

TAXONOMY

Taxonomical implications.—We have adopted a conservative approach to accommodate findings from recent phylogenies and propose a revised classification that reflects changes based on substantial evidence. The following outline adds no additional suborders, families or genera to the Boletales, however, excludes Serpulaceae and Hygrophoropsidaceae from the otherwise polyphyletic suborder Coniophorineae. Major changes on family level concern the Boletineae including Paxillaceae (incl. Melanogastraceae) as an additional family. The Strobilomycetaceae E.-J. Gilbert is here synonymized with Boletaceae in absence of characters or molecular evidence that would suggest maintaining two separate families. Chamonixiaceae Jülich, Octavianiaceae Loq. ex Pegler & T. W. K. Young, and Astraeaceae Zeller ex Jülich are already recognized as invalid names by the Index Fungorum (www.indexfungorum.com). In addition, Boletinellaceae Binder & Bresinsky is a homonym of Boletinellaceae P. M. Kirk, P. F. Cannon & J. C. David. The current classification of Boletales is tentative and includes 16 families and 75 genera. For 16 genera (marked with asterisks) are no sequences available. Several taxa listed in the current GenBank classification (www.ncbi.nlm.nih.gov) or in the 9th edition of the Dictionary of the Fungi (Kirk et al 2001) are excluded from the Boletales based on evidence from recent phylogenies (e. g. Binder et al 2005, Peintner et al 2001).

Synopsis of the Boletales:

Boletineae Rea emend. E.-J. Gilbert

Boletaceae Chevall. (*Afroboletus** Pegler & T.W.K. Young, *Aureoboletus* Pouzar, *Austroboletus* (Corner) Wolfe, *Boletellus* Murrill, *Boletochaete** Singer, *Boletus* Dill. ex Fr., *Buchwaldoboletus* Pilát, *Chalciporus* Bataille, *Chamonixia* Rolland, *Fistulinella* Henn., *Gastroboletus* Lohwag, *Gastroleccinum** Thiers, *Gastrotylopilus** T.H. Li & Watling, *Heimiella* Boedijn, *Heimioporus** E. Horak, *Leccinellum* Bresinsky & Binder, *Leccinum* S.F. Gray, *Mycoamaranthus* Castellano, Trappe & Malajczuk, *Octaviania* O. Kuntze, *Paxillogaster** E. Horak, *Phylloboletellus* Singer, *Phyllobolites** Singer, *Porphyrellus* E.-J. Gilbert, *Pseudoboletus* Sutara, *Pulveroboletus* Murrill, *Retiboletus* Binder & Bresinsky, *Rhodactina* Pegler & T.W.K. Young, *Royoungia* Castellano, Trappe & Malajczuk, *Rubinoboletus* Pilát & Dermek, *Setogyroporus** Heinem. & Rammeloo, *Singeromyces** M.M. Moser, *Sinoboletus* M. Zang, *Strobilomyces* Berk., *Tubosaeta** E. Horak, *Tylopilus* P. Karst., *Velopor-*

*phyrellus** L.D. Gómez & Singer, *Xanthoconium* Singer, *Xerocomus* Quél.)

Paxillaceae Lotsy (*Alpova* C. W. Dodge, *Austrogaster** Singer, *Gyrodon* Opat., *Meiorganum** Heim, *Melanogaster* Corda, *Paragyrodon*, (Singer) Singer, *Paxillus* Fr.)

Boletineae incertae sedis: *Hydnomerulius* Jarosch & Besl

Sclerodermatineae Binder & Bresinsky

Sclerodermataceae E. Fisch. (*Chlorogaster** Laessøe & Jalink, *Horakiella** Castellano & Trappe, *Scleroderma* Pers, *Veligaster* Guzman)

Boletinellaceae P. M. Kirk, P. F. Cannon & J. C. David (*Boletinellus* Murrill, *Phlebopus* (R. Heim) Singer)

Calostomataceae E. Fisch. (*Calostoma* Desv.)

Diplocystaceae Kreisel (*Astraeus* Morgan, *Diplocystis* Berk. & M.A. Curtis, *Tremelloogaster* E. Fisch.)

Gyroporaceae (Singer) Binder & Bresinsky (*Gyroporus* Quél.)

Pisolithaceae Ulbr. (*Pisolithus* Alb. & Schwein.)

Suillineae Besl & Bresinsky

Suillaceae (Singer) Besl & Bresinsky (*Suillus* S.F. Gray)

Gomphidiaceae R. Maire ex Jülich (*Brauniellula* A.H. Smith & Singer, *Chroogomphus* (Singer) O.K. Mill., *Gomphidius* Fr., *Gomphogaster** O.K. Mill.)

