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Amylocorticiales ord. nov. and Jaapiales ord. nov.: Early diverging clades of Agaricomycetidae were dominated by corticioid forms

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Abstract: The Agaricomycetidae is one of the most morphologically diverse clades of Basidiomycota that includes the well known Agaricales and Boletales, which are dominated by pileate-stipitate forms, and the more obscure Atheliales, which is a relatively small group of resupinate taxa. This study focused taxon sampling on resupinate forms that may be related to these groups, aimed at resolving the early branching clades in the major groups of Agaricomycetidae. A specific goal was to resolve with confidence sister group relationships among Agaricales, Boletales and Atheliales, a difficult task based on conflicting results concerning the placement of the Atheliales. To this end we developed a six-locus nuclear dataset (nuc-ssu, nuc-lsu, 5.8S, *rpb1*, *rpb2* and *tef1*) for 191 species, which was analyzed with maximum parsimony, maximum likelihood and Bayesian methods. Our analyses of these data corroborated the view that the Boletales are closely related to athelioid forms. We also identified an additional early branching clade within the Agaricomycetidae that is composed primarily of resupinate forms, as well as a few morphologically more elaborate forms including *Plicaturopsis* and *Podoserpula*. This clade, which we describe here as the new order Amylocorticiales, is the sister group of the Agaricales. We introduce a second order, the Jaapiales, for the lone resupinate genus *Jaapia* consisting of two species only. The Jaapiales is supported as the sister group of the remainder of the Agaricomycetidae, suggesting that the greatest radiation of pileate-stipitate mushrooms resulted from the elaboration of resupinate ancestors.

Key words: morphological evolution, multigene datasets, *rpb1* and *rpb2* primers

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INTRODUCTION

The Agaricomycetes includes approximately 21 000 described species (Kirk et al. 2008) that are dominated by taxa with complex fruiting bodies, including agarics, polypores, coral fungi and gasteromycetes. Intermixed with these forms are numerous lineages of corticioid fungi, which have inconspicuous, resupinate fruiting bodies (Binder et al. 2005; Larsson et al. 2004, Larsson 2007). No fewer than 13 of the 17 currently recognized orders of Agaricomycetes contain corticioid forms, and three, the Atheliales, Corticiales, and Trechisporales, contain only corticioid forms (Hibbett 2007, Hibbett et al. 2007). Larsson (2007) presented a preliminary classification in which corticioid forms are distributed across 41 families of Agaricomycetes.

The present study concerned corticioid lineages in the Agaricomycetidae, which includes the Agaricales and Boletales. The Agaricales is by far the largest order of Agaricomycetes (ca. 13 000 described species) and contains roughly 85% pileate-stipitate species, while Boletales (ca. 1300 spp.) contains roughly 77% pileate-stipitate species. Early phylogenetic analyses of Agaricomycetes (Hibbett et al. 1997; Moncalvo et al. 2000) demonstrated a sister group relationship of Agaricales and Boletales and suggested that the common ancestor of the Agaricomycetidae could have been pileate-stipitate (Binder and Hibbett 2002), but these studies did not sample corticioid forms extensively. Among the first phylogenetic studies to include corticioid taxa in Agaricomycetidae were those of Bruns et al. (1998), which suggested that *Piloderma croceum* might be closely related to the Agaricales, and Hibbett et al. (2000), which showed that *Amphinema byssoides* is closely related to the Boletales. As more sequences of corticioid forms were included in phylogenetic studies it became apparent that several early diverging lineages in the Agaricomycetidae are largely composed of corticioid taxa, raising the possibility that the common ancestor of the group could have been

corticioid. Multiple lineages of corticioid forms also were found to be nested within the Agaricales, suggesting that there have been repeated reversals from complex forms to corticioid forms (Binder et al. 2005; Hibbett 2004, Hibbett and Binder 2002; Larsson 2007).

Approximately three groups of Agaricomycetidae contain corticioid forms and appear to be outside both the Agaricales and Boletales. The smallest of these is the corticioid genus *Jaapia*, which we propose to call the Jaapiales. Analyses of nuclear and mitochondrial large and small subunit (nuc-lsu, nuc-ssu, mt-lsu, mt-ssu) rRNA genes (Binder et al. 2005) placed *Jaapia* as the sister group of the rest of the Agaricomycetidae, although analyses of the nuc-lsu rRNA alone (Larsson 2007) placed *Jaapia* as a close relative of the Gloeophyllales, Corticiales and Thelephorales. *Jaapia* includes two species, *J. argillacea* and *J. ochroleuca*, which produce thin, fully resupinate fruiting bodies and function as saprotrophs.

