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Phylogenetic overview of the *Boletineae*

Mitchell E. NUHN[a,1], *Manfred BINDER*[b,1], *Andy F. S. TAYLOR*[c,e], *Roy E. HALLING*[d], *David S. HIBBETT*[a,*]

[a]*Dept. of Biology, Clark University, 950 Main St., Worcester, MA 01610, USA*
[b]*CBS-KNAW Fungal Biodiversity Centre, Evolutionary Phytopathology, Institute of the Royal Netherlands Academy of Arts and Sciences, Uppsalalaan 8, 3584 CT Utrecht, Netherlands*
[c]*The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, Scotland, UK*
[d]*Institute of Systematic Botany, New York Botanical Garden, 2900 Southern Blvd., Bronx, NY 10458-5126, USA*
[e]*Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Dr., Aberdeen AB24 3UU, UK*

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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-alpha (*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.

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 multilocus 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.

* *Corresponding author*. Tel.: +1 5087937420.
E-mail address: dhibbett@clarku.edu (D. S. Hibbett).
[1] These authors contributed equally to the 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]	***Boletoideae***	*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*
	Veloporphyrellus	*Heimioporus*
	Xanthoconium[a]	*Heliogaster*
	Gyrodontoideae	*Hemileccinum*
	Gyrodon	*Leccinellum*
	Paragyrodon	*Leccinum*
	Meiogranum	*Mycoamaranthus*[f]
	Strobilomyceloideae	*Notholepiota*
	Strobilomyces	*Paxillogaster*[f]
	Xerocomoideae	*Phylloboletellus*
	Phylloporus	*Phyllobolites*
	Tubosaeta	*Phylloporus*
	Xerocomus[a]	*Porphyrellus*
	Paxillaceae	*Pseudoboletus*
	Paxillus	*Pulveroboletus*
		Retiboletus
		Rhodactina
		Rossbeevera
		Royoungia
		Rubinoboletus
		Sinoboletus[f]
		Spongiforma
		Strobilomyces
		Sutorius
		Tubosaeta
		Tylopilus
		Xanthoconium
		Xerocomellus
		Xerocomus
		Zangia
		Paxillaceae
		Alpova
		Austrogaster[f]
		Gyrodon
		Hoehnelogaster[f]
		Meiorganum[f]
		Melanogaster
		Paragyrodon
		Paxillus

Table 1 – (*continued*)

Boletaceae of Smith & Thiers (1971)	Boletineae of Singer (1986)	Current
		Incertae sedis
		Hydnomerulius

a Species of *Boletus sensu* Smith & Thiers (1971) are distributed across four genera of Singer (1986).
b Currently classified in *Sclerotermatineae*.
c Species of *Leccinum sensu* Singer are distributed across *Leccinum* and *Tylopilus* (pro parte) by Smith & Thiers (1971).
d Species of *Tylopilus sensu* Smith & Thiers (1971) are distributed across *Tylopilus* and *Porphyrellus sensu* Singer (1986). Single Smith and Thiers.
e Currently classified in *Suillineae*.
f No molecular data available or no *nuc-lsu*, *tef1*, and *RPB1*.

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 $FeSO_4$. 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 *Paragyrodon* 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; Drehmel *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-alpha (*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′-GAATGY ATATGYGTWAACTGTGG-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).

Table 2 – Morphological features of selected *Boletineae* genera.

Genera	Spore ornamentation				Stipe ornamentation					Veil		
	Smooth	Bacil-late	Longitu-ninally ridged	Other	Smooth	Longitu-dinally striated	Reticu-late	Scabr-ous	Other	None	Partial	Double
Boletaceae												
Afroboletus	○	○	●	○	○	○	○	○	●		●	○
Aureoboletus	●	○	○	○	●	○	○	○	○	●	●**	○
Austroboletus	○	○	●	○	●	●	○	○	○	●	○	○
Boletellus	●	○	●	●	●	○	●	○	○	●	●	○
Boletus	●	○	○	○	●	●	●	○	○	●	○	○
Bothia	●	○	○	○	●	○	●	○	○	●	○	○
Buchwaldoboletus	●	○	○	○	●	○	○	○	○	●	○	○
Chalciporus	●	○	○	○	●	○	○	○	○	●	○	○
Gastroboletus	●	○	○	○	●	●	●*	○	○	●	○	○
Hemileccinum	●	○	○	○	○	○	○	○	●	●	○	○
Leccinellum	●	○	○	○	○	○	○	●	○	●	○	○
Leccinum	●	○	○	○	○	○	○	●	○	●	○	○
Notholepiota	●	○	○	○	●	○	○	○	○	●	○	○
Phylloporus	●	●	○	○	●	●	○	○	○	●	○	○
Porphyrellus	●	○	○	○	●	●	○	○	○	●	○	○
Pseudoboletus	●	○	○	○	●	○	○	○	●	●	○	○
Retiboletus	●	○	○	○	○	○	●	○	○	●	○	○
Royoungia	●	○	○	○	○	○	○	○	○	●	○	○
Spongiforma	●	○	○	○	○	○	○	○	○	●	○	○
Strobilomyces	○	○	○	●	○	○	○	○	●	○	●	○
Tylopilus	●	○	○	○	●	○	●	○	○	●	○	○
Xanthoconium	●	○	○	○	●	○	●	○	○	●	○	○
Xerocomellus	●	○	○	○	●	●	○	○	○	●	○	○
Xerocomus	●	●	○	○	●	●	○	○	○	●	○	○
Paxillaceae												
Paragyrodon	●	○	○	○	○	○	○	○	○	○	●	●
Paxillus	●	○	○	○	○	○	○	○	○	●	○	○
Gyrodon	●	○	○	○	○	○	○	○	○	●	○	○
Incertae sedis												
Hydnomerulius	●	○	○	○	○	○	○	○	○	○	○	○

● – Feature present in at least one species in genus.
○ – Feature absent in all species.
? – unknown.
* Stipe is highly reduced, nearly not present.
** Veil is present in the form of a gelatinous mass that disappears with age, and is not considered to be a veil by all mycologists.

For sequencing, 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;

Hymenophore			Fruiting body morphology				Hypogeal	Clamp connections	Caulobasidium	Caulohymenium	Cystida			
Lamellate	Poroid		Pileatestipitate	Secotioid	Gasteroid	Resupinate					Caulocystida	Cheilocystida	Chrysocystidia	Pleurocystida
	Regularly poroid	Irregularly poroid												
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	○	○	○	●	●	●
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	●	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
●	○	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	●	○	●	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	●	●	○		○	●
○	○	○	●	○	○	○	○	○	●	●	●	●	○	●
○	●	○	●	○	○	○	○	○	●	●	●	●	○	●
○	○	●	○	●	○	○	○	○	○	○	?	?	?	?
●	○	○	●	○	○	○	○	○	○	○	○	●	●	●
○	●	○	●	○	○	○	○	○	○	○	○	●	○	●
○	●	○	●	○	○	○	○	○	○	○	○	○	○	●
○	●	○	●	○	○	○	○	○	○	○	○	●	●	●
○	○	○	○	○	●	○	●	○	○	○	○	○	○	○
○	○	○	○	○	●	○	○	○	○	○	Cystidia Present			
○	●	○	●	○	○	○	○	○	○	○		●		●
	●		●	○	○	○	○	○	●	●	●	●	●	●
○	●	○	●	○	○	○	○	○	○	○	●		●	●
○	●	●	●	○	○	○	○	○	●	●	●	●	●	●
○	●	●	●	○	○	○	○	○	●	●	●	●	●	●
○	○	●	●	○	○	○	○	●	○	○	●	●	○	●
●	○	○	●	○	○	○	○	●	○	○	○	●	○	●
○	○	●	●	○	○	○	○	●	○	○	○	●	○	●
○	○	○	○	○	○	●	○	●	○	○	Cystidioles			

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, the proportion

Table 3 – GenBank accession numbers—Missing.

