola</i> 4249 <i>Boletus edulis</i> Be1 ^a <i>Boletus semigastroides</i> PBM3076 ^a <i>Boletus separans</i> DPL2704	N/A	<i>Boletus edulis</i> <i>Boletus pinophilus</i> AF462358 <i>Boletus subcarulescens</i> 4070 <i>Boletus pinophilus</i> AF462356 <i>Boletus edulis</i> AF462356 <i>Boletus edulis</i> AF291300 <i>Boletus rex veris</i> EU232005 <i>Boletus fibrillosus</i> Thiers 6995 <i>Boletus fibrillosus</i> PBM1342 <i>Boletus cf edulis</i> AF462357 <i>Boletus cf edulis</i> AF462355 <i>Boletus quercophilus</i> EU232001 <i>Boletus edulis</i> AY612802 <i>Boletus gertrudiae</i> AF457407 <i>Boletus nobilis</i> EU232002 <i>Boletus violaceofuscus</i> AF457403 <i>Boletus separans</i> AF457404 <i>Tylopius rhaodsiae</i> AY612836 <i>Boletus variipes</i> BD378 HQ161846 <i>Boletus variipes</i> BD245 EU232003 <i>Boletus aurantioruber</i> MEN11-0 MB
	Xerocomellus	<i>Xerocomellus zelleri</i> 8724 <i>Xerocomellus chrysenteron</i> Xch1 ^a <i>Xerocomellus cisalpinus</i> AT2005034	N/A	<i>Xerocomellus chrysenteron</i> IM200000405 <i>Xerocomellus cf porosporus</i> MB00-017 <i>Xerocomellus chrysenteron</i> 8723 <i>Xerocomellus porosporus</i> IB19880304 <i>Xerocomellus porosporus</i> IB19990957 <i>Xerocomus porosporus</i> AF050645 <i>Xerocomellus truncates</i> <i>Octaviania columelifera</i> EF183541

Octaviania columelifera EF183544
 Xerocomellus fennicus H126
 Xerocomellus fennicus RJ126
 Xerocomellus ripariellus GR22465
 Xerocomellus ripariellus IB19980360
 Xerocomellus pruinatus AF050644
 Xerocomellus pruinatus IB19980368
 Xerocomellus cf. chrysenteron PBM925
 Xerocomus truncates 8718
 Xerocomus dryophilus IB19991057
 Xerocomus dryophilus IB19990901
 Xerocomellus zelleri 8724
 Xerocomus cisalpinus
 Xerocomus cisalpinus IB20000701
 Xerocomellus chrysenteron AF071537
 Xerocomus spadiceus AY612842
 Xerocomellus rubellus GS961
 Xerocomellus rubellus IB19990917
 Xerocomellus armeniacus
 Xerocomus bubalinus MB05-008
 Xerocomus badius MB03-00
 Boletus glabellus 00194
 Xerocomus badius AF050648
 Tylopilus sp 00-311
 Tylopilus sp 00-388
 Tylopilus sp 00-366
 Tylopilus atronicotianus WV
 Tylopilus atronicotianus PA
 Tylopilus alboater AY612832
 Porphyrellus sordidus DQ534644
 Porphyrellus porphyrosporus MB97-023
 Porphyrellus pseudoscaber EU685107
 Porphyrellus porphyrosporus DQ534642
 Porphyrellus sp. 00-348
 Vateriopsis sp. EcM L2481 AM412263
 Xanthoconium affine var. maculosum HQ161845
 Xanthoconium affine AY612838
 Xanthoconium affine AF457406
 Xanthoconium sp. MB06-049
 Tylopilus badiceps AY612833
 Leccinum manzanitae
 Leccinum aurantiacum AY612810
 Leccinum cerinum AF139692
 Leccinum piceinum
 Leccinum quercinum
 Leccinum monticola HQ161869
 Leccinum percandidum AF139702
 Leccinum aurantiacum AF139689

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	rubellus	Xercomellus rubellus cf. East Coast MB03-033	Xerocomellus rubellus cf. West Coast PBM1331
	badius	No representative	Xerocomus badius Xb2 Xerocomus badius MB03-098 Boletus pallidus 179-97
	No clade designated	Xanthoconium stramenium ^a Porphyrellus porphyrosporus ^a	N/A
Leccinoid	Leccinum	No representative	Leccinum scabrum Ls1 Leccinum albellum MB06-040

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Table 5 – (continued)				
Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
				<i>Leccinum callitrichum</i> AF139695 <i>Leccinum nigellum</i> ined AF139699 <i>Leccinum aerugineum</i> <i>Leccinum rigidipes</i> <i>Leccinum rotundifoliae</i> AF139704 <i>Leccinum versipelle</i> AF139707 <i>Leccinum schistophilum</i> <i>Leccinum scabrum</i> AY612814 <i>Leccinum flavostipitatum</i> AF139696 <i>Leccinum variicolor</i> AF139706 <i>Leccinum palustre</i> AF139701 <i>Leccinellum corsicum</i> AF139693 <i>Leccinellum lepidum</i> AF139698 <i>Leccinellum carpini</i> AF139691 <i>Leccinum rugosiceps</i> AY612813 <i>Chamonixia</i> sp DQ218598 <i>Rossbeevera westraliensis</i> HQ647164 <i>Rossbeevera vittatispora</i> HQ647163 <i>Rossbeevera pachyderma</i> HQ647157 <i>Rossbeevera pachyderma</i> DQ534620 <i>Leccinum albellum</i> AY612811 <i>Chamonixia caespitosa</i> AF336245 <i>Boletus longicurvipes</i> AF139688 <i>Boletus longicurvipes</i> AY612812 <i>Octaviania asterosperma</i> <i>Octaviania tasmanica</i> HQ547147 <i>Austroboletus flavidus</i> DPL7541 <i>Durianella echinulata</i> EU293062 <i>Tylopilus aff chromapes</i> 01-513 <i>Tylopilus aff chromapes</i> 01-549 <i>Tylopilus aff virens</i> DQ534621 <i>Tylopilus virens</i> DQ534621 <i>Harrya chromapes</i> HQ161856 <i>Harrya chromapes</i> AY612834 <i>Harrya chromapes</i> AF139709 <i>Retiboletus retipes</i> AF456808 <i>Retiboletus</i> sp MAN-053 <i>Retiboletus retipes</i> AF456830 <i>Retiboletus retipes</i> AF456807 <i>Retiboletus retipes</i> AF456811 <i>Retiboletus retipes</i> AY612821 <i>Retiboletus griseus</i> Bothsn1 <i>Tylopilus</i> sp AF456813 <i>Retiboletus nigerrimus</i> AF456832 <i>Retiboletus flavoniger</i> AF456829
	Leccinellum	<i>Leccinellum corsicum</i> BUF4507	<i>Leccinellum crocipodium</i> 9382809 ^a	
	No clade designated	<i>Spongiforma thailandia</i> DED7873 ^a <i>Retiboletus griseus</i> Both	N/A	