Truncocolumellaceae Agerer (*Truncocolumella* Zeller)

Rhizopogonaceae Gäum. & C. W. Dodge (*Rhizopogon* Fr. & Nordholm)

Coniophorineae Agerer & Ch. Hahn

Coniophoraceae Ulbr. (*Coniophora* DC., *Gyrodontium* Pat., *Leucogyrophana arizonica* Ginns, *Paxillus chalybaeus* E. Horak, *P. gymnopus* Ch. Hahn)

Tapinellineae Agerer

Tapinellaceae Ch. Hahn (*Bondarceomyces* Parmasto, *Pseudomerulius* Jülich, *Tapinella* E.-J. Gilbert)

Without subordinal placement:

Hygrophoropsidaceae Kühner (*Hygrophoropsis* (J. Schröt.) R. Maire ex Martin-Sans, *Leucogyrophana* Pouzar)

Serpulaceae Jarosch & Bresinsky (*Austropaxillus* Bresinsky & Jarosch, *Gymnopaxillus* Horak emend. Claridge, Trappe & Castellano, *Neopaxillus* Singer ###, *Serpula* (Pers.) S. F. Gray)

Boletales incertae sedis: *Leucogyrophana olivascens* (Berk. & M.A. Curtis) Ginns & Weresub, *L. romellii* Ginns

Taxa excluded: Gastrosporiaceae Pilát (Phallales), Hymenogasteraceae Vittad. (Agaricales), Leucogasteraceae Moreau ex Fogel (Russulales), *Stephanosporaceae* Oberw. & E. Horak (Agaricales).

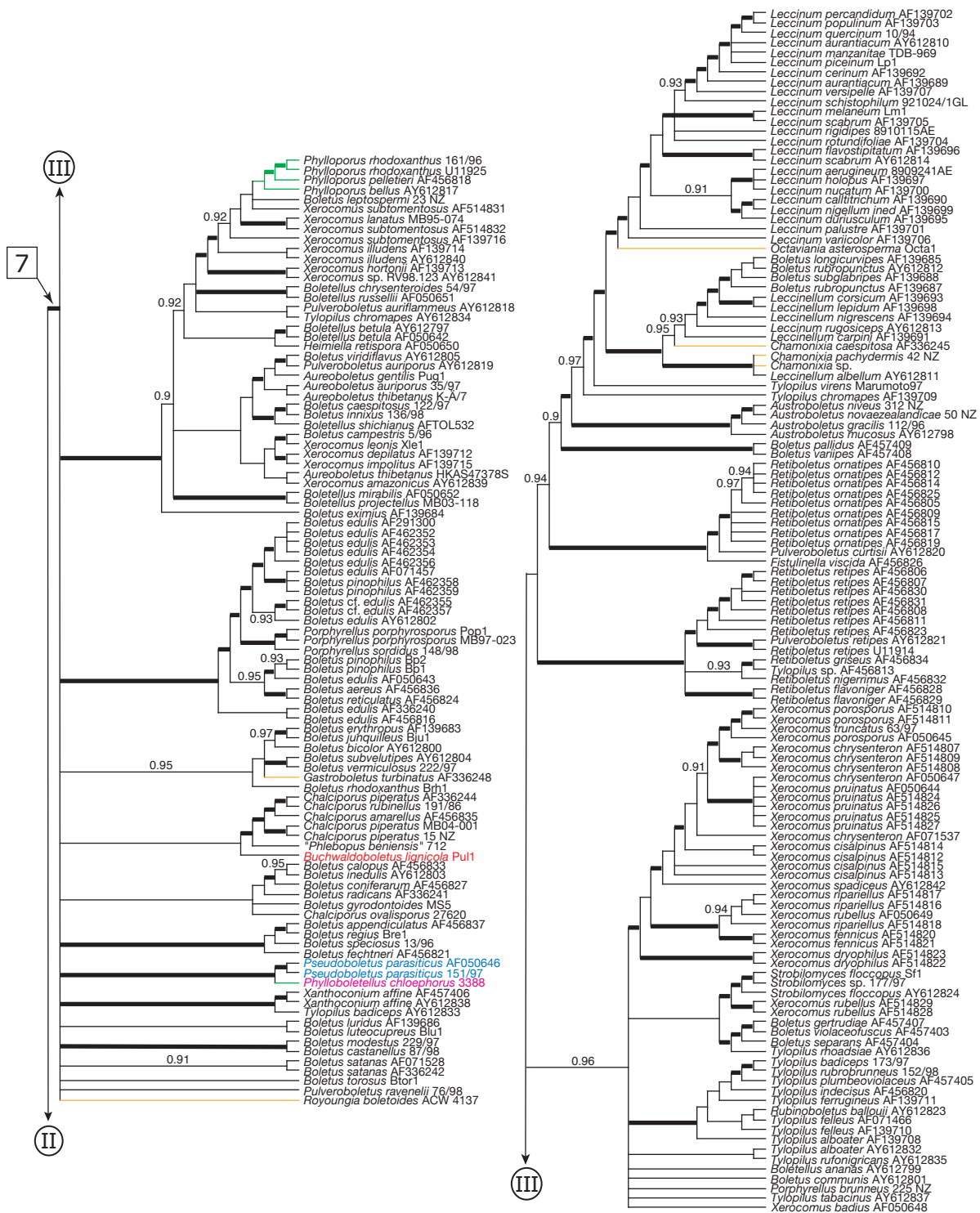
[### Note on the placement of *Neopaxillus*. Blast searches using the ITS sequence of *Neopaxillus echinospermus* (Speg.) Singer (AJ419194; Martin and Raidl 2002) retrieve distinct hits in the Agaricales].