The second basal clade of corticioid forms in the Agaricomycetidae contains the Atheliales, which includes corticioid genera *Amphinema*, *Athelia*, *Athelopsis*, *Byssocorticium*, *Fibulorhizoctonia*, *Leptosporomyces*, *Piloderma* and *Tylospora* (Binder and Hibbett 2006; Larsson et al. 2004, Larsson 2007) and a recently proposed family, the Lepidostromataceae (containing three species in *Lepidostroma*), which is supported by nuc-lsu and nuc-ssu rRNA sequences as the sister group of Atheliales (Ertz et al. 2008). Members of the Atheliales all have simple corticioid fruiting bodies, while species in the Lepidostromataceae have minute clavarioid fruiting bodies. Diverse ecological strategies have evolved in the Atheliales-Lepidostromataceae clade. *Amphinema*, *Byssocorticium*, *Piloderma* and *Tylospora* spp. form ectomycorrhizal symbioses with Pinaceae and Fagaceae (Agerer 1987–1998; Danielson and Pruden 1989; Erland and Taylor 1999; Horton et al. 2005; Lilleskov et al. 2004), whereas *Athelia* and *Athelopsis* spp. are associated with a white rot and degrade primarily decayed wood, woody debris, mosses and leaf litter (Eriksson and Ryvarden 1973; Lindsey and Gilbertson 1978). Some *Athelia* species parasitize lichens or function as symbionts of cyanobacteria (Gilbert 1988; Jülich 1978; Oberwinkler 1970; Yurchenko and Golubkov 2003), while *Fibulorhizoctonia* forms symbioses with termites that involve mimicry of termite eggs by fungal sclerotia (Matsuura et al. 2000). Species of *Lepidostroma* are basidiolichens (Ertz et al. 2008).

The focus of this paper concerned the third assemblage of basal Agaricomycetidae, which has been referred to as the Amylocorticiaceae (Larsson 2007) or Atheliaceae pro parte (Matheny et al. 2006; this is not the same group that Larsson [2007] referred to as Atheliaceae sensu stricto, which is placed correctly in the Atheliales). Here we propose to call this clade the Amylocorticiales. Several studies have suggested that the Amylocorticiales is in the Agaricomycetidae, but its precise placement has not been well resolved. Analyses with nuc-lsu rRNA genes (alone or as the dominant component of a supermatrix including nuc-ssu, mt-lsu and mt-ssu rRNA genes) have placed the Amylocorticiales as the sister group of the Agaricales or as the sister group of a clade containing Agaricales, Boletales and Atheliales (Binder et al. 2005, Hibbett and Binder 2002; Larsson et al. 2004, Larsson 2007). Analyses of a large dataset focused on Agaricales with nuc-lsu, nuc-ssu and 5.8S rRNA genes, and genes that encode two subunits of RNA polymerase II (*rpb1*, *rpb2*) suggested that the Amylocorticiales is the sister group of the Agaricales, possibly along with a clade containing certain clavarioid (*Clavaria*, *Clavulinopsis*) and pileate-stipitate agaricoid forms (*Camarophyllop-Camarophyllopsis*) (Matheny et al. 2006).

The most taxonomically inclusive sampling of the Amylocorticiales so far was by Niemelä et al. (2007), who analyzed 15 species with nuc-lsu and 5.8S rRNA genes. Based on the work of Niemelä et al. and others (Binder et al. 2005, Hibbett and Binder 2002; Larsson et al. 2004, Larsson 2007; Matheny et al. 2006), taxa that could be members of the Amylocorticiales include corticioid (*Amylocorticium*, *Amylocorticiellum subillaqueatum* and *Ceraceomyces*), resupinate poroid (*Anomoporia* and *Anomoloma*), coralloid (*Deflexula*, *Lentaria*), pileate (*Plicapturopsis*, *Podoserpula*) and even gasteroid taxa (*Stephanospora*). However there has yet to be a multilocus analysis that combines all taxa reported to be members of the Amylocorticiales, along with adequate representatives of the other clades of Agaricomycetidae, which makes it difficult to assess the limits of the group.