Retiboletus flavoniger AF456828
 Retiboletus ornatipes AF456819
 Retiboletus ornatipes AF456825
 Pulveroboletus curtisii AY612820
 Spongiforma squarepantsii LHBf14 HQ724509
 Spongiforma squarepantsii LHBf01 HQ724510
 Aureoboletus auriporus AY612819
 Aueroboletus viridiflavus
 AY612805
 Aureoboletus auriporus
 Boletus campestris
 Aureoboletus citrinoporus 8719
 Boletellus russellii AF050651
 Boletellus russellii DPL6698
 Aureoboletus innixus
 Aureoboletus innixus 136
 Pulveroboletus auriflammeus AY612818S
 Boletellus shichianus
 Boletus atkinsonianus 4471
 Boletus atkinsonianus 4557
 Boletellus mirabilis AF050652
 Boletellus mirabilis 8717
 Boletellus projectellus sn2 Hor
 Hemileccinum depilaturm AF139712 ^a
 Boletus rubropunctus MB05-003
 Boletus tenax 6641
 Xerocomus chrysonemus JAM0539
 Xerocomus subtomentosus AF514831
 Boletus leptospermi
 Xerocomus illudens AY612840
 Xerocomus illudens AF139714
 Xerocomus lanatus
 Xerocomus subtomentosus AF514832
 Xerocomus silwoodensis JAM0612
 Phylloporus arenicola 27954
 Phylloporus phaeoxanthus MAN-017
 Phylloporus alborufus MAN-022
 Phylloporus leucomycelinus MB05-007
 Phylloporus rhodoxanthus 16 196
 Phylloporus rhodoxanthus U11925
 Phylloporus purpureus MAN-050
 Phylloporus centroamericanus MAN-037
 Phylloporus bellus AY612817
 Xerocomus amazonicus AY612839
 Xerocomus sp AY612841
 Xerocomus hortonii AF139713
 Boletus sp 4698

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Hypoboletus	Aureoboletus	Aureobotelus thibetanus Aureoboletus roxanae DS629-07 Boletus abruptibulbus 4588 Boletellus projectellus	Aureoboletus moravicus Xleo1 Aureoboletus gentilis Pug1 ^a Boletellus shichianus Aureoboletus innixus MB03-101	
	Hemileccinum	Hemileccinum impositum Bn1 ^a Boletus subglabripes 72206bs	N/A	
	Xerocomus	Xerocomus subtomentosus Xs1 ^a Phylloporus pelletieri Pp1 ^a	Xerocomus perplexus MB00-005 Boletus tenax 6871	
	No clade designated	Boletellus chrysenteroides 3838	Boletus morrisii 8206	

Table 5 – (continued)				
Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
No group	Royoungia	No representative	Royoungia boletoides 27 546 ^a Royoungia sp 24836	Boletellus betula AF050642 Boletellus betula AY612797 Gastroboletus vividus 27480 Boletus smithii 8727 Boletus miniatoolivaceus 4091 Boletellus ananas AY612799 Boletellus ananas HQ161853 Cf Boletellus TAA195080 Intsia Boletellus cf ananiceps 00-335 Phophyrellus sp 00-356 Heimioporus retispora AF050650 Boletellus chrysenteroides Boletellus dicymbophilus HQ161852 Boletellus piakarii HQ161861 Boletus morrisii 8206
	No clade	Bothia castanella MB03-053 ^a Austroboletus gracilis 112-96	N/A	Tylopilus aff eximius 01528 Boletus eximius AF139684 Austroboletus niveus Austroboletus novaezealandicae Austroboletus mucosus AY612798 Tylopilus aff conicus 01533 Fistulinella viscida AF456826 Pulveroboletus sp 00317 Pulveroboletus sp 00507 Pulveroboletus sp 00428 Boletus modestus Boletus subluridellus 3737 Boletus bicolor MB06-037 Boletus bicolor var subreticulatus 3818 Boletus sp bicolor 3921 Boletus sensibilis var subviscidus 3929
No group	Bicolor clade	Boletus rufomaculatus4414 Boletus bicolor var. bicolor MB07-001	N/A	Boletus inedulis AY612803 Boletus roseipes 4515 Boletus coniferarum AF456827 Pulveroboletus ravenelii 64-96 Boletus rubripes 8722 Boletus calopus 112606 Boletus gyrodontoides MS5 Boletus inedulis sn2002 Chalciporus ovalisporus Boletus satanas AF336242 Porphyrellus brunneus DQ534630 Boletus pulcherrimus 00291
No group	Dupainii clade	Boletus inedulis MB06-044 Boletus firmus MB06-060 Boletus dupanii JAM0607 Boletus amygdalinus 112605ba	Boletus calopus Bc1 Boletus rhodosanguineus 4254 Boletus luridiformis AT2001807	