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

Table 3 – (*continued*)

Species	Isolate ID	Location	Date	Collector/ Identifier	*nuc-lsu*	*tef1*	*rpb1*
Boletus subglabripes	72 206	Jefferson, NH	22-Jul-06	B. Neill	**KF030303**	**KF030404**	**KF030374**
Boletus tenax	REH6871	New York Botanical Garden, NY, USA	25-Jul-95	R.E. Halling	**KF030320**	**KF030437**	–
Boletus variipes var *fagicola*	4249	Cheboygan Co., MI, USA	10-Aug-68	A. H. Smith	JQ327014	JQ327017	**KF030378**
Bothia castanella	MB 03-053	Massachussetts	25-Jun-05	M. Binder	DQ867117	**KF030421**	**KF030382**
Buchwaldoboletus lignicola	Pul1	Maindreieck, Germany	9-Sep-95	J. Schreiner	JQ326997	JQ327040	–
Chalciporus rubinus	DS4640-3	Germany	–	J. Schreiner	**KF030283**	**KF030440**	–
Chalciporus piperatus	MB 04-001	Rutland, MA, USA	28-Sep-04	M. Binder	DQ534648	GU187690	GU187453
Chalciporus pseudorubinellus	4302	Deer Meadows, Tulet, NY	14-Sep-98	E. Both	**KF030284**	**KF030441**	–
Gyrodon lividus	Gl1	Bavaria, Germany	11-Aug-95	H. Besl, W. Helfer	AF098378	GU187701	GU187461
Hemileccinum impolitum	Bim1	Bavaria, Germany	21-Sep-95	J. Schreiner	AF139715	JQ327034	**KF030375**
Hydnomerulius pinastri	CFMR:MD312	USDA Forest Products Laboratory	13-Nov-19	–	GU187580	GU187708	GU187462
Leccinellum corsicum	Buf 4507				**KF030347**	**KF030435**	**KF030389**
Leccinellum crocipodium	930809/1	France	9-Aug-93	G. Lannoy	AF139694	**KF030434**	–
Leccinum albellum	MB 06-040	Erie Co., NY, USA	30-Jul-06	M. Binder	JQ327007	JQ327038	–
Leccinum scabrum	Ls1	Austria	14-Sep-95	M. Binder	AF139705	JQ327039	–
Paragyrodon sphaerosporus	MB 06-066	Iowa City, IA, USA	22-Spt-06	M. Binder	GU187593	GU187737	–
Paxillus filamentosus	Pf1	Bavaria, Germany	21-Aug-95	L. Krieglsteiner	AF167680	GU187736	–
Paxillus obscurisporus	Po1	Bavaria, Germany	21-Aug-98	Ch. Hahn	AY177256	**KF030442**	–
Paxillus vernalis	Pv2	Canada	31-Aug-97	T. Lohmeyer	AY645059	DQ457629	–
Phylloporus pelletieri	Pp1	Bavaria, Germany	9-Sep-95	M. Kronfeldner	AF456818	JQ327036	**KF030390**
Porphyrellus porphyrosporus	MB 97-023	Bavaria, Germany	9-Sep-96	M. Beisenherz	DQ534643	GU187734	GU187475
Pseudoboletus parasiticus	Xpa1	Bavaria, Germany	9-Jul-95	A. Bresinksy	AF050646	**KF030443**	**KF030394**
Retiboletus griseus	202/97	MA, USA	30-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456834	**KF030414**	**KF030373**
Royoungia boletoides	27 546	New South Wales, Australia	25-Oct-99	J. Trappe	JX889655	JX889696	–
Royoungia boletoides	AWC4137	Victoria, Australia	28-May-01	T. Lebel *et al.*	DQ534663	JX889700	–
Spongiforma thailandica	DED 7873	Thailand	7-Jul-05	D.E. Desjardin	EU685108	**KF030436**	**KF030387**
Strobilomyces floccopus	Sf1	Bavaria, Germany	12-Aug-95	J. Enzmann	DQ534626	JQ327037	AY858963
Strobilomyces sp	REH8514	Cayo District, Belize	3-Oct-03	R.E. Halling	EU685109	**KF030398**	–
Tylopilus badiceps	78 206	Holliston, MA, USA	28-Jul-06		**KF030335**	**KF030429**	–
Tylopilus felleus	AT2001011	Stadsskogen, Uppsala, Sweden	17-Sep-01	A. F. S. Taylor	JQ326993	JQ327015	**KF030380**
Tylopilus ferrugineus	MB 06-053	Erie Co., NY, USA	3-Aug-06	E. Both	JQ326994	JQ327016	–
Tylopilus plumbeoviolaceus	MB 06-056	Chestnut Ridge Park, NY, USA	4-Aug-06	E. Both	**KF030350**	**KF030439**	**KF030395**
Xanthoconium stramineum	3518	Lake Mize, Gainsville, FL, USA	10-Aug-92	E. Both	**KF030353**	**KF030428**	**KF030386**
Xerocomellus chrysenteron	Xch1	Bavaria, Germany	8-Aug-95	M. Binder	AF050647	**KF030415**	**KF030365**
Xerocomellus rubellus cf West Coast	PBM 1331	West Coast, USA	–	P.B. Matheny	**KF030297**	**KF030420**	–

(*continued on next page*)

Table 3 – (*continued*)