The Amylocorticiales, Jaapiales and Atheliales-Lepidostromataceae clades are important for understanding the ancestral condition and early events in the diversification of the Agaricomycetidae, which includes more than half of all known Agaricomycetes. The goals of the present study were to address the higher-level relationships and limits of the basal clades of Agaricomycetidae, with a focus on Amylocorticiales. We analyzed a six-gene dataset emphasizing taxa that have been reported to be members of Amylocorticiales and Atheliales, along with Agaricales, Boletales, Jaapiales and other Agaricomycetes. We also compiled and analyzed a dataset containing all available nuc-lsu rRNA gene sequences of putative Amylocorticiales. This study reports 386 new gene sequences and includes formal taxonomic proposals for family Jaapiaceae fam. nov. and orders Amylocorticiales ord. nov. and Jaapiales ord. nov.

MATERIALS AND METHODS

Fungal isolates and DNA extraction.—The 127 fungal isolates used in this study were obtained from the Forest Products Laboratory (FPL, USDA Forest Service), wherefrom voucher specimens are available, BCCM/MUCL (Belgium), or they were already at hand from our studies (Binder et al. 2005; Binder and Hibbett 2006; Matheny et al. 2006, 2007). Isolates were grown up to 4 wk under ambient conditions on solid media, including MEA (20 g malt extract, 0.5 g yeast extract, 20 g agar) and modified vitamin medium (4 g glucose, 1 g malt extract, 1 g ammonium tartrate, 0.2 g KH_2PO_4, 0.1 g $MgSO_4$, 0.02 g NaCl, 0.026 g $CaCl_2$, 0.88 mg $ZnSO_4$, 0.81 mg $MnSO_4$, 0.8 mg $FeCl_3$, 20 g agar, 1 mL BME vitamins 100× solutions from Sigma) based on the original recipe by Fries (1978).

Approximately 25 mg tissue was scraped from Petri dishes with sterile forceps and scalpels, transferred to precooled mortars and ground with pestles to a fine powder with liquid nitrogen. The samples were transferred into 2 mL reaction tubes and cell lysis proceeded 1 h at 65 C with the addition of 800 mL extraction buffer (50 mM EDTA, 50 mM Tris-HCl, 3% SDS, pH 8). Cell debris, polysaccharides and proteins were separated from aqueous DNA portions through two purification steps with equal volumes of phenol:chloroform (1:1) and chloroform:isomylalcohol (24:1). Total DNA was precipitated with the addition of 3 M sodium acetate (0.1 Vol.%) and isopropanol (0.54 Vol.%) at −18 C. The DNA pellets were washed in 1 mL 70% EtOH, air dried and resuspended in 100 µL sterile H_2O. Crude extractions were purified with the GeneClean II Kit (MP Biomedicals, Santa Ana, California) before PCR experiments.

PCR, primer design and sequencing.—Genes and products amplified in this study were ribosomal nuc-ssu (amplified with primers PNS1–NS8), ribosomal nuc-lsu (LR0R–LR7), ITS (ITS1-F–ITS4), DNA-directed RNA polymerase II subunit one *rpb1* (A_f–C_r), DNA-directed RNA polymerase II subunit two *rpb2* (fRPB2-5F–bRPB2-7.1R and bRPB2-6.9F–bRPB2-11R1) and translation elongation factor 1-alpha *tef1* (EF1-983F–EF1-2218R). The specifications and sequences for these primers have been published (Gardes and Bruns 1993; Matheny et al. 2002, 2006; Matheny 2005; Rehner and Buckley 2005; Vilgalys and Hester 1990; White et al. 1990; http://faculty.washington.edu/benhall/). In addition we designed three new primers for *rpb1* and one new primer for *rpb2* more specific to Agaricomycetidae with an in silico approach with Amplify 3× 3.1.4 (http://engels.genetics.wisc.edu/amplify/). These were *rpb1*: aA_f (5′-GAGTGTCCGGGGCATTTYGG), i2.2F (5′-CGTTTTCGRTCGCTTGAT), aC_r (5′-ARAARTCBACHCGYTTBCCCAT) and *rpb2*: a-8.0R (5′-TCTCKGAAYTTVAGRTAYTCCAT). The new oligonucleotides were used in PCR and sequencing experiments.