Boletus eastwoodiae AT2003096
Boletus satanas AF071528
Boletus rodoxanthus
Boletus erthyropus 3131 USA
Boletus torosus
Boletus sp 4696
Boletus rhodopus 4590
Boletus cf luridus MAN-011 Costa Rica
Boletus luteocupreus
Boletus luridus AF139686
Boletus erythropus AF139863
Boletus junquilleus
Boletus regius
Boletus speciosus var *brunneus* 4258
Boletus abieticola 26763
Boletus appendiculatus 8720
Boletus roseopurpureus 3765
Boletus roseopurpureus 4497
Boletus speciosus
Boletus fechtneri 2003097
Boletus fechtneri AF456821
Boletus floridanus HQ161859
Boletus bicolor AY612800
Boletus pallidoroeseus sn Hor
Boletus subvelutipes AY612804
Boletus vermiculosus
Gastroboletus turbinatus AF3362448
Boletus billieae 4588
Boletus rubrofibrillosus 3094
Phylloboletellus chloephorus
Boletus bicolor snOH
Boletus carminipes 4591
Boletus pallidoroeseus HQ161860
Boletus hypocarcinus DPL6899
Boletus oliveisporus 6823
Pseudoboletus parasiticus 151^a
Chalciporus piperatus NSL-15^a
Chalciporus piperatus AF336244^a
Chalciporus rubinellus
Chalciporus amarellus AF456835
Tylopilus tabacinus 6789
Chalciporus pseudorubinellus DS612-07
Chalciporus pseudorubinellus BN07
Chalciporus amarellus 8434
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Paxillus involutus AF167683^a
Paxillus involutus AF167684^a

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No group	Regius clade	<i>Boletus appendiculatus</i> Bap1 <i>Boletus pulchriceps</i> DS4514 <i>Boletus roseopurpureus</i> MB04-040	<i>Boletus peckii</i> 3959 <i>Boletus regius</i> 11265
No group	No clade	N/A	N/A
No group	Carminipes clade	<i>Boletus carminipes</i> MB06-061 <i>Boletus pulverulentus</i> 9606	<i>Boletus bicolor</i> var <i>borealis</i> 2858 <i>Boletus pseudosensibilis</i> DS615-07
No group 1Chalciporus	No clade Chalciporus	<i>Pseudoboletus parasiticus</i> Xpa1 ^a <i>Chalciporus piperatus</i> MB04-001 ^a	N/A <i>Chalciporus rubinus</i> 4640-3 <i>Chalciporus pseudorubinellus</i> 4302
	No clade designated	No representative	<i>Buchwaldoboletus lignicola</i> ^a Pul1 ^a
Paxillus	Paxillus	No representative	

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Table 5 – (continued)				
Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
	No clade designated	Gyrodon lividus g11 ^a Paragyrodon sphaerosporus MB06-066 ^a	Paxillus vernalis Pv2 Paxillus obscurisporus Po1 Paxillus filamentosus Pf1 N/A	Paxillus involutus AF167682 ^a Paxillus involutus AY612815 ^a Paxillus involutus AF1677018 Paxillus involutus AF167686 ^a Paxillus filamentosus AF167681 Gyrodon lividus AF167677 ^a Gyrodon monticola AF352040 Alpova trappei 28042 Alpova trappei AF071456 Melanogaster variegates Melanogaster broomeianus AF098383 Melanogaster tuberiformis AF167679 Melanogaster tuberiformis AF074919 Melanogaster ambiguous AF352046 Alpova diplophloeus AF071454 Alpova diplophloeus AF352035 Paragyrodon sphaerosporus ^a AF071531 Hydnomerulius pinastri AF352044 ^a Hydnomerulius pinastri China Wang ^a N/A
No group	No clade	Hydnomerulius pinastri ^a	N/A	
Outgroup	N/A	Boletinellus merulioides ^a	N/A	
a Type species for genus.				

separate *Tylopilus* from *Boletus* s.str. The context is often bitter tasting in the type, *T. felleus* and a few other species, but not everyone is able to detect this (personal experience; [Arora 1986](#)).

The species in the three-gene extended *Tylopilus* clade are members of either the stirps *felleus* or *indecisus* of Smith and Thiers, and Singer's *Tylopilus* subsections *Tylopilus* or *Oxydables* ([Singer 1986](#); [Smith & Thiers 1971](#)). The morphological concept of *Tylopilus* circumscribes a non-monophyletic grouping of species, despite recent works that have removed several species previously assigned to either *Boletus*, *Tylopilus* or *Leccinum*, and created the new genera *Australopilus*, *Harrya*, and *Sutorius* ([Halling et al. 2012a, b](#)). [Smith and Thiers's \(1971\)](#) morphological concept of the stirps *felleus* and *indecisus* is species that have smooth spores that are pinkish to vinaceous in print, pileus that is violaceous, vinaceous-brown or tan-ish, with a reticulate stipe. [Singer's \(1986\)](#) concept of the *Tylopilus* sections *Tylopilus* and *Oxydables* encompasses smooth spored species, with a spore print that is pinkish to a grey-brown colour, a stipe that is typically reticulate, and a pileus colour that is highly similar to [Smith and Thiers's \(1971\)](#) concept. Further sampling is needed to know if these stirps and sections are sufficient to circumscribe the members of the least inclusive clade containing *T. felleus*.

Strobilomyces clade [100/95/1.0]—This placement of the *Strobilomyces* clade in relation to other Boletineae genera is novel. *Afroboletus luteolus* receives strong support as the sister group of *Strobilomyces* [100/95/1.0]. *Afroboletus* was first recognized as unique by [Singer \(1945\)](#) and placed in *Strobilomyces* section *Pterospori*; the only other section in *Strobilomyces* besides section *Strobilomyces*. Later, [Pegler & Young \(1981\)](#) recognized *Afroboletus* as a distinct genus based on unique spore morphology. As with *Tylopilus*, the clade containing all of the three-gene *Strobilomyces* clade members (including *Afroboletus*) receives no support in the constrained analysis. However, a clade containing the three-gene *Strobilomyces* spp, and not *Afroboletus luteolus*, receives 99 BS support in the constrained analysis and includes *S. dryophilus*, a different *S. floccopus* exemplar and a different *Strobilomyces* sp. exemplar.