LITERATURE CITED

- Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (homobasidiomycetes). *Syst Biodiv* 3: 113–157.
- Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. *Ainsworth and Bisby's Dictionary of the Fungi*. 9th ed. Cambridge, United Kingdom: CAB International University Press.
- Martin MP, Raidl S. 2002. The taxonomic position of *Rhizopogon melanogastroides* (Boletales). *Mycotaxon* 84: 221–228.
- Peintner U, Bougher NL, Castellano M, Moncalvo J-M, Moser MM, Trappe JM, Vilgalys R. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae) *Am J Bot* 88:2168–2179.



SUPPLEMENTARY FIG. 1.



SUPPLEMENTARY FIG. 1. Phylogenetic analyses of the nuc-*lsu* data set. Shown is a majority-rule consensus tree of 575300 trees sampled from stationary tree distributions of two independent MC³ analyses. Likelihood scores range from $-\ln L = 36197.381$ to 36134.785. Branches printed in bold type indicate posterior probability ranges from 0.98 – 1.0, regardless of the color. Lower PP values (0.9 – 0.97) are written along branches. Character coding for ancestral state reconstructions is shown by branch shading (morphology) and shading of species names (nutritional mode). The eight nodes that were reconstructed in the MRCA analyses are indicated in boxes. Roman numerals connect the partitioned parts of the tree. GenBank accession numbers are provided for published sequences and strain numbers of taxa are provided, for which new sequences were generated in this study. Terminals marked with “REG” only are unpublished sequences originating from the study of Jarosch (2001).

SUPPLEMENTARY TABLE I. List of newly generated sequences used in the multigene dataset analyses. Sequences marked with asterisks are deposited in the AFTOL database (<http://ocid.nacse.org/research/aftol/data.php>) including earlier entries.

Species	Strain	Origin	GenBank accession number				
			nuc-ssu	nuc-lsu	mt-lsu	ITS	<i>atp6</i>
<i>Aureoboletus thibetanus</i>	HKAS 47378S	China	AY654882*	AY700189*	DQ534577	DQ200917*	DQ534600*
<i>Austropaxillus</i> sp.	HN3434	Tasmania	DQ534673	DQ534670	DQ534578		
<i>Austropaxillus</i> sp.	HN3440	Tasmania	DQ534674		DQ534579	DQ534571	
<i>Austropaxillus</i> sp.	HN3458	Tasmania		DQ534671	DQ534580	DQ534572	
<i>Boletellus projectellus</i>	MB 03-118	U.S.A., MA	AY662660*	AY684158*	DQ534582	AY789082*	DQ534604*
<i>Boletinellus meruliooides</i>	MB 02-199	U.S.A., MA	AY662668*	AY684153*	DQ534581	DQ200922*	DQ534601*
<i>Boletus edulis</i>	REG Be3	Germany	DQ534675				
<i>Boletus pallidus</i>	179/97	U.S.A., NY	DQ534676			DQ534564	
<i>Boletus satanas</i>	REG Bs2	Germany				DQ534567	
<i>Bondarcevomyces taxi</i>	Dai2524	China	DQ534677	DQ534672	DQ534583	DQ534575	DQ534611
<i>Calostoma cinnabarinum</i>	MB 04-007	U.S.A., MA			DQ534584		DQ534599
<i>Chamonixia caespitosa</i>	92/83	Germany	DQ534678			DQ534565	
<i>Chalciporus piperatus</i>	MB 04-001	U.S.A., MA	DQ534679	DQ534648			
<i>Coniophora marmorata</i>	DAOM178982	Canada			DQ534585		
<i>Gyroporus cyanescens</i>	REG Gcy2	Germany	DQ534680				
<i>Gyrodon lividus</i>	REG Gll	Germany	DQ534681			DQ534568	
<i>Gomphidius roseus</i>	MB 95-038	Germany	DQ534682*	DQ534669*	DQ534587	DQ534570*	DQ534610*
<i>Hydnomerulius pinastri</i>	DAOM147762	Canada	DQ534683		DQ534588		DQ534595
<i>Hygrophoropsis aurantiaca</i>	MB 03-127	Germany	AY662663*	AY684156*		AY854067*	DQ534605
<i>Leucogyrophana mollusca</i>	DAOM138006	Canada	DQ534684		DQ534590		
<i>Melanogaster variegatus</i>	REG384	Germany	DQ534685	DQ534668			DQ534596
<i>Paxillus filamentosus</i>	REG304	Germany	DQ534686				
<i>Paxillus vernalis</i>	MB-062	China	AY662662*	AY645059*		DQ647827*	DQ534606*
<i>Phlebopus portentosus</i>	REG Php1	Botswana	DQ534687			DQ534569	
<i>Phylloporus pelletieri</i>	REG Pp1	Germany				DQ534566	
<i>Pisolithus arrhizus</i>	REG588	U.S.A.	DQ534688				
<i>Porphyrellus porphyrosporus</i>	MB 97-023	Germany	DQ534689*	DQ534643*		DQ534563*	DQ534609*
<i>Pseudomerulius aureus</i>	FP-103859-sp	U.S.A.			DQ534591		
<i>Rhizopogon olivaceotinctus</i>	OSC8245	U.S.A., OR	DQ534690				
<i>Strobilomyces floccopus</i>	MB 03-102	U.S.A., MA	AY662661*	AY684155*		AY854068*	DQ534607*
<i>Suillus granulatus</i>	REG Sg1	Germany	DQ534691		DQ534592		
<i>Suillus lakei</i>	PDD7	New Zealand	DQ534692				
<i>Suillus spraguei</i>	MB 03-93	U.S.A., MA	AY662659*	AY684154*		AY854069*	DQ534608*
<i>Suillus variegatus</i>	REG Sv3	Germany	DQ534693		DQ534593		
<i>Scleroderma laeve</i>	27936	U.S.A., OR	DQ534694				
<i>Tapinella atrotomentosa</i>	Ta86	U.S.A.	DQ534695			DQ534573	
<i>Tapinella panuoides</i>	REG318	Germany			DQ534594	DQ534574	
<i>Truncocolumella citrina</i>	Tci1	U.S.A.	DQ534696				
<i>Botryobasidium isabellinum</i>	GEL2109	Germany					DQ534597
<i>Gloeophyllum sepiarium</i>	DAOM137861	Canada					DQ534598
<i>Fibulorhizoctonia</i> sp.	LA082103L	U.S.A.			DQ534586		DQ534602*
<i>Fomitiporia mediterranea</i>	3/22 #7	Germany	AY662664*	AY684157*		AY854080*	DQ534603*
<i>Hygrocybe conica</i>	PBM918	U.S.A., CA			DQ534589		
<i>Plicaturopsis crispa</i>	FP-101310-sp	U.S.A.				DQ534576	