A standardized PCR program (initial denaturation 95 C for 2 min, denaturation 94 C for 45 s, annealing 50 C for 1 min 10 s, extension 72 C for 2 min, 34 cycles) with prolonged extension times was chosen for all loci except *tef1*, for which the original protocol designed by Rehner and Buckley (2005) was found to be superior. *rpb1* and *rpb2* amplifications frequently resulted in more than one product. In this case the appropriate products were identified on 2% TAE gels in comparison to a 1 kb DNA-ladder (Promega, Madison, Wisconsin), excised with sterile spatulas and purified with the GeneClean II Kit. Multiple PCR products that could not be separated by electrophoresis were cloned with TOPO TA (Invitrogen, Carlsbad, California). The cleaned products were inserted into pCR 2.1-TOPO vectors and transformed with 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 analyzed directly using PCR with M13 forward and reverse primers. All PCR products were sequenced with BigDye 3.1 terminator sequencing chemistry (Applied Biosystems, Foster City, California) and run on an Applied Biosystems 3130 Genetic Analyzer. Contiguous sequences were assembled and edited with Sequencher 4.8 (GeneCodes Corp., Ann Arbor, Michigan). Automated alignments generated by Clustal X (Thompson et al. 1997) were manually adjusted in MacClade 4.08 (Maddison and Maddison 2005).

Dataset assembly.—Three hundred eighty-six sequences were newly generated for this study (TABLE I, SUPPLEMENTARY TABLES I–II), including 51 nuc-ssu, 63 nuc-lsu, 62 ITS, 59 *rpb1*, 58 *rpb2* and 93 *tef1* sequences. Initially the data were assembled separately by locus, complemented with published sequences (Binder and Hibbett 2006; Garnica et al. 2007; James et al. 2006; Matheny et al. 2006, 2007) and extended to 191 taxa. To assess topological congruence preliminary single-locus bootstrap analyses were run in PAUP* 4.0b10 (Swofford 2002) under the maximum parsimony criterion and inspected for potential conflicts, indicated by bootstrap support 70% and higher. Some supported conflicts occurred, comparing trees inferred from rDNA and protein-coding genes, which were overcome largely by omitting all intron regions (1711 base pairs [bp]) except the 5′ end of intron 2 in *rpb1*. Systematic errors were minimized further by contrasting parsimony bootstrapped trees inferred from alignments with and without third-codon positions, which are prone to saturation (Matheny et al. 2007). The direct comparison indicated that the exclusion of 1331 third-codon positions enhanced the phylogenetic signal and support values in general. Seven data partitions were imposed on the dataset, comprising combined rDNA loci and protein-coding genes by divided first and second positions, and the optimal models for each were estimated with Modeltest 3.06 (Posada and Crandall 2001).

Phylogenetic analyses.—All analyses were performed on a Linux Pro 9.2 Opteron AMD 246 cluster (Microway, Plymouth, Massachusetts). We inferred phylogenetic trees based on the combined six-gene dataset with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML 7.0.4 (Stamatakis 2006). The general time reversible model (GTR), using proportion of invariant sites and distribution of rates at variable sites modeled on a discrete gamma distribution with four rate classes, was estimated as the best-fit likelihood model for all partitions. Bayesian analyses used

TABLE I. Species list of Amylocorticiales, Atheliales, Jaapiales, resupinate Boletales and resupinate Agaricales used in this study and GenBank entries. Accession numbers for newly generated sequences are highlighted in boldface. See SUPPLEMENTARY TABLES I AND II for a complete list of isolates and GenBank accessions