Species	Isolate ID	Location	Date	Collector/ Identifier	*nuc-lsu*	*tef1*	*rpb1*
Xerocomellus rubellus cf *East Coast*	MB 03-033	Worcester, MA, USA	23-Jul-03	M. Binder	**KF030294**	**KF030419**	**KF030371**
Xerocomellus zelleri	REH8724	Redwood National Park, Humboldt Co., CA, USA	18-Nov-05	R.E. Halling	**KF030271**	**KF030416**	**KF030366**
Xerocomus badius	MB 03-098a	Rutland, MA, USA	19-Aug-03	M. Binder	**KF030355**	**KF030423**	**KF030393**
Xerocomus badius	Xb2	Bavaria, Germany	Sep-95	A. Bresinksy	**KF030357**	**KF030422**	–
Xerocomus cisalpinus	AT2005034	Upsala, Uppland, Finland	30-Jul-05	A.F. S. Taylor	**KF030354**	**KF030417**	**KF030367**
Xerocomus perplexus	MB 00-005	Rutland, MA, USA	5-Aug-00	M. Binder	JQ003702	**KF030438**	–
Xerocomus subtomentosus	Xs1	Bavaria, Germany	10-Aug-95	J. Enzmann, A. Bresinsky	AF139716	JQ327035	**KF030391**
Constrained data set taxa							
Alpova diplophloeus	17685	–	–	J. Trappe	AF071454		
Alpova diplophloeus	Adi1	Canada	Sep-87	W. Steglich, B. Sfeffan	AF352035		
Alpova trappei	28 042	–	–	J. Trappe	**KF030307**		
Alpova trappei	16 394	–	–	J. Trappe	AF071456		
Aureoboletus auriporus	35/94	MA, USA	28-Aug-97	H. Besl	DQ534636		
Aureoboletus auriporus	DD971	Orange Co, NC, USA	–	–	AY612819		
Aureoboletus citrinoporus	REH8719	Humboldt Co, CA, USA	16-Nov-05	R.E. Halling	**KF030298**		
Auroboletus innixus	136	–	–	–	**KF030240**		
Aureoboletus viridiflavus	DD972	Orange Co, NC, USA	–	–	AY612805		
Austroboletus flavidus	DPL7541	–	–	–	**KF030351**		
Austroboletus mucosus	TH6300	Pakaraima Mnts, Guyana	–	–	AY612798		
Austroboletus niveus	M312	New Zealand	10-May-95	A. Bresinksy	DQ534622		
Austroboletus novaezealandicae	M50	New Zealand	17-Mar-95	A. Bresinksy	DQ534623		
Boletellus ananas	TH8819	Guyana	–	T.W. Henkel	HQ161853		
Boletellus ananas	TH6264	Pakaraima Mnts, Guyana	–	T.W. Henkel	AY612799		
Boletellus cf *ananiceps*	00-335	Africa	2000	D. Arora	**KF030330**		
Boletellus betula	134/96	MA, USA	31-Aug-96	N. Arnold	AF050642		
Boletellus betula	DD9852	Orange Co, NC, USA	–	–	AY612797		
Boletellus chrysenteroides	54/97	MA, USA	21-Aug-97	N. Arnold, H. Besl	DQ534634		
Boletellus dicymbophilus	TH8840	Guyana	–	T.W. Henkel	HQ161852		
Boletellus mirabilis	CBS 136.60	–	–	–	AF050652		
Boletellus mirabilis	REH8717	Big Lagoon Park, Humboldt Co, CA, USA	15-Nov-05	R.E. Halling	**KF030299**		
Boletellus piakaii	TH8077	Guyana	–	T.W. Henkel	HQ161861		
Boletellus projectellus	sn2 Hor	–	–	–	**KF030300**		
Boletellus russellii	12/96	MA, USA	6-Aug-96	N. Arnold	AF050651		
Boletellus russellii	DPL6698	Texas	2000	D. Lewis	**KF030325**		
Boletellus sp	TAA195080	Valle de Mai, Praslin, Seychelles	–	–	AM412293		
Boletineae sp EcM Vateriopsis	L2481_Bol5	L'Abondance, Mahe, Seychelles	–	–	AM412263		
Boletus abieticola	26 763	–	–	J. Trappe	**KF030268**		
Boletus appendiculatus	REH8720	Shasta/Trinity National Forest, Trinity Co, CA, USA	15-Nov-05	R.E. Halling	**KF030269**		
Boletus atkinsonianus	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*
Boletus atkinsonianus	4557	Tucker Co, WV, USA	28-Aug-00	W.C. Woody, E. Both	**KF030242**		
Boletus aurantioruber	MEN11-0 MB	Cape Cod, MA, USA		M. Binder	**KF030342**		
Boletus bicolor	TH6933	Watuaga Co., NC, USA	–	–	AY612800		
Boletus bicolor	snOH	Ohio, USA	–	D. Lewis	**KF030258**		
Boletus bicolor	MB 06-037	Chestnut Ridge Park, NY, USA	3-Aug-06	M. Binder	**KF030250**		
Boletus bicolor var *subreticulatus*	3818	North Collins Town Park, NY, USA	10-Aug-95	E. Both	**KF030247**		
Boletus billieae	4558	Well Fleet, Cape Cod, MA, USA	25-Aug-01	B. Neill, E. Both	**KF030265**		
Boletus calopus	112606	Mendocino Co., CA, USA	26-Nov-05	B. Neill	**KF030279**		
Boletus campestris	5/96	Eastabrook Woods, MA, USA	9-Aug-96	N. Arnold	DQ534640		
Boletus carminipes	4591	Berea, Cuyalioga Co, OH, USA	22-Jul-01	P. Monk, E. Both	**KF030259**		
Boletus cf *edulis*	–	Turkey	–	–	AF462355		
Boletus cf *edulis*	197/84	China	–	–	AF462357		
Boletus cf *luridus*	MAN 011	Costa Rica	1-Jun-04	M.A. Neves	**KF030288**		
Boletus coniferarum	7/94	CA, USA	Oct-94	W. Steglich	AF456827		
Boletus eastwoodiae	AT2003096	–	–	A.F. S. Taylor	**KF030253**		
Boletus eastwoodiae	TDB-1000C	Marin Co., CA, USA	28-Nov-87	T. D. Bruns	AF071528		
Boletus edulis	OSC FAC 1615	–	–	–	AF071457		
Boletus edulis	No. 15	Yugoslavia	–	–	AF462356		
Boletus edulis	HN141	Watuaga Co., NC, USA	–	–	AY612802		
Boletus edulis	FO 46874	Germany	–	–	AF291300		
Boletus erthyropus	3131	Raquette Lake, NY, USA	21-Jul-98	E. Both	**KF030291**		
Boletus erythropus	Ber1	Bavaria, Germany	1-Oct-95	J. Enzmann, H. Besl	AF139683		
Boletus fechtneri	AT2003097	–	–	A.F. S. Taylor	**KF030270**		
Boletus fechtneri	Bf1	Bavaria, Germany	1-Sep-94	M. Beisenherz	AF456821		
Boletus fibrillosus	PBM1342	WA, USA	–	P.B. Matheny	**KF030343**		
Boletus fibrillosus	Thiers 6995	Jackson Forest, Mendocino Co, CA, USA	21-Nov-92	R.E. Halling, E. Both	**KF030344**		
Boletus floridanus	BD368	Parque Nacional de Guanacaste, Area de Conservacion Guanacaste, Costa Rica	–	B. Dentinger	HQ161859		
Boletus gertrudiae	86/97	MA, USA	23-Aug-97	N. Arnold	AF457407		
Boletus glabellus	00-194	Boston Cemitary, Boston, MA, USA	–	B. Neill	**KF030318**		
Boletus gyrodontoides	MS5	Malaysia	8-Apr-91	N. Arnold, H. Besl	DQ534651		
Boletus hypocarcinus	DPL6899	USA	–	D. Lewis	**KF030260**		
Boletus inedulis	NCJ14	Orange Co, NC, USA	–	D. Dremel	AY612803		
Boletus inedulis	sn2002	NY, USA	–	E. Both	**KF030281**		
Boletus junquilleus	Bju1	Keilberg-Waldmichbach, Salzbuckel, Germany	9-Nov-96	J. Schreiner	DQ534645		
Boletus leptospermi	M23	New Zealand	15-Mar-95	A. Bresinksy	DQ534632		
Boletus longicurvipes	8/97	USA	14-Aug-97	N. Arnold	AF139688		
Boletus longicurvipes	TH6944	Watuaga Co., NC, USA	–	–	AY612812		
Boletus luridus	Bl2	Bavaria, Germany	14-Aug-95	M. Beisenherz	AF139686		
Boletus luteocupreus	Blu1	Karlburg, Hagwald, Germany	8-Aug-95	J. Schreiner	DQ534657		
Boletus minato-olivaceus	4091	Chestnut Ridge Park, NY, USA	26-Jun-97	E. Both	**KF030243**		
Boletus modestus	229/97	MA, USA	1-Sep-97	N. Arnold	DQ534659		
Boletus nobilis	BD239 (MIN)	Duke Forest, Durham, NC, USA	–	–	EU232002		
Boletus oliveisporus	DPL6823	Texas	2000	D. Lewis	**KF030254**		
Boletus pallidoroseus	BD396	Pine Bend Bluff Scientific and Natural Area, MS, USA	–	B. Dentinger	HQ161860		
Boletus pallidoroseus	sn Hor	Suffolk Co, NY, USA	2000	J. Horman	**KF030305**		
Boletus pinophilus	No. 4	Bavaria, Germany	20-Jul-92	H. Besl	AF462358		
Boletus pinophilus	42/93	–	–	–	AF462359		
Boletus pulcherrimus	00-291	Mendocino Co., CA, USA	–	A. Mohr, E. Both	**KF030256**		
Boletus quercophilus	BDCR0417 (MIN)	along Rio Savegre, San Gerardo de Dota, San Jose, Costa Rica	–	–	EU232001		
Boletus radicans	Brad1	–	–	–	AF336241		
Boletus regius	Bre1	Bavaria, Germany	24-Aug-95	L. Krieglsteiner	DQ534653		