SUPPLEMENTARY TABLE II. List of newly generated sequences used in the nuc-lsu dataset analyses

Species	Authority	Strain	Country	GenBank No.
<i>Aureoboletus auriporus</i>	(Peck) Pouzar	35/97	U.S.A., MA	DQ534636
<i>Aureoboletus cf. thibetanus</i>	(Pat.) Hongo & Nagas.	K-Å/7	Japan	DQ534637
<i>Aureoboletus gentilis</i>	(Quél.) Pouzar	REG Pug1	Germany	DQ534635
<i>Austroboletus gracilis</i>	(Peck) Wolfe	112/96	U.S.A., MA	DQ534624
<i>Austroboletus niveus</i>	(McNabb) Wolfe	312	New Zealand	DQ534622
<i>Austroboletus novaezealandiae</i>	(McNabb) Wolfe	15	New Zealand	DQ534623
<i>Austropaxillus</i> sp.		HN3434	Tasmania	DQ534670
<i>Austropaxillus</i> sp.		HN3458	Tasmania	DQ534671
<i>Boletellus chrysenderoides</i>	(Snell) Snell	54/97	U.S.A., MA	DQ534634
<i>Boletus caespitosus</i>	Peck sensu Singer	122/97	U.S.A., NC	DQ534638
<i>Boletus campestris</i>	A.H. Smith & Thiers	5/96	U.S.A., MA	DQ534640
<i>Boletus cf. modestus</i>	Peck	229/97	U.S.A., MA	DQ534659
<i>Boletus gyrodontoides</i>	Corner	MS5	Malaysia	DQ534651
<i>Boletus innixus</i>	Frost	136/98	U.S.A., MA	DQ534639
<i>Boletus junquilleus</i>	(Quél.) Boud.	REG Bju1	Germany	DQ534645
<i>Boletus leptospermi</i>	McNabb	23	New Zealand	DQ534632
<i>Boletus luteocupreus</i>	Bertéa & Estadès	REG Blu1	Germany	DQ534657
<i>Boletus regius</i>	Krombh.	REG Bre1	Germany	DQ534653
<i>Boletus rhodoxanthus</i>	(Krombh.) Kallenb.	Brh1	U.S.A.	DQ534647
<i>Boletus speciosus</i>	Frost	13/96	U.S.A., MA	DQ534654
<i>Boletus torosus</i>	Fr. in Fr. & Hók	REG Btor1	Germany	DQ534661
<i>Boletus vermiculosus</i>	Peck	222/97	U.S.A., MA	DQ534646
<i>Bondarceomyces taxi</i>	(Bondartsev) Parmasto	Dai2524	China	DQ534672
<i>Calostoma cinnabarinum</i>	Desv.	MB 04-007	U.S.A., MA	DQ534666
<i>cf. Chalciaporus</i> sp.		712	Chile	DQ534650
<i>Chalciaporus ovalisporus</i>	(Cleland) Grgur.	27620	Australia	DQ534652
<i>Chalciaporus piperatus</i>	(Bull.) Bataille	MB 04-001	U.S.A., MA	DQ534648
<i>Chalciaporus piperatus</i>		15	New Zealand	DQ534649
<i>Chamonixia pachydermis</i>	(Zeller & C.W. Dodge) G.W. Beaton, Pegler & T.W.K. Young	42	New Zealand	DQ534620
<i>Diplocystis wrightii</i>	Berk. & M.A. Curtis	DSH s.n.	Puerto Rico	DQ534665