The species of the *Strobilomyces* clade are morphologically distinct, with a veil and coarsely fibrillose to conspicuously scaly pileus with scales turning grey to black ([Smith & Thiers 1971](#)). *Strobilomyces* spores are globose, with reticulate or variously echinate to cristate ornamentation (see [Table 2](#)), never having longitudinal costae and a dark rusty brown to blackish brown spore print ([Smith & Thiers 1971](#); [Pegler & Young 1981](#)). [Pegler & Young \(1981\)](#) moved members of [Singer's](#) section *Pterospori* to *Afroboletus* based on spore shape and ornamentation, subglobose to ellipsoid, longitudinally costate, with a basal thickened rim (around apiculus), and the restriction of these species to Africa. Because recent works have described both morphological affinities and clades containing a genus or closely related genera with species from multiple continents, e.g. *Sutorius* spp., *Harrya chromapes* and *H. chromapes* like species and *T. ballouii*, it seems plausible that *Afroboletus* may be restricted to Africa and could either be the sister genus of *Strobilomyces* or merely a geographically isolated *Strobilomyces* lineage ([Wolfe & Bougher 1993](#); [Osmundson & Halling 2010](#); [Halling et al. 2012a, b, 2008](#)).

Xanthoconium stramineum and *Porphyrellus porphyrosporus*—Both are type species of their respective genera, are strongly supported as members of the anaxoboletus group, but their placement within the anaxoboletus group in respect to the other members is not resolved. This is in conflict with the topology presented by [Hosen et al. \(2012\)](#), but there is no support for nodes along the backbone of that phylogeny either.

In the constrained analysis, *P. porphyrosporus* forms a clade with 99 % BS support with five other *Porphyrellus* taxa. *Xanthoconium stramineum* forms a clade with 80 % BS containing four other *Xanthoconium* taxa and *Tylopilus badiceps*, which may be misidentified. The placement of the clade containing *X. stramineum* and *P. porphyrosporus* in the three-gene analysis is topologically similar to the results of other studies ([Desjardin et al. 2008, 2009](#); [Dentinger et al. 2010](#)).

Morphologically, *Xanthoconium* was separated from *Boletus* based on unique golden yellow colour of the spores under microscopic view and in deposit, and the absence of certain pigments, though [Singer](#) neither mentions nor provides a citation for these different pigments ([Singer 1944, 1947, 1986](#)). Furthermore, [Singer \(1986\)](#) separated *Xanthoconium* from *Tylopilus* based on the colour of the spores, the unchanging white, mild context, and the non-reticulate, non-pustulate-floccose stipe. [Smith and Thiers's \(1971\)](#) concept did not recognize *Xanthoconium* as unique, instead placing the species in *Boletus* subsection *Versicolores* strips *Affinis*.

Later works included reticulate species in an expanded concept of *Xanthoconium* ([Smith 1973](#); [Wolfe 1987](#); [Halling & Both 1998](#)). [Singer \(1944\)](#) originally placed *Xanthoconium stramineum* and *X. affine* in *Xanthoconium*, both of which were described as having a glabrous stipe, but other authors have reported reticulation at the apex of the stipe of these two species ([Wolfe 1987, 1988, 1989](#); [Halling & Both 1998](#); [Bessette et al. 2000](#)). [Halling & Both \(1998\)](#) placed *Boletus separans* in the genus, partially based on previous inclusions of reticulate species in the genus. Molecular data does not support the inclusion of *B. separans* in *Xanthoconium* (see [Figs 1 and 2](#)). *Xanthoconium separans* is described as reticulate on at least the upper portion of the stipe or finely reticulate as in *Boletus edulis*, while all members of *Xanthoconium* with molecular support, *X. stramineum* and *X. affine* in this study and *Xanthoconium purpureum* (= *Boletus purpureofuscus* H.V. Smith & A.H. Smith) in [Dentinger et al. \(2010\)](#), are described as glabrous or reticulate only at the very apex of the stipe ([Smith & Thiers 1971](#); [Singer 1986](#); [Wolfe 1987, 1988](#); [Bessette et al. 2000](#)). Additionally, *X. separans* shares the 'stuffed' pore feature found in all non-secotioid members of the 'porcini' group ([Smith & Thiers 1971](#); [Singer 1986](#); [Dentinger et al. 2010](#)).

'porcini' Clade [99/93/1.0]— This group is recovered with similar placement in the Boletaceae as reported by [Dentinger et al. \(2010\)](#) and [Li et al. \(2011\)](#). Following the terminology outlined by [Dentinger et al. \(2010\)](#), the clade containing *Boletus edulis* but not *Xanthoconium separans* is *Boletus sensu stricto*, and the clade containing *X. separans* but not *B. edulis* is 'alloboletus.' *Notholepiota areolata* is placed in the 'porcini' clade for the first time. *Notholepiota areolata* was originally described as a secotioid member of the Agaricaceae by [Horak \(1971\)](#), but was later shown to have affinities with the Boletaceae ([Vellinga 2004](#)). *Xanthoconium separans* was previously placed

in *Boletus* and removed based on morphological characters, but is not closely related to the type of *Xanthoconium* (see Figs 1 and 2; Halling & Both 1998).

An equivalent 'porcini' clade is not supported in the constrained analysis, but the two major clades inside the 'porcini' are supported in the constrained analysis, with 89 % BS for *Boletus* s.str. clade and 95 % BS for the 'alloboletus' clade, including *N. areolata*.