(*continued on next page*)

Table 3 – (*continued*)

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

Table 3 – (*continued*)

Species	Isolate ID	Location	Date	Collector/ Identifier	*nuc-lsu*	*tef1*	*rpb1*
Fistulinella viscida	238	New Zealand	26-Apr-95	A. Bresinksy	AF456826		
Gastroboletus turbinatus	19/95	WA, USA	18-Aug-95	N. Arnold, J. Ammirati	AF336248		
Gastroboletus vividus	27 480	–	–	J. Trappe	**KF030245**		
Gyrodon lividus	GlM1	Bavaria, Germany	16-Sep-98	A. Bresinksy	AF167677		
Gyrodon monticola	30/96	Colombia	27-Apr-96	Ch. Hahn	AF352040		
Harrya chromapes	BD377	USA	–	B. Dentinger	HQ161856		
Harrya chromapes	sn1	–	–	–	AF139709		
Harrya chromapes	sn2	–	–	–	AY612834		
Heimioporus retispora	MS6	Malaysia	8-Apr-91	H. Besl	AF050650		
Heliogaster columellifera	TNS-F-11696	Mt. Kuram, Kyoto Pref., Kyoto-shi, Sakyo-ku, Japan	–	T. Orihara	EF183541		
Heliogaster columellifera	TNS-F-11700	Aichi Pref., Okazaki-shi	–	T. Orihara	EF183544		
Hemileccinum depilatum	Bd1	Bavaria, Germany	11-Sep-94	A. Bresinksy	AF139712		
Hydnomerulius pinastri	412	Canada	10-Oct-73	J.H. Ginns	AF352044		
Hydnomerulius pinastri	Z. Wang sn	CA, USA	22-Aug-04	Z. Wang	DQ534667		
Leccinellum carpini	930808	France	8-Aug-93	G. Lannoy	AF139691		
Leccinellum corsicum	931101/1 GL	France	1-Nov-93	G. Lannoy	AF139693		
Leccinellum lepidum	110684	Italy	11-Jun-84	H. Besl	AF139698		
Leccinum aerugineum	8909241AE	Belledonne 38, Seiglieres, France	24-Sep-84	A. Estades	DQ534618		
Leccinum albellum	TH6968	Watuaga Co., NC, USA	–	–	AY612811		
Leccinum aurantiacum	La1	Bavaria, Germany	11-Sep-94	M. Beisenherz	AF139689		
Leccinum aurantiacum	HN1573	Giles Co, VA, USA	–	–	AY612810		
Leccinum calltitrichum	GR92103	–	–	–	AF139690		
Leccinum cerinum	MK11800	–	–	–	AF139692		
Leccinum duriusculum	880904/4 GL	France	4-Sep-88	Wolfer	AF139695		
Leccinum flavostipitatum	24/98	USA	23-Aug-98	N. Arnold	AF139696		
Leccinum manzanitae	TDB-969	CA, USA	21-Nov-94	T. D. Bruns	DQ534613		
Leccinum monticola	BDCR14	Cerro de la Muerte summit, Costa Rica	–	B. Dentinger	HQ161869		
Leccinum nigellum ined	GPL4676	France	11-Sep-79	G. Redeuilh	AF139699		
Leccinum palustre	MK11107	Finland	2-Sep-92	M. Korhonen	AF139701		
Leccinum percandidum	9210040	France	10-Apr-92	A. Estades	AF139702		
Leccinum piceinum	Lp1	Pruz in Tyrol, Austria	14-Sep-94	M. Binder, H. Besl	DQ534614		
Leccinum quercinum	196695	NGS Ludwigshain, Kelheim, Germany	5-Sep-94	A. Reisinger	DQ534612		
Leccinum rigidipes	196696	Massif Belledonne 38, Prabert, France	11-Oct-89	A. Estades	DQ534617		
Leccinum rotundifoliae	MK7676:251	Finland	–	M. Korhonen	AF139704		
Leccinum rugosiceps	TH6967	Watuaga Co., NC, USA	–	–	AY612813		
Leccinum scabrum	NCJ26	Watuaga Co., NC, USA	–	–	AY612814		
Leccinum schistophilum	921024/1 GL	59 Waredin, Rost, France	24-Oct-92	G. Lannoy	DQ534615		
Leccinum variicolor	Lvar1	Bavaria, Germany	19-Sep-95	J. Enzmann	AF139706		
Leccinum versipelle	Lv2	Bavaria, Germany	6-Sep-94	H. Besl	AF139707		

(*continued on next page*)

Table 3 – (*continued*)

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

Table 3 – (*continued*)