<i>Gomphidius roseus</i>	(Fr.) P. Karst.	MB 95-038	Germany	DQ534669
<i>Hydnomerulius pinastri</i>	(Fr.) Jarosch & Besl	Z. Wang s.n.	U.S.A., CA	DQ534667
<i>Leccinum aerugineum</i>	(Fr.) Lannoy & Estadès	8909241AE	France	DQ534618
<i>Leccinum manzanitae</i>	Thiers	TDB-969	U.S.A., CA	DQ534613
<i>Leccinum melaneum</i>	(Smotl.) Pilát & Dermek	REG Lm1	Germany	DQ534616
<i>Leccinum picinum</i>	Pilát & Dermek	REG Lp1	Austria	DQ534614
<i>Leccinum quercinum</i>	(Pilát) E.E. Green & Watling	REG Lq1	Germany	DQ534612
<i>Leccinum rigidipes</i>	P.D. Orton	8910115AE	France	DQ534617
<i>Leccinum schistophilum</i>	Bon	921024/1 GL	France	DQ534615
<i>Melanogaster variegatus</i>	(Vittad.) Tul.	REG384	Germany	DQ534668
<i>Octaviania asterosperma</i>	Vittad.	REG Octa1	France	DQ534619
<i>Phylloboletellus chloephorus</i>	Singer	3388	Mexico	DQ534658
<i>Phylloporus rhodoxanthus</i>	(Schwein.) Bres.	161/96	U.S.A., MA	DQ534631
<i>Porphyrellus brunneus</i>	McNabb	225	New Zealand	DQ534630
<i>Porphyrellus porphyrosporus</i>	(Fr. & Hók) Gilbert	REG Pop1	Germany	DQ534642
<i>Porphyrellus porphyrosporus</i>		MB97-023	Germany	DQ534643
<i>Porphyrellus sordidus</i>	(Frost) Snell	148/98	U.S.A., MA	DQ534644
<i>Pseudoboletus parasiticus</i>	(Bull. :Fr.) Sutara	151/97	U.S.A., NC	DQ534655
<i>Pseudoboletus parasiticus</i>		11/98	U.S.A., MA	DQ534656
<i>Pulveroboletus ravenelii</i>	(Berk. & M.A. Curtis) Murrill	76/98	U.S.A., MA	DQ534662
<i>Royoungia boletoides</i>	Castellano, Trappe & Malajczuk	ACW 4137	Australia	DQ534663
<i>Strobilomyces floccopus</i>	(Vahl :Fr.) P. Karst.	REG Sf1	Germany	DQ534626
<i>Strobilomyces</i> sp.		177/97	U.S.A., MA	DQ534627
<i>Tremellogaster surinamensis</i>	E. Fisch.	MCA1985	Guyana	DQ534664
<i>Tylopilus badiceps</i>	(Peck) A.H. Smith & Thiers	173/97	U.S.A., MA	DQ534628
<i>Tylopilus rubrobrunneus</i>	Mazzer & A.H. Smith	152/98	U.S.A., MA	DQ534629
<i>Tylopilus virens</i>	(W.F. Chiu) Hongo	Marumoto s.n.	Japan	DQ534621
<i>Xerocomus castanellus</i>	(Peck) Snell & Dick	87/98	U.S.A., MA	DQ534660
<i>Xerocomus lanatus</i>	(Rostk.) Singer	MB 95-074	Germany	DQ534633
<i>Xerocomus leonis</i>	(D.A. Reid) Bon	REG Xle1	Germany	DQ534641
<i>Xerocomus truncatus</i>	(Singer, Snell & E.A. Dick) Pouzar	63/97	U.S.A.	DQ534625