Morphologically, *Boletus* s.str. has been united by a variety of characters: white (or pale yellow) unchanging context tissue that is mild tasting, smooth spores that are yellow-brown or olive-brown in deposit, stipe with enlarged base and reticulum, a hyphal mat (cheilocystidia) that covers the tubes of immature specimens, leading to the 'plugged' or 'stuffed' pore feature (Coker & Beers 1943; Smith & Thiers 1971; Singer 1986; Dentinger et al. 2010). The 'stuffed' pore feature has been reported in species that are distantly related to *Boletus* s.str., including a *Phlebopus* sp. (Sclerodermatineae) (Singer 1986; Miller Jr et al. 2000) and is mimicked in *Xanthoconium* sensu Singer by compressed pore mouths in young specimens (Singer 1986). Despite variations in morphological features, a combination of white context, reticulated stipe, smooth spores, pore surface that is white or nearly white when young, basal mycelium that is white, and a 'stuffed' pore appearance due to a mycelium layer growing over the pore surface appear unique to the 'porcini' clade (Coker & Beers 1943; Smith & Thiers 1971; Singer 1986; Dentinger et al. 2010). Dentinger et al. (2010) provide an in-depth overview of morphological traits in the 'porcini' group.

Xerocomellus clade [100/100/1.0]—The placement of the *Xerocomellus* clade in the 'anaxoboletus' is mirrored in Li et al. (2011), though there is no support for a group corresponding to the 'anaxoboletus' in Li et al. (2011). The *Xerocomellus* clade contains the type species, *Xerocomellus chrysenteron*, and the division between *Xerocomellus* and *Xerocomus subtomentosus* based on morphological features is supported by a host–parasite study of the group and the phylogeny in Figs 1 and 2 (Douhan & Rizzo 2003; Šutara 2008). Further, Peintner et al. (2003) support the division of the *Xerocomellus* and 'rubellus' clades. The *Xerocomus/Xerocomellus* containing clades (*Xerocomellus*, 'rubellus', and 'badius') in the 'anaxoboletus' group do form an unsupported clade, but are morphologically more similar to each other than other members of the 'anaxoboletus'.

'rubellus' clade [100/100/1.0]—Two 'rubellus' specimens were included, one from Eastern USA and one from Western USA. The two specimens form a clade separate from other *Xerocomellus* species. Inclusion of 'rubellus' clade in the *Xerocomellus* clade is only supported by 71 % BS support in the ML analysis. Further, the constrained analysis gives 96 % BS support to the clade containing *Xerocomellus rubellus* species.

'badius' clade [79/-/0.96]—The two *Xerocomus badius* specimens form a clade with *Boletus pallidus*, albeit with weak support. *Boletus pallidus* has been previously recovered in a close relationship to *X. rubellus* (Desjardin et al. 2009). Inside this group the *X. badius* clade receives maximal support. *Xerocomus badius* species form a clade with 100 % BS support in the constrained analysis including one non-*Xerocomellus* species, *Boletus glabellus* 00-194.

Šutara (2008) placed the members of the *Xerocomellus*, 'badius', and 'rubellus' clades in the genus *Xerocomellus* based on

morphology, however, they form a clade that lacks support in our analyses. Members of these clades were distinguished from *Boletus* based on a hymenophore trama that is intermediate between boletoid and phylloporoid types, a lateral stipe trama that is reduced, no thicker than 30–40 µm and non-gelatinized or absent lateral stipe trama, and spores that are longitudinally striate or smooth, never bacillate (Šutara 2008). *Boletus* has spores that are always smooth, boletoid type hymenophore trama, and lateral stipe stratum that is 60–90 µm thick and often gelatinized (Šutara 2008). The gelatinized trama appears as viscid fluid and/or partially dissolved hyphae surrounding the normal hyphae in the trama.

Additionally, Šutara (2008) separated *Xerocomellus* from *Xerocomus* on the lack of bacillate spores, an intermediate form of hymenophore trama that is never of the phylloporoid trama type, and a pileipellis that is a palisadoderm that typically is heavily encrusted; not a trichoderm with little or no encrustations. See Table 1 for the overview of the morphological features of *Boletus*, *Xerocomellus*, *Xerocomus*.

'leccinoid' group [95/-/0.99]—There are two clades, *Leccinum* and *Leccinellum*, and two additional species, *Spongiforma thailandica* (type specimen) and *Retiboletus griseus* in this group.

Leccinum clade [100/100/1.0]—The clade contains *Leccinum albellum* and *Leccinum scabrum*. The clade in the constrained analysis containing the two *Leccinum* species receives 100 % BS support and contains 20 extra *Leccinum* taxa. A more inclusive clade in the constrained analysis contains *Octaviania asterosperma* and *Octaviania tasmanica*, but only receives 60 % BS support.

Leccinellum clade—The separation of *Leccinellum corsicum* and the type species of *Leccinellum*, *Leccinellum crocipodium*, from the genus *Leccinum* is supported in our analysis [81/95/-], and the clade containing both *Leccinellum* and *Leccinum* receives maximum support in all analysis methods.

Previous studies support the division of *Leccinum* and *Leccinellum*, and the close relationship between the two genera (Binder & Besl 2000; Bresinsky et al. 2003; Dentinger et al. 2010). The branch leading to *Leccinum* and *Leccinellum* in the constrained analysis receives 81 % BS support. There is no support for a *Leccinellum* clade in the constrained analysis.

Leccinum and *Leccinellum* species have been circumscribed based on Singer's (1986) broad classification, or Smith and Thiers's (1971) more strict classification. Smith & Thiers (1971) used the darkening stipe scabrosities and white context as the defining characteristic of the genus *Leccinum*. Sequence and chemotaxonomic analysis supports a *Leccinum* concept sensu Smith and Thiers over that of Singer's (Singer 1986; Binder & Besl 2000; Bakker & Noordeloos 2005; Binder & Hibbett 2006). Bresinsky et al. (2003) separated *Leccinellum* from *Leccinum* based on a yellow context, a difference that Smith & Thiers (1971) noted as well.