Species	Isolate ID	Location	Date	Collector/ Identifier	*nuc-lsu*	*tef1*	*rpb1*
Retiboletus flavoniger	REH7247	San José, Costa Rica	8-Jun-98	R.E. Halling	AF456828		
Retiboletus flavoniger	REH7189	San José, Costa Rica	24-Nov-93	R.E. Halling	AF456829		
Retiboletus griseus	Both sn	NY, USA	–	E. Both	**KF030308**		
Retiboletus nigerrimus	Tyni1	Japan	15-Jul-96	R. Marumoto	AF456832		
Retiboletus ornatipes	93/97	USA	23-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456825		
Retiboletus ornatipes	186/97	USA	30-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456819		
Retiboletus retipes	96/97	USA	23-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456830		
Retiboletus retipes	Japan1	Japan	10-Jul-97	H. Shibata	AF456807		
Retiboletus retipes	REH7567	Cartago, Costa Rica	28-May-96	R.E. Halling	AF456808		
Retiboletus retipes	55/97	USA	21-Aug-97	N. Arnold, W. Helfer, W. Steglich	AF456811		
Retiboletus sp	MAN 053	Costa Rica	12-Jun-04	M.A. Neves	**KF030327**		
Rossbeevera pachyderma	MEL2079350	New Zealand	–	–	HQ647157		
Rossbeevera pachyderma	M42	New Zealand	15-Mar-95	A. Bresinksy	DQ534620		
Rossbeevera vittatispora	MEL2293673	Australia	–	–	HQ647163		
Rossbeevera westraliensis	MEL2233755	Australia	–	–	HQ647164		
Rubinoboletus sp	01-590	Africa	2001	–	**KF030314**		
Tylopilus ballouii	TH6385	Pakaraima Mnts, Guyana	–	–	AY612823		
Spongiforma squarepantsii	LHFB14	Malaysia	–	T.D. Bruns, D. E. Desjardin	HQ724509		
Spongiforma squarepantsii	LHFB01	Malaysia	–	T.D. Bruns, D. E. Desjardin	HQ724510		
Strobilomyces dryophilus	72 106	–	–	–	**KF030345**		
Strobilomyces floccopus	HN0027	Orange Co, NC, USA	–	–	AY612824		
Strobilomyces sp	177/97	MA, USA	26-Aug-97	N. Arnold	DQ534627		
Sutorius eximius	40/97	MA, USA	20-Aug-97	N. Arnold	AF139684		
Tylopilus aff *chromapes*	01-513	Africa	2001	D. Arora	JX889672		
Tylopilus aff *conicus*	01-533	Africa	2001	D. Arora	**KF030328**		
Tylopilus aff *eximius*	01-528	Africa	2001	D. Arora	JQ327002		
Tylopilus aff *virens*	01-541	Africa	2001	D. Arora	JX889677		
Tylopilus alboater	TH6941	Durham, Co, NC, USA	–	–	AY612832		
Tylopilus alboater	TDB-1207	Duncombe, Co., NC, USA	3-Sep-88	T. D. Bruns	AF139708		
Tylopilus atronicotianus	WV	Camp Creek State Forest, WV, USA	14-Aug-02	W.C. Woody, E. Both	**KF030293**		
Tylopilus atronicotianus	–	Clarion Public Hunting Area, Clarion Co, PA, USA	18-Sep-04	W. Sturgeon, E. Both	EU685110		
Tylopilus badiceps	NCJ20	Watuaga Co., NC, USA	–	–	AY612833		
Tylopilus badiceps	MB 03-052	MA, USA	Aug-03	M. Binder	**KF030336**		
Tylopilus cf *chromapes*	01-549	Africa	2001	D. Arora	JX889671		
Tylopilus felleus	–	–	–	–	AF071466		
Tylopilus felleus	HKAS54926	Marburg, Germany	–	–	HQ326933		
Tylopilus indecisus	98/98	USA	26-Aug-98	N. Arnold, W. Helfer	AF456820		
Tylopilus intermedius	BD277	Nerstrand-Big Woods State Park, MN, USA	–	B. Dentinger	HQ161875		
Tylopilus rhoadsiae	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*
Tylopilus rubrobrunneus	152/98	MA, USA	28-Aug-98	N. Arnold	DQ534629		
Tylopilus sp	00-311	Africa	2000	D. Arora	**KF030309**		
Tylopilus sp	190/83	USA	18-Sep-83	W. Steglich	AF457405		
Tylopilus sp	00-366	Africa	2000	D. Arora	**KF030337**		
Tylopilus sp	00-388	Africa	2000	D. Arora	**KF030338**		
Tylopilus sp	204/97	MA, USA	30-Aug-97	N. Arnold	AF456813		
Tylopilus variobrunneus	9306tv	Plymouth, MA, USA	3-Sep-06	B. Neill	**KF030315**		
Tylopilus variobrunneus	sn Hor	–	–	–	**KF030316**		
Tylopilus virens	–	Japan	1997	R. Marumoto	DQ534621		
Xanthoconium affine	78/94	USA	31-Aug-94	W. Steglich	AF457406		
Xanthoconium affine	RV98.112	Giles Co, VA, USA	–	–	AY612838		
Xanthoconium affine var *maculosum*	BD217	Mnt. Lake Biological Station, Mountain Lake, VA, USA	–	B. Dentinger	HQ161854		
Xanthoconium sp	MB 06-049	Dutch Grove, Erie Co, NY, USA	4-Aug-06	M. Binder	**KF030292**		
Xerocomus amazonicus	TH6304	Pakaraima Mnts, Guyana	–	–	AY612839		
Xerocomellus armeniacus	Xar1	Germany	6-Sep-95	L. Krieglsteiner	**KF030295**		
Xerocomus badius	Xb1	Bavaria, Germany	Sep-95	M. Binder	AF050648		
Xerocomus badius	MB 03-098(a|b)	Rutland, MA, USA	19-Aug-03	M. Binder	**KF030355, KF030356**		
Xerocomus bubalinus	MB 05-008	Beaver St., Worcester, Worcester Co, MA, USA	5-Oct-05	M. Binder	**KF030296**		
Xerocomellus cf *chrysenteron*	PBM925	USA	–	–	**KF030272**		
Xerocomellus cf *porosporus*	MB 00-017	Woodland St., Worcester, Worcester Co, MA, USA	2-Aug-00	M. Binder	**KF030277**		
Xerocomellus chrysenteron	IB20000405	–	–	–	AF514809		
Xerocomellus chrysenteron	REH8723	Redwood National Park, Humboldt Co., CA, USA	17-Nov-05	R.E. Halling	**KF030276**		
Xerocomellus chrysenteron	TDB-635	Crawford, Co., MI, USA	8-Aug-83	T. D. Bruns	AF071537		
Xerocomellus porosporus	Xpor1	Bavaria, Germany	17-Sep-95	M. Binder	AF050645		
Xerocomellus porosporus	IB19880304	–	–	–	AF514810		
Xerocomellus porosporus	IB19990957	–	–	–	AF514811		
Xerocomellus pruinatus	Xpru1	Bavaria, Germany	21, Aug-98	L. Krieglsteiner	AF050644		
Xerocomellus pruinatus	IB19980368	–	–	–	AF514827		
Xerocomellus ripariellus	GR22465	–	–	–	AF514818		
Xerocomellus ripariellus	IB19980360	–	–	–	AF514816		
Xerocomellus rubellus	GS961	–	–	–	AF514829		
Xerocomellus rubellus	IB19990917	–	–	–	AF514828		
Xerocomellus zelleri	27 888	–	–	J. Trappe	**KF030275**		
Xerocomus chrysonemus	JAM0539	Cantabria, Las Rozas, Spain	21-Sep-04	J.A. Munoz	**KF040544**		
Xerocomus cisalpinus	5823-130998	Germany	13-Sep-98	J. Schreiner	**KF030274**		
Xerocomus cisalpinus	IB20000701	–	–	–	AF514812		
Xerocomus dryophilus	IB19990901	–	–	–	AF514823		
Xerocomus dryophilus	IB19991057	–	–	–	AF514822		
Xerocomus fennicus	H126	–	–	–	AF514820		
Xerocomus fennicus	RJ126	–	–	–	AF514821		
Xerocomus hortonii	84/94	MA, USA	23-Aug-94	W. Steglich	AF139713		