MATERIALS AND METHODS, SUPPLEMENT

DNA extraction, cloning, sequencing, and sequence alignment.—DNA was extracted from herbarium specimens and cultures using a phenol/chloroform extraction protocol (Lee and Taylor, 1990). The crude extracts were purified using GeneClean (Q-BIOgene, Irvine, California). DNA was diluted up to 100-fold with deionized water for use as PCR template. PCR reactions were performed for three nuclear and two mitochondrial rDNA regions using the primer combinations ITS1-F-ITS4 (ITS region including the 5.8S gene), LR0R-LR5 (nuc-lsu), PNS1-NS41 and NS19b-NS8 (nuc-ssu), ML5-ML6 (mt-lsu), and ATP6-1 – ATP6-2 (*atp6*). Sequences of primers used in this study have been described elsewhere (Vilgalys and Hester, 1990; White et al., 1990; Hibbett, 1996; Moncalvo et al., 2000). The amplifications were run in 35 cycles on a PTC-200 thermal cycler (MJ Research, Waltham, Massachusetts) using the following parameters: denaturation 94°C (1 min), annealing 50°C (45 sec), extension 72°C (1.5 min). PCR products were purified with Pellet Paint (Novagen, EMB Biosciences, San Diego, California). *Atp6* products were amplified using the protocol in Kretzer and Bruns (1999). In addition, some *atp6* products were cloned using TOPO TA cloning (Invitrogen, Carlsbad, California). Cleaned PCR products were inserted into the pCR 2.1-TOPO vector and transformed using the One Shot competent cell kit (Invitrogen). The cells were plated and incubated overnight on LB medium containing 50 µg/mL kanamycin, which was saturated with 50 µL X-gal. Three positive transformants each were directly analyzed with PCR using M13 Forward (-20) and M13 Reverse primers.

All PCR products were sequenced using BigDye terminator sequencing chemistry (Applied Biosystems, Foster City, California), purified with Pellet Paint, and run on an Applied Biosystems 3730 automated DNA sequencer. Contiguous sequences were assembled and edited using Sequencher 4.1 (GeneCodes Corp., Ann Arbor, Michigan). Automated alignments obtained by using ClustalX (Thompson et al 1997) were manually adjusted in MacClade 4.0 (Maddison and Maddison 2000).

Phylogenetic analyses of the nuc-lsu data set and the multi-gene dataset.—The following section describes how maximum parsimony analyses, maximum likelihood analyses, and Bayesian MC³ analyses were run in this study:

[-----NUC-LSU DATA SET, MAXIMUM PARSIMONY ANALYSIS-----]

BEGIN ASSUMPTIONS;

```

EXSET * stored_2 = 9 13 39 44 49 63 70-72 79 80 89 115 116 119 126 131
140 144 153 160 196 200 203 205 211 212 235 264 296 301 347 356 362 372 378 391
406 413 420 435 444-460 485-487 494 498 505 506 516 517 553-559 613-637 651-702
710 711 774 775 802-804 834-836 857 858 885-895;
END;
```

BEGIN PAUP;

set criterion=parsimony;

```

delete/only;
outgroup Botryobasidium_isabellinum Gautieria_otthii/only;
assume ancstates=standard;
set maxtrees=2000 increase=no;
log file=allboletaleshs.log;
```

```

hsearch addseq=random nreps=10000 nchuck=2 chuckscore=1000;
```

```

rootTrees;
```

```

savetrees brlens=yes File=allboletaleshs.tre replace=no;
```

end;

[-----NUC-LSU DATA SET, BAYESIAN ANALYSIS-----]

BEGIN mrbayes;

```

charset all = 1-1071;
set partition = all;
```

```

exclude 9 13 39 44 49 63 70-72 79 80 89 115 116 119 126 131 140 144 153
160 196 200 203 205 211 212 235 264 296 301 347 356 362 372 378 391 406
413 420 435 444-460 485-487 494 498 505 506 516 517 553-559 613-637 651-
702 710 711 774 775 802-804 834-836 857 858 885-895;
```

```

outgroup Botryobasidium_isabellinum;
```

```

lset nst=6 rates=invgamma;
```

```

set autoclose = yes;
```

```

mcmc ngen=50000000 printfreq=1000 samplefreq=100 nchains=4
savebrlens=yes filename=allboletales_bay;
```

```

mcmc;
sumt filename=allboletales_bay burnin=150000 contype=halfcompat;
```

```

[The final burnin proportion of trees was estimated plotting likelihood
scores as a function of the number of generations in Excel and sumt was
run again using the accurate value]
```

END;

[*****END*****NUC-LSU DATA SET*****]
[-----MULTIGENE DATA SET, PARSIMONY BOOTSTRAP-----]

BEGIN ASSUMPTIONS;

```

OPTIONS DEFTYPE=unord PolyTcount=MINSTEPS;
```

```

charset ATOL_BOLETALES = 1-3939;
```



```
charset atp6 = 1-705;
charset mt_lsu = 706-1021;
charset 28S = 1022-1977;
charset ITS = 1978-2162;
charset 18S = 2163-3939;
charset ambiguous_alignment = 106-108 250-255 828-836 1090 1091 1094-1098
1163 1167 1389 1413 1414 1417 1418 1430-1444 1459 1465 1481 1497-1500 1512 1529-
1531 1555-1557 1570 1582 1596-1626 1724 1727 1728 1729 1756-1759 1767 1776 1781
1804 1824 1825 2145-2147 2198 2207 2796 2802-2806 2842-2845 3135 3190 3421 3534-
3538 3640-3642 3926-3939;
```

```
Taxset no_atp6 = Athelia_arachnoidea Austropaxillus_sp.
Coniophora_marmorata Leucogyrophana_mollusca Pseudomerulius_aureus;
Taxset no_mt_lsu = Fomitiporia_mediterranea Melanogaster_variegatus
Coniophora_marmorata Suillus_pictus Porphyrellus_porphyrosporus;
Taxset no_ITS = Dendrocorticium_roseocarneum;
Taxset no_18S = Scleroderma_hypogaeum Suillus_ochraceoroseus;
```

```
EXSET * stored_2 = 106-108 250-255 828-836 1090 1091 1094-1098 1163
1167 1389 1413 1414 1417 1418 1430-1444 1459 1465 1481 1497-1500 1512 1529-1531
1555-1557 1570 1582 1596-1626 1724 1727 1728 1729 1756-1759 1767 1776 1781 1804
1824 1825 2145-2147 2198 2207 2796 2802-2806 2842-2845 3135 3190 3421 3534-3538
3640-3642 3926-3939;
```