The placement of *S. thailandica* as sister to the clade containing 'leccinoid' species is inconsistent with the studies of Desjardin et al. (2009), Li et al. (2011), and Hosen et al. (2012) where it is sister to *Porphyrellus* and *Strobilomyces*. However, Desjardin et al. (2009) used only nuc-18S sequence data, which is too conserved to resolve the *Boletineae*. Despite this, Li et al. (2011) have support for a clade containing *Spongiforma*, *Porphyrellus*, and *Strobilomyces*, and Hosen et al. (2012) have support for a clade containing *Spongiforma* and *Porphyrellus*. In

the constrained analysis *S. thailandica* forms a strongly supported clade [100] with the only other species in the genus, *Spongiforma squarepantsii*. Recently, a new genus, *Borofutius*, was shown to be related to *Spongiforma* (Hosen et al. 2012).

Morphologically, *Spongiforma* species are distinct. The two species known are both gasteroid, appearing like a sponge and are unlike any other members of the Boletales. There is no gross morphological similarity to place *Spongiforma* in the 'leccinoid' clade, or in any other Boletineae clade.

Retiboletus griseus is the sister group of the rest of the 'leccinoid' group, which is congruent with previous studies (Dentinger et al. 2010; Lebel et al. 2011; Li et al. 2011). In the constrained analysis *R. griseus* is in a distinct, albeit unsupported, clade that contains all *Retiboletus* species in the analysis and three taxa that are not *Retiboletus* (See Sup. Fig 1).

Retiboletus species are highly reticulate along the entire stipe, but morphologically very similar to the classical description of *Boletus*; e.g. smooth spores, olive or brownish spore print (See Table 1; Smith & Thiers 1971; Singer 1986). *Retipolides*, a unique pigment group, was reported in *Retiboletu ornatipes*/*Retiboletu retipes* (Herman 1980), and with additional molecular analysis, the occurrence of this unique pigment provided the description of *Retiboletus* (Binder & Bresinsky 2002b). Not all *Retiboletus* spp. that were placed in the genus with molecular methods have been tested for the occurrence of retipolides, but in all *Retiboletus* species chrysocystidia, which are rare or absent in *Boletus*, are present (Smith & Thiers 1971; Singer 1986; Binder & Bresinsky 2002b; Watling 2008).

While the type species of *Leccinum* is not included in core analyses, the topology of the *Leccinum*, *Leccinellum*, and *Retiboletus* containing clade is identical to the topology of the combined analysis in Dentinger et al. (2010). All members of the 'leccinoid' clade (not including *Spongiforma*) have a boletoid type tube trama, and most have smooth spores. The exception is *S. thailandica* which has rough spores in SEM (Desjardin et al. 2009).

'hypoboletus' group[89/73/0.99]—The 'hypoboletus' group contains four supported clades, three of which we have given designations: *Aureoboletus*, *Hemileccinum*, and *Xerocomus*.

Aureoboletus clade [95/77/0.99]—The *Aureoboletus* clade contains eight taxa, five *Aureoboletus* species, two species currently classified in the genus *Boletellus* by some authors, and one *Boletus* species. The type species, *Aureoboletus gentilis* is nested in the clade. The placement of *Boletellus* species within the *Aureoboletus* clade has been reported in previous studies (Binder & Hibbett 2006; Desjardin et al. 2009; Dentinger et al. 2010). Dentinger et al. (2010) included the type species of *Boletellus*, *Boletellus ananas*, which is placed outside of the clade containing the *Aureoboletus* species in their analysis. The phylogeny shown by Li et al. (2011), gives indirect support for this relationship, with *Boletellus mirabilis* and *Boletellus projectellus* as sister group to *Xerocomus illudens* and *X. subtomentosus*, a relationship that has been recovered in the three-gene extended analysis with taxon pruning.

Aureoboletus was circumscribed by Pouzar (1957), but Singer's (1986) concept placed all the *Aureoboletus* spp. included in our analysis in the genus *Pulveroboletus* sect. *Auripori*, based on the gelatinized pileus, voluminous cheilocystidia with yellow sap in ammonia solutions, and an ochraceous

buff spore print as the defining characteristics. The classification of Smith & Thiers (1971) placed the majority of the *Aureoboletus* clade in *Boletus* section *Subtomentosi* subsection *Versicolores stirps Roxanae*, but placed *Boletellus projectellus* in the Subsection *Mirabiles* of section *Subtomentosi*. Singer's (1986) concept placed *B. projectellus* in section *Mirabilis* of *Boletellus*, based on the smooth spores, not bluing but often staining lemon yellow tissue, and association with *Pinaceae* and *Fagaceae*. This is in conflict with Smith & Thiers (1971) *Boletellus* concept, which excluded *B. mirabilis* and *B. projectellus*, placing them in *Boletus* instead. However, here we use the Singer taxonomy as both species are shown to be distantly related to *B. edulis* (Fig 2). There are no apparent morphological characters uniting the *Aureoboletus* clade. Both smooth and ornamented (only *Boletellus schichianus*) spores occur in the clade; the hymenophore colour is variable: bright lemon yellow to 'pale olive-fresh'; stipe ornamentation ranges from non-reticulate, to longitudinally striated, to highly or coarsely reticulate; spore print colour is reported as a pale ochraceous buff to dark olive brown; gelatinization of the stipe and pileus is found in some species; and non-veiled species and veiled species are present: gelatinous veil, *Aureoboletus thibetanus* and typical veil, *B. projectellus* are present (Smith & Thiers 1971; Singer 1986). In depth taxon sampling is necessary to resolve phylogenetic relationships in this clade.

Hemileccinum clade [100/100/1.0]—This well-supported clade is sister to the *Aureoboletus* clade. It contains the type species of *Hemileccinum*, *Hemileccinum impositum* and *Boletus subglabripes*. This is the first analysis containing both *H. impositum* and *B. subglabripes*. Previously, *B. subglabripes* had been placed as a close relative of *Leccinellum*, *Leccinum* and *Chamonixia* (Desjardin et al. 2008) and *Rossbeevera* (Lebel et al. 2011). Desjardin et al. (2009) placed *H. impositum* and *Hemileccinum depilatum* as sister to a clade equivalent to the three-gene extended analysis *Xerocomus* clade. Inclusion of *H. depilatum* in this analysis would provide a more conclusive view of the *Hemileccinum* clade. *Hemileccinum* species have been placed in *Boletus*, *Xerocomus*, and *Leccinum* using different generic concepts (Smith & Thiers 1971; Singer 1986; Binder & Besl 2000). Šutara (2008) erected *Hemileccinum* to accommodate species that are smooth spored, with hymenophore trama that is boletoid, lateral stipe stratum that is gelatinous, and stipe scarbrosities.