Table 3 – (*continued*)

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

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

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

Boletaceae remains poorly resolved. Nonetheless, there are 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

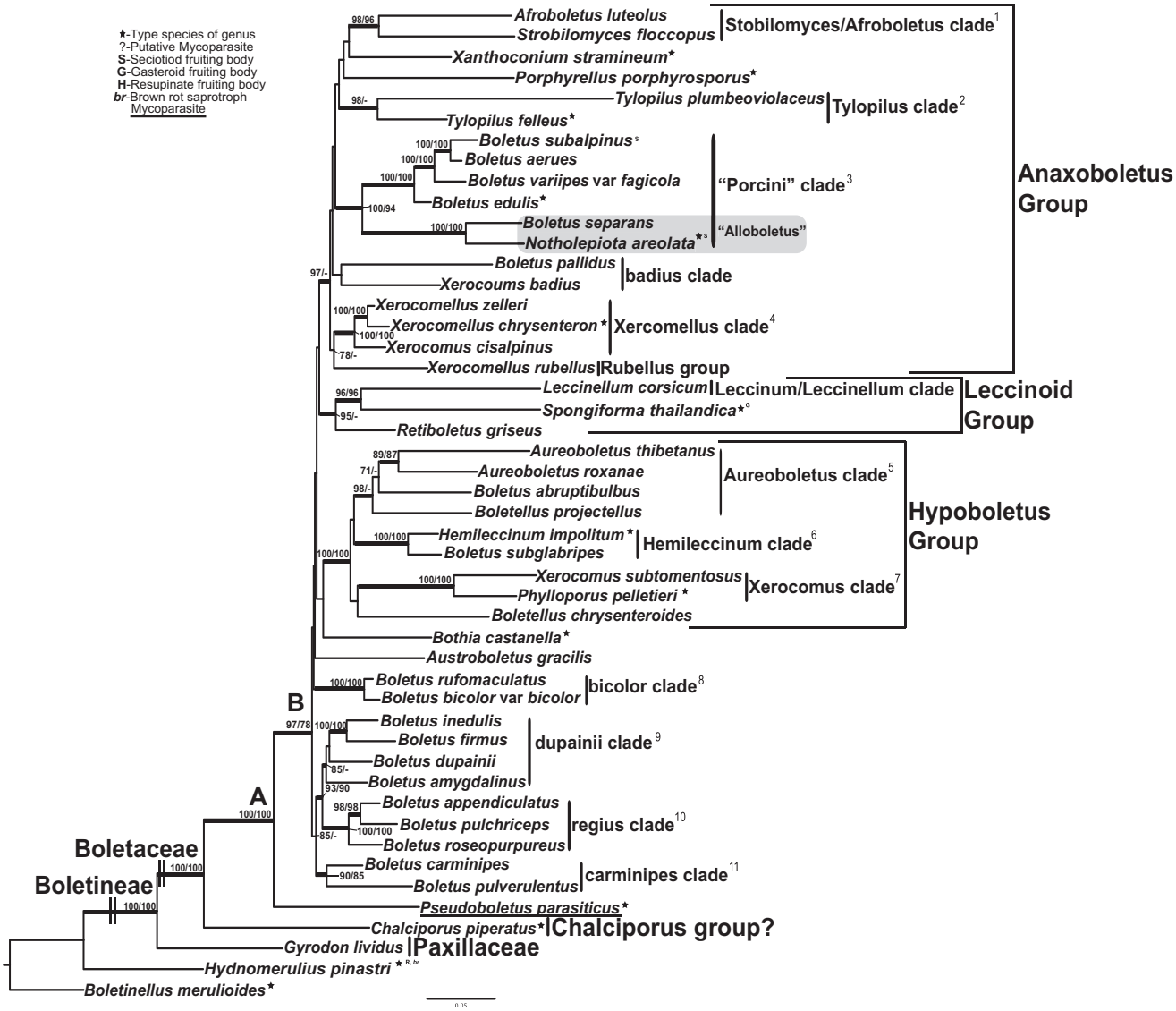

Fig 1 – Phylogenetic relationships of the *Boletineae* inferred from *nuc-lsu*, *tef1*, and *RPB1* using RAxML, PhyloBayes, and PAUP*. This is referred to as the three-gene core analysis in the text. The tree topology corresponds to the optimal ML tree. Support values ≥70 % BS for ML and MP are displayed [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.

are members of the 'porcini' group as defined by Dentinger *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; Šutara 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 boletelogists 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; Högberg *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 (Högberg *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; Högberg *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 (Szczepka & Sokól 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).