END;

BEGIN PAUP;

set criterion=parsimony;

```
delete/only;
outgroup Botryobasidium_isabellinum Gautieria_otthii/only;
assume ancstates=standard;
set maxtrees=10000 increase=no;
log file=atolboletales2.log;
bootstrap treefile=atolboletales2.out nreps=1000 conlevel=50
search=heuristic/ addseq=random nreps=100;
rootTrees;
savetrees from=1 to=1 file=atolboletales2.tre;
```

end;

[-----MULTIGENE DATA SET, MAXIMUM LIKELIHOOD ANALYSIS-----]

Begin trees;

```
tree usertree = [&U]
(Botryobasidium_isabellinum,(Gautieria_otthii,((((Ceriporia_viridans,Gloeophyllum_separium),((Echinodontium_tinctarium,Plicaturopsis_crispa),(Sarcodon_imbricatus,Dendrocorticium_roseocarneum))),((Schizophyllum_commune,Cortinarius_iodes),Hygrocybe_conica),(Athelia_arachnoidea,Fibulorhizactonia_sp.),((((((((Calostoma_cinnabarinum,Gyroporus_cyanescens),(Scleroderma_laeve,Scleroderma_hypogaeum)),Pisolithus_arrhizus),(Phlebobius_portentosus,Boletinellus_merulioides)),((((((((Boletellus_projectellus,Aureoboletus_thibetanus),Phylloporus_rhodoxanthus),Xerocomus_chrysenteron),((Boletus_edulis,(Strobilomyces_floccopus,Porphyrellus_porphyrosporus)),Boletus_pallidus)),Boletus_satanas),Chamonixia_caespitosa),Chalciporus_piperatoides),(Hydnomerulius_pinastri,((Paxillus_filamentosus,Paxillus_involutus),Melanogaster_variegatus),Gyrodon_lividus))),((Hygrophoropsis_aurantiaca,Leucogyrophana_mollusca),(Coniophora_arida,Coniophora_marmorata)),((((Suillus_pictus,Suillus_variegatus),(Suillus_granulatus,Suillus_lakei)),Suillus_ochraceoroseus),Truncocolumella_citrina),Rhizopogon_olivaceotinctus),(Gomphidius_roseus,Chroogomphus_vinicolor)),((Tapinella_atrotomentosa,Tapinella_panuoides),Pseudomerulius_aureus),Bondarcevomyces_taxi),Austropaxillus_sp.),Serpula_himantioides))),Fomitiporia_mediterranea));
```

end;

BEGIN PAUP;

set criterion=likelihood;

set autoclose=yes warnreset=no;

```

delete/only;
outgroup Botryobasidium_isabellinum Gautieria_otthii/only;
set maxtrees=1000 increase=no;

lset nst=6 rates=gamma ncat=4 shape=0.4 basefreq=empirical;
lset pinvar=estimate;
lset tratio=estimate;

hsearch start = 1;

savetrees brlens=yes file=output.ml.trees replace=no;
end;

[-----MULTIGENE DATA SET, BAYESIAN ANALYSIS-----]

Begin mrbayes;

charset all = 1-3939;
charset atp6 = 1-705;
charset mt_lsu = 706-1021;
charset 28S = 1022-1977;
charset ITS = 1978-2162;
charset 18S = 2163-3939;

partition all = 5: atp6,mt_lsu,28S,ITS,18S;

set partition = all;

outgroup 1;

exclude 106-108 250-255 828-836 1090 1091 1094-1098 1163 1167 1389 1413 1414
1417 1418 1430-1444 1459 1465 1481 1497-1500 1512 1529-1531 1555-1557 1570 1582
1596-1626 1724 1727 1728 1729 1756-1759 1767 1776 1781 1804 1824 1825 2145-2147
2198 2207 2796 2802-2806 2842-2845 3135 3190 3421 3534-3538 3640-3642 3926-3939;

unlink revmat = (all);
unlink Tratio = (all);
unlink statefreq = (all);
unlink shape = (all);
unlink pinvar = (all);

lset nst=6 rates=invgamma;

set autoclose = yes;

mcmc ngen=10000000 printfreq=1000 samplefreq=100 nchains=4 savebrlens=yes
filename=Boletales_bay;

mcmc;
sumt filename=Boletales_bay.t burnin=15000;

[The final burnin proportion of trees was estimated plotting likelihood scores
as a function of the number of generations in Excel and sumt was run again using
the accurate value]

end;

[=====MODELS=====]

[-----MULTIGENE ANALYSES, MODELS ESTIMATED WITH MRMODELTEST 3.06-----]

[atp6 model:

Model selected: TVM+G
-lnL = 11664.4287