Xerocomus clade [100/100/1.0]—This clade contains the type species of *Xerocomus* and *X. subtomentosus*. Previous studies have reported the distant relationship among the members of the *Xerocomus* clade and the *Xerocomellus*, 'badius,' and 'rubellus' clades (Douhan & Rizzo 2003; Peintner et al. 2003). As previous studies have found, Singer's view of the close relationship between *Xerocomus* and *Phylloporus* is upheld (Singer 1986; Binder & Hibbett 2006; Hosen et al. 2012; Neves et al. 2012).

Smith and Thiers's (1971) concept did not differentiate (at the generic level) between *Xerocomus* s.l. (*Xerocomus* and *Xerocomellus* including the 'badius' and 'rubellus' clades) and *Boletus* s.l., stating that the differences in the hypha and hymenophore trama, when combined with an ambiguous definition from other authors (including Singer), caused them to place *Xerocomus* s.l. species in the genus *Boletus*. Molecular work has helped to differentiate between the two genera,

but Šutara (2008) provides a clear morphological framework, which is supported by molecular data, to separate the problematic genera.

The key characteristics of *Xerocomus* are: a non-gelatinized lateral stipe stratum, bacillate ornamented spores, and hymenophore trama of the phylloporoid type (Šutara 2008). This is different from *Boletus* s.str. which generally has a gelatinized lateral stipe stratum with hyphae that are distant from each other, smooth spores, and a boletoid type hymenophore trama. Furthermore, it is separated from *Xerocomellus* based on the presence of longitudinally striate or smooth spores that are never bacillate in *Xerocomellus*, hymenophore trama that is intermediate between boletoid and phylloporoid type, the lack of a lateral stipe stratum (or a very reduced lateral stipe stratum, 30–40 μM compared to 80–200 μM in *Xerocomus* s.str.), and a pileipellis constructed of hyphae with encrusted walls (Šutara 2008).

Xerocomus s.str. is separated from *Hemileccinum* by having bacillate spores, a hymenophore trama that is of the phylloporoid type and a lack of stipe scabers or scabrousness that are typical of *Hemileccinum* (Šutara 2008). The morphological distinctions between *Boletus*, *Hemileccinum*, *Xerocomellus*, and *Xerocomus* are succinctly and sufficiently described by Šutara (2008).

Royoungia clade [100/100/1.0]—The monotypic *Royoungia* clade contains two specimens of *Royoungia boletoides* and has previously been shown as closely related, without support, to *Harrya chromapes* (as *Tylopilus*, Desjardin et al. 2009) and to the new genus *Zangia* (Li et al. 2011). Li et al. (2011) demonstrated a sister taxa relationship between *Zangia* species and *T. chromapes* using nuc-lsu, tef1, mitochondrial large subunit, mitochondrial small subunit, and atp6.

Recent work by Halling et al. (2012b) has shown strong support for a clade containing *Zangia*, *Royoungia*, *Harrya*, *Australopilus*, and *Tylopilus* species which are not *Tylopilus* s.str. All things equal, this clade would be sister to the clade B in Figs 1 and 2. The clade appears to be united by pigment in the base of the stipe for stipitate species, and in the peridium of *Royoungia*, though the chemical work to identify the pigment to test for homology across the genera has not been performed. **bicolor Clade [100/100/1.0]**—This clade contains two species, *Boletus bicolor* var. *bicolor* sensu Peck and *Boletus rufomaculatus*. The two members of the clade are characterized by distinct yellow pore surface, with minute pore openings, and stipe colouration and are closely related based on morphology (Bessette et al. 2000; Both 1998; Smith & Thiers 1971). Peck's *B. bicolor* is an illegitimate name; *B. bicolor* was originally described by Raddi in 1807, though the details of what species Raddi named *B. bicolor* have been lost (Peck 1870; Raddi 1807; Snell & Dick 1941). We have chosen to use *Boletus bicolor* sensu Peck, despite the illegitimate naming, as two of the three synonyms available would create a different polytomy in *Xerocomus* and *Boletus* and the third places the species in the now defunct *Ceromyces* (Murrill 1909; Singer 1947 1986). Correcting this taxonomic confusion necessitates the description of a new genus, but at this time it would be premature to describe a new species based on the small amount of data on hand.

dupainii clade [71/–/–]—While this clade has support in only ML methods, it is separated from residual regius clade

by strong support [94/81/0.99]. Only *Boletus calopus* and *Boletus inedulius* species have been previously published in phylogenetic studies (Binder & Hibbett 2006; Desjardin et al. 2009, 2008; Li et al. 2011; Halling et al. 2012a). A clade containing both *B. calopus* and *B. inedulius* is supported in all analysis except for Desjardin et al. (2008), which did not include *B. inedulius*. Species relationships inside dupainii are poorly resolved.

regius clade [100/100/1.0]—Inside this clade only *Boletus regius* and *Boletus appendiculatus* have been published in molecular studies (Binder & Hibbett 2006; Halling et al. 2012a).

carminipes clade [82/–/0.96]—This group contains *Boletus carminipes*, *B. bicolor* var. *borealis*, *Boletus pseudosensibilis*, and *Boletus pulverulentus*. It is a mixture of different sections of both Singer's (1986) concept of the genus *Boletus* and the sections of Smith and Thiers's (1971) concept of the genus *Boletus*.