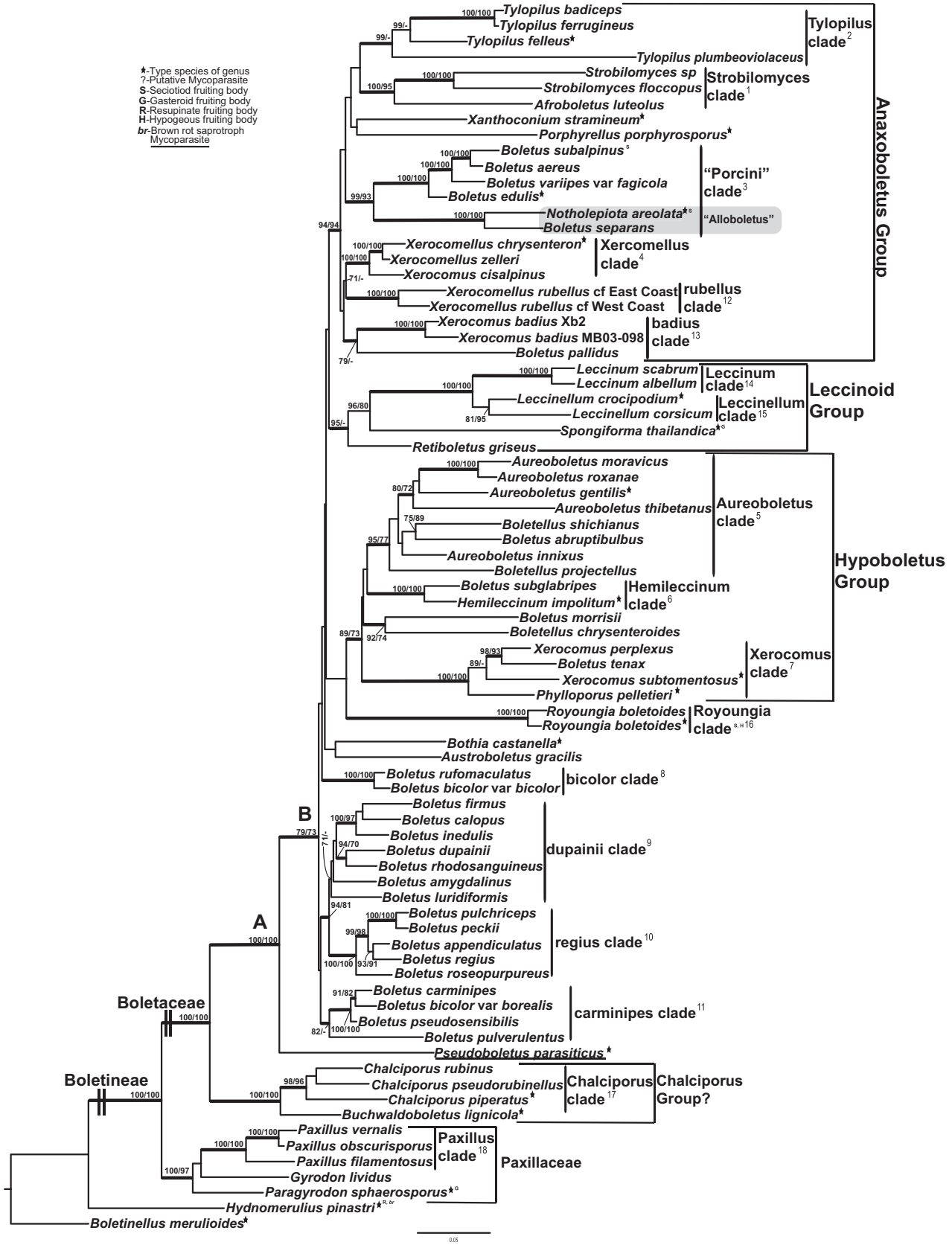

Fig 2 – Phylogenetic relationships of the *Boletineae* inferred from *nuc-lsu*, *tef1*, and *RPB1* using RAxML, PhyloBayes, and PAUP; all taxa have nuc-lsu and *tef1*, but not all taxa have *RPB1*.This is referred to as the three-gene extended analysis in the text. The tree topology corresponds to the 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

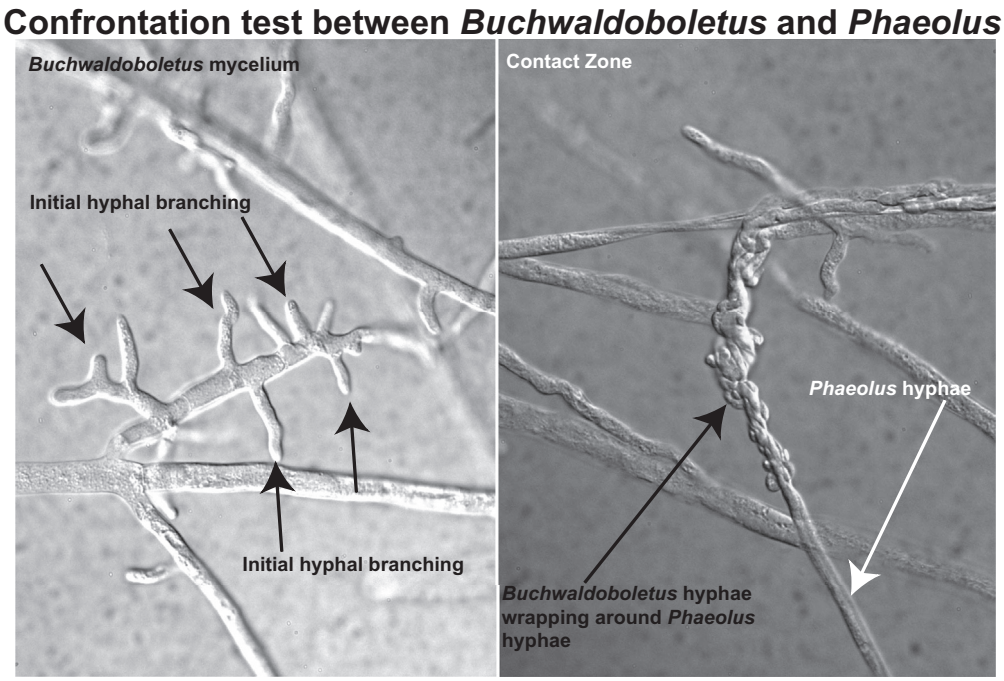

Fig 3 – Confrontation analysis between the hyphae of *Buchwaldoboletus lignicola* and *Phaeolus schweinitzii*. Initial hyphal branching of *Buchwaldoboletus lignicola* is seen in the image on the left and indicated by blue arrows. In the image on the right, the black arrow indicates the *P. schweinitzii* 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

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.

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

				Octavania 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
	rubellus	*Xercomellus rubellus* cf. East Coast MB03-033	*Xerocomellus rubellus* cf. West Coast PBM1331	*Xerocomellus rubellus* GS961
				Xerocomellus rubellus IB19990917
				Xerocomellus armeniacus
				Xerocomus bubalinus MB05-008
	badius	No representative	*Xerocomus badius* Xb2	*Xerocomus badius* MB03-00
			Xerocomus badius MB03-098	*Boletus glabellus* 00194
			Boletus pallidus 179-97	*Xerocomus badius* AF050648
				Tylopilus sp 00-311
				Tylopilus sp 00-388
				Tylopilus sp 00-366
	No clade designated	*Xanthoconium stramenium*[a]	N/A	*Tylopilus atronicotianus* WV
		Porphyrellus porphyrosporus[a]		*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
Leccinoid	Leccinum	No representative	*Leccinum scabrum* Ls1	*Leccinum manzanitae*
			Leccinum albellum MB06-040	*Leccinum aurantiacum* AY612810
				Leccinum cerinum AF139692
				Leccinum piceinum
				Leccinum quercinum
				Leccinum monticola HQ161869
				Leccinum percandidum AF139702
				Leccinum aurantiacum AF139689

(*continued on next page*)

Table 5 – (*continued*)

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

				Retiboletus flavoniger AF456828
				Retiboletus ornatipes AF456819
				Retiboletus ornatipes AF456825
				Pulveroboletus curtisii AY612820
				Spongiforma squarepantsii LHBF14 HQ724509
				Spongiforma squarepantsii LHBF01 HQ724510
Hypoboletus	Aureoboletus	*Aureobotelus thibetanus*	*Aureoboletus moravicus* Xleo1	*Aureoboletus auriporus* AY612819
		Aureoboletus roxanae DS629-07	*Aureoboletus gentilis* Pug1[a]	*Aueroboletus viridiflavus*
		Boletus abruptibulbus 4588	*Boletellus shichianus*	AY612805
		Boletellus projectellus	*Aureoboletus innixus* MB03-101	*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	*Hemileccinum impolitum* Bn1[a]	N/A	*Hemileccinum depilaturm* AF139712 [a]
		Boletus subglabripes 72206bs		*Boletus rubropunctus* MB05-003
	Xerocomus	*Xerocomus subtomentosus* Xs1[a]	*Xercomus perplexus* MB00-005	*Boletus tenax* 6641
		Phylloporus pelletieri Pp1[a]	*Boletus tenax* 6871	*Xerocomus chrysonemus* JAM0539
				Xerocomus subtomentosus AF514831
				Boletus leptospermi
				Xerocomus illudens AY612840
				Xerocomus illudens AF139714
				Xerocomus lanatus
				Xerocomus subtomentosus AF514832
				Xerocomous 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
	No clade designated	*Boletellus chrysenteroides* 3838	*Boletus morrisii* 8206	