```

AIC = 23344.8574

Base frequencies:

freqA = 0.3640
freqC = 0.0865
freqG = 0.0715
freqT = 0.4780

Substitution model:

Rate matrix

R(a) [A-C] = 1.1314
R(b) [A-G] = 3.4024
R(c) [A-T] = 1.1990
R(d) [C-G] = 5.5481
R(e) [C-T] = 3.4024
R(f) [G-T] = 1.0000

Among-site rate variation

Proportion of invariable sites = 0

Variable sites (G)

Gamma distribution shape parameter = 0.3907

Likelihood settings from best-fit model (TVM+G) selected by AIC in Modeltest Version 3.06

BEGIN PAUP;

Lset Base=(0.3640 0.0865 0.0715) Nst=6 Rmat=(1.1314 3.4024 1.1990 5.5481 3.4024) Rates=gamma Shape=0.3907 Pinvar=0;
END;]

[mt-lsu model:

Model selected: TVM+I+G

-lnL = 2548.5554
AIC = 5115.1108

Base frequencies:

freqA = 0.3183
freqC = 0.1805
freqG = 0.2293
freqT = 0.2719

Substitution model:

Rate matrix

R(a) [A-C] = 1.2879
R(b) [A-G] = 3.0849
R(c) [A-T] = 1.6795
R(d) [C-G] = 0.6866
R(e) [C-T] = 3.0849
R(f) [G-T] = 1.0000

Among-site rate variation

Proportion of invariable sites (I) = 0.4408

Variable sites (G)

Gamma distribution shape parameter = 1.2415

BEGIN PAUP;

Lset Base=(0.3183 0.1805 0.2293) Nst=6 Rmat=(1.2879 3.0849 1.6795 0.6866 3.0849) Rates=gamma Shape=1.2415 Pinvar=0.4408;
END;]

[nuc-lsu model:

Model selected: GTR+I+G

-lnL = 9461.9609
AIC = 18943.9219

Base frequencies:

freqA = 0.2470
freqC = 0.2279
freqG = 0.2897
freqT = 0.2354

Substitution model:

Rate matrix

```

R(a) [A-C] = 1.0873
R(b) [A-G] = 3.4091
R(c) [A-T] = 1.0670
R(d) [C-G] = 0.5938
R(e) [C-T] = 9.3781
R(f) [G-T] = 1.0000
Among-site rate variation
Proportion of invariable sites (I) = 0.4128
Variable sites (G)
Gamma distribution shape parameter = 0.6052

BEGIN PAUP;
Lset Base=(0.2470 0.2279 0.2897) Nst=6 Rmat=(1.0873 3.4091 1.0670 0.5938
9.3781) Rates=gamma Shape=0.6052 Pinvar=0.4128;
END;]

```

[ITS 5.8S model:

```

Model selected: GTR+I+G
-lnL = 9461.9609
AIC = 18943.9219

Base frequencies:
freqA = 0.2470
freqC = 0.2279
freqG = 0.2897
freqT = 0.2354
Substitution model:
Rate matrix
R(a) [A-C] = 1.0873
R(b) [A-G] = 3.4091
R(c) [A-T] = 1.0670
R(d) [C-G] = 0.5938
R(e) [C-T] = 9.3781
R(f) [G-T] = 1.0000
Among-site rate variation
Proportion of invariable sites (I) = 0.4128
Variable sites (G)
Gamma distribution shape parameter = 0.6052

```

```

BEGIN PAUP;
Lset Base=(0.2470 0.2279 0.2897) Nst=6 Rmat=(1.0873 3.4091 1.0670 0.5938
9.3781) Rates=gamma Shape=0.6052 Pinvar=0.4128;
END;]

```

[18S model:

```

Model selected: GTR+I+G
-lnL = 9461.9609
AIC = 18943.9219

Base frequencies:
freqA = 0.2470
freqC = 0.2279
freqG = 0.2897
freqT = 0.2354
Substitution model:
Rate matrix
R(a) [A-C] = 1.0873
R(b) [A-G] = 3.4091
R(c) [A-T] = 1.0670
R(d) [C-G] = 0.5938
R(e) [C-T] = 9.3781
R(f) [G-T] = 1.0000
Among-site rate variation
Proportion of invariable sites (I) = 0.4128
Variable sites (G)
Gamma distribution shape parameter = 0.6052

```

BEGIN PAUP;

```
Lset Base=(0.2470 0.2279 0.2897) Nst=6 Rmat=(1.0873 3.4091 1.0670 0.5938  
9.3781) Rates=gamma Shape=0.6052 Pinvar=0.4128;  
END;]
```