The above groups and clades are supported [79/73/0.99] as a clade, and are more closely related to each other than the remaining members of the Boletineae, *Pseudoboletus parasiticus*, the *Chalciporus* clade, and the *Paxillaceae*.

Pseudoboletus parasiticus—*Pseudoboletus parasiticus* is placed as sister to the species indicated by node B in Figs 1 and 2 [100/100/1.0]. This is consistent with the study of Desjardin et al. (2009). This bolete is distinctive and difficult to misidentify when found on its host, *Scleroderma citrinum* (Order Boletales, Suborder Sclerodermatineae), and more rarely on other *Scleroderma* spp (Smith & Thiers 1971; Singer 1986). *Pseudoboletus parasiticus* is characterized by yellow–brown fibrils similar in appearance to the scabers of *Leccinum* and *Lecicinellum*, an instant cinnabar-orange KOH reaction of the stipe, orange-ochraceous KOH reaction of pileus, and Phylloporus-type trama (Smith & Thiers 1971; Singer 1986; Šutara 1991). However, the defining characteristic is growth from a fruiting body from *Scleroderma* spp.

This clade may contain multiple species. Singer (1986) mentions *Xerocomus astraicola* as a possible sister species, and the fruiting bodies of *P. parasiticus* were collected near, but not on, *Scleroderma* spp. formed ECM in culture with *Pinus resinosa*, possibly indicating a new species (Richter & Bruhn 1989).

Chalciporus group [100/100/1.0]—The *Chalciporus* group is the earliest branching lineage of the family Boletaceae based on sampling used in this study, which is indicated at node A on Figs 1 and 2. The *Chalciporus* group includes the monophyletic genus *Chalciporus* and *Buchwaldoboletus lignicola*. The placement of the *Chalciporus* group in Figs 1 and 2 is supported by previous studies (Binder & Bresinsky 2002b; Binder & Hibbett 2006; Desjardin et al. 2009; Li et al. 2011).

Singer (1986) defined *Chalciporus* as having a solid stipe, a hymenophore that is dull cinnamon red, carmine to wine red or pink, having basal mycelium that is bright yellow in part or in all, and a taste that is either mild or acrid peppery. Furthermore, Singer (1986) describes *Chalciporus* as lacking clamp connections and having adnate to subdecurrent hymenophore, but never arcuate. Smith & Thiers (1971) use similar characters to describe *Chalciporus* species, but place them in the section *Piperati* of *Boletus*.

Buchwaldoboletus was described based on the lignicolous habitat, decurrent and arcuate hymenophore, bluing, yellow flesh and yellow basal mycelium (Pilát 1969). In addition, *Buchwaldoboletus* spp. lack clamp connections in the basidocarp,

but clamp connections are present in hyphae of cultures (Pilát 1969; Singer 1986; Watling 2008). This is similar to the description of *Chalciporus*, and the two genera are united by the yellow basal mycelium, lack of veil, and overall hymenophore structure (Pilát 1969); the respective type species of the genera may also share a mycoparasitic nutritional mode.

Paxillaceae [100/97/0.99]—The Paxillaceae comprises members of the genus *Paxillus*, and the type species of *Gyrodon* and the monotypic *Paragyrodon sphaerosporus*. *Paxillus* is monophyletic in the Paxillaceae[100/100/1.0] and the Paxillaceae currently contains only ECM fungi.

The family includes morphologically diverse taxa. The genus *Paxillus* comprises only lamellate fungi, while *Gyrodon lividus* produces a tubular hymenophore resembling that of *Boletinellus merulioides* (Sclerodermatineae, Boletales), and *Paragyrodon sphaerosporus* is one of the few species in the Boletineae with a veil (Smith & Thiers 1971; Singer 1986; Binder & Bresinsky 2002a; Binder & Hibbett 2006). Despite the large morphological differences found in the Paxillaceae, all members in this study have clamp connections in the basidiocarp, an uncommon occurrence in the Boletineae (Singer 1986; Binder & Bresinsky 2002a; Watling 2008).

Hydnomerulius pinastri—This resupinate, brown-rot fungus was considered as a member of the Boletineae incertae sedis (Binder & Hibbett 2006), and our analyses lack support for its placement in both the three-gene core and extended analyses. It is similar to the members of the Paxillaceae in spore shape and ornamentation, and the presence of clamp connections (Watling 2008).

Conclusions

The analyses presented here identify clades containing the type species for the following genera: *Aureoboletus*, *Boletus*, *Chalciporus*, *Hemileccinum*, *Leccinellum*, *Leccinum*, *Paxillus*, *Royoungia*, *Strobilomyces*, *Tylopilus*, *Xerocomellus*, and *Xerocomus*. The type species of *Buchwaldoboletus*, *Gyrodon*, *Notholepiota*, *Phylloporus*, *Paragyrodon*, *Pseudoboletus*, *Spongiforma*, and *Xanthoconium* have also been sampled. Three new, strongly supported lineages within the Boletineae were recovered: the anaxoboletus, leccinoid, and hypoboletus groups. These results could form the basis for a genus-level reclassification of the Boletineae. However, to achieve a comprehensive phylogenetic classification of the Boletineae, many more taxa need to be sampled with multiple loci, including taxa from the *Xerocomellus/Xerocomus* containing clades, the leccinoid group and *Aureoboletus* clade.

The genus *Boletus* should be restricted to the 'porcini' clade; this would reduce the magnitude of *Boletus* from approximately 300 to approximately 30 species (Kirk et al. 2008; Dentinger et al. 2010). We return *Boletus separans* to the genus from *Xanthoconium*, present *Boletus subalpinus* comb. nov, formally transferring *Gastroboletus subalpinus* to *Boletus*, and transfer *Notholepiota areolata* from the Agaricales to *Boletus* s.str., *Boletus semigastroideus* nom. nov. By far the largest challenge to future phylogenetic studies in the Boletineae is resolving the relationships of the roughly 270 species that have been classified in *Boletus* but are not likely to be placed in the 'porcini' clade using molecular markers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funbio.2013.04.008>.

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