(*continued on next page*)

Table 5 – (*continued*)

Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
				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 piakaii* HQ161861 *Boletus morrisii* 8206
No group	Royoungia	No representative	*Royoungia boletoides* 27 546[a] *Royoungia* sp 24836	
	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*
No group	Bicolor clade	*Boletus rufomaculatus*4414 *Boletus bicolor* var. *bicolor* MB07-001	N/A	*Boletus subluridellus* 3737 *Boletus bicolor* MB06-037 *Boletus bicolor* var *subreticulatus* 3818 *Boletus* sp *bicolor* 3921 *Boletus sensibilis* var *subviscidus* 3929
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 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

				Boletus eastwoodiae AT2003096
				Boletues 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
	Regius clade	*Boletus appendiculatus* Bap1 *Boletus pulchriceps* DS4514 *Boletus roseopurpureus* MB04-040	*Boletus peckii* 3959 *Boletus regius* 11265	*Boletus regius* *Boletus speciosus* var *brunneus* 4258 *Boletus abieticola* 26763 *Boletus appendiculatus* 8720 *Boletus roseopurpureus* 3765 *Boletus roseopurpureus* 4497
	No clade	N/A	N/A	*Boletus speciosus* *Boletus fechtneri* 2003097 *Boletus fechtneri* AF456821 *Boletus floridanus* HQ161859
No group	Carminipes clade	*Boletus carminipes* MB06-061 *Boletus pulverulentus* 9606	*Boletus bicolor* var *borealis* 2858 *Boletus pseudosensibilis* DS615-07	*Boletus bicolor* AY612800 *Boletus pallidoroseus* 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 pallidoroseus* HQ161860 *Boletus hypocarcinus* DPL6899 *Boletus oliveisporus* 6823
No group 1Chalciporus	No clade Chalciporus	*Pseudoboletus parasiticus* Xpa1[a] *Chalciporus piperatus* MB04-001[a]	N/A *Chalciporus rubinus* 4640-3 *Chalciporus pseudorubinellus* 4302	*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
	No clade designated	No representative	*Buchwaldoboletus lignicola*[a] Pul1[a]	N/A
Paxillus	Paxillus	No representative		*Paxillus involutus* AF167683[a] *Paxillus involutus* AF167684[a]

(*continued on next page*)

Table 5 – (*continued*)

Group	Clade	Species in core dataset	Additional species in extended dataset	Additional Species in constrained nLSU dataset
			Paxillus vernalis Pv2 *Paxillus obscurisporus* Po1 *Paxillus filamentosus* Pf1	*Paxillus involutus* AF167682[a] *Paxillus involutus* AY612815[a] *Paxillus involutus* AF167701 *Paxillus involutus* AF167686[a] *Paxillus filamentosus* AF167681
	No clade designated	*Gyrodon lividus* g11[a] *Paragyrodon sphaerosporus* MB06-066[a]	N/A	*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
No group	No clade	*Hydnomerulius pinastri*[a]	N/A	*Hydnomerulius pinastri* AF352044[a] *Hydnomerulius pinastri* China Wang[a]
Outgroup	N/A	*Boletinellus merulioides*[a]	N/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; Aurora 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 reticulated, 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-lsu* 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, *Borofutus*, 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 *Retiboletus/Retiboletus 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 only because both species are shown to be distantly related to *B. edulis*, not because both species are expected to be closely related to the type of *Boletellus*, *B. ananas* (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 impolitum* and *Boletus subglabripes*. This is the first analysis containing both *H. impolitum* 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. impolitum* 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 scrabrosities.

Xerocomus clade [100/100/1.0]—This clade contains the type species of *Xerocomus*, *X. subtomentosus*. Previous studies have reported the distant relationship among the members of the *Xerocomus* clade and members of 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 scabrousity 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 *Tylopilius*, 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 *Ceriomyces* (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 inedulis* 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. inedulis* is supported in all analysis except for Desjardin *et al.* (2008), which did not include *B. inedulis*. Species relationships inside dupainii are poorly resolved.

regius clade [100/100/1.0]—Inside this clade only *Boletus reguis* 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 *Leccinellum*, 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 astraeicola* 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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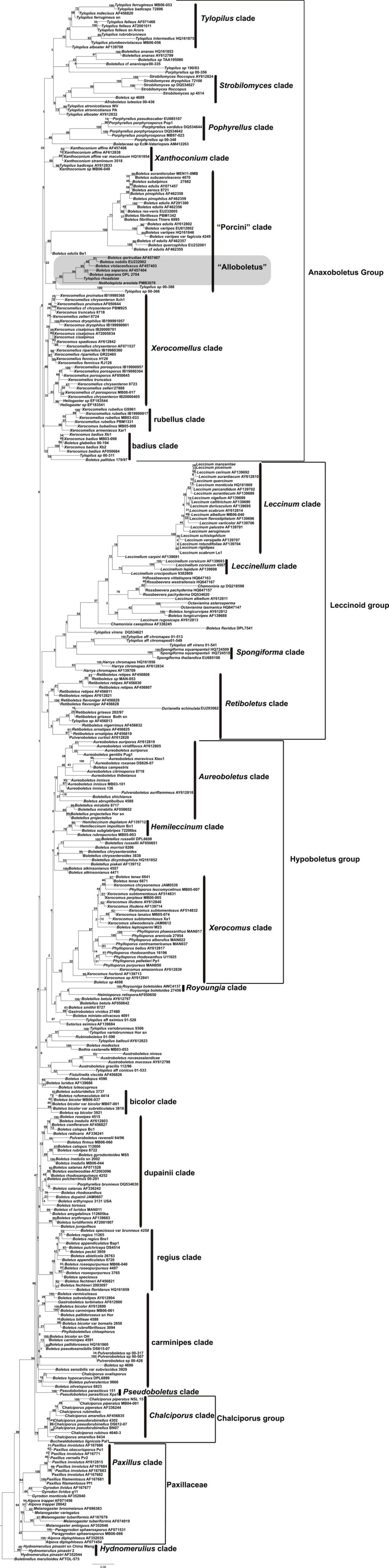
Suppl. Fig 1. Phylogenetic relationships among the Boletineae inferred from nuclear large-subunit (nuc-lsu) using RAxML. The tree topology corresponds to the optimal ML topology using the three-gene extended analysis tree topology as a backbone constraint. All BS support values are shown.


Tylopilus clade
Strobilomyces clade
Pophyrellus clade
Xanthoconium clade
"Porcini" clade
"Alloboletus"
Anaxoboletus Group
Xerocomellus clade
rubellus clade
badius clade
Leccinum clade
Leccinellum clade
Leccinoid group
Spongiforma clade
Retiboletus clade
Aureoboletus clade
Hemileccinum clade
Hypoboletus group
Xerocomus clade
Royoungia clade
bicolor clade
dupainii clade
regius clade
carminipes clade
Pseudoboletus clade
Chalciporus clade
Chalciporus group
Paxillus clade
Paxillaceae
Hydnomerulius clade
0.06