Species	Isolate ID	GenBank accession numbers					
		ITS, 5.8S	nuc-lsu	nuc-ssu	*rpb1*	*rpb2*	*tef1*
Agaricales							
Aphanobasidium pseudotsugae	HHB-822	**GU187509**	**GU187567**	**GU187620**	**GU187455**	**GU187781**	**GU187695**
Cristinia rhenana	344311	**GU187496**	**GU187663**	—	—	—	—
Cristinia sp.	FP-100305	**GU187526**	**GU187585**	**GU187637**	**GU187470**	**GU187793**	**GU187718**
Amylocorticiales							
Amyloathelia crassiuscula	GB/K169-796	DQ144610	DQ144610	—	—	—	—
Amylocorticium cebennense	HHB-2808	**GU187505**	**GU187561**	**GU187612**	**GU187439**	**GU187770**	**GU187675**
Amylocorticium subincarnatum	AS_95	AY463377	AY586628	—	—	—	—
Amylocorticium subsulphureum	HHB-13817	**GU187506**	**GU187562**	**GU187617**	**GU187443**	**GU187773**	**GU187680**
Amyloxenasma allantosporum	KHL s. n.	**GU187498**	**GU187666**	—	—	—	—
Anomoloma myceliosum	MJL-4413	**GU187500**	**GU187559**	**GU187614**	**GU187441**	**GU187766**	**GU187677**
Anomoloma albolutescens	L-6088	**GU187507**	**GU187563**	**GU187618**	**GU187438**	**GU187768**	**GU187671**
Anomoporia bombycina	L-6240	**GU187508**	**GU187564**	**GU187611**	—	**GU187765**	**GU187674**
Anomoporia kamtschatica	KHL11072	AY463379	AY586630	—	—	—	—
Athelia rolfsii	AFTOL-664	DQ484061	AY635773	AY665774	—	**GU187821**	**GU187681**
Ceraceomyces tessulatus	KHL8474	AY463391	AY586642	—	—	—	—
Hypochniciellum subillaqueatum	KHL8493	AY463431	AY586679	—	—	—	—
Leptosporomyces septentrionalis	JS16122	**GU187497**	**GU187664**	—	—	—	—
Plicaturopsis crispa	AFTOL-1924	DQ494686	DQ470820	AY293148	—	**GU187816**	—
Podoserpula pusio	AFTOL-1522	DQ494688	DQ470821	—	—	**GU187804**	—
Serpulomyces borealis	L-8014	**GU187512**	**GU187570**	**GU187624**	**GU187446**	**GU187782**	**GU187686**
Atheliales							
Amphinema byssoides	EL11_98	GQ162810	GQ162810	—	—	—	—
Athelia arachnoidea	CBS418.72	**GU187504**	**GU187557**	**GU187616**	**GU187436**	**GU187769**	**GU187672**
Athelia epiphylla	FP-100564	**GU187501**	**GU187558**	**GU187613**	**GU187440**	**GU187771**	**GU187676**
Athelia sp.	FP-133442	**GU187503**	**GU187560**	**GU187615**	**GU187442**	**GU187772**	**GU187679**
Athelia sp.	L-10567	**GU187537**	**GU187592**	**GU187645**	**GU187477**	**GU187802**	**GU187739**
Athelia sp.	HHB-15599	**GU187502**	**GU187565**	**GU187619**	**GU187437**	**GU187767**	**GU187678**
Athelopsis glaucina	KHL11901	**GU187495**	**GU187662**	—	—	—	—
Athelopsis subinconspicua	KHL8490	AY463383	AY586634	—	—	—	—
Fibulorhizoctonia sp.	AFTOL-576	AY854062	AY635779	AY654887	AY857985	AY885161	AY879115
Leptosporomyces raunkiaerii	HHB-7628	**GU187528**	**GU187588**	**GU187640**	**GU187471**	**GU187791**	**GU187719**
Piloderma fallax	S-12	**GU187535**	**GU187591**	**GU187644**	pseudogene	**GU187797**	**GU187738**
Piloderma byssinum	KHL8501	DQ469282	DQ469282	—	—	—	—
Piloderma lanatum	JS24861	AY463454	AY586700	—	—	—	—

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TABLE I. Continued

Species	Isolate ID	GenBank accession numbers					
		ITS, 5.8S	nuc-lsu	nuc-ssu	*rpb1*	*rpb2*	*tef1*
Boletales							
Coniophora arida	FP-104367	**GU187510**	**GU187573**	**GU187622**	**GU187445**	**GU187775**	**GU187684**
Coniophora arida v. suffocata	MUCL30844	**GU187511**	**GU187568**	**GU187623**	**GU187457**	**GU187779**	**GU187685**
Coniophora cerebella	HK'8'	**GU187513**	**GU187569**	**GU187625**	**GU187447**	**GU187776**	**GU187687**
Coniophora marmorata	MUCL31667	**GU187515**	**GU187571**	**GU187626**	**GU187448**	**GU187780**	**GU187688**
Coniophora olivacea	FP-104386	**GU187516**	**GU187572**	**GU187627**	**GU187452**	—	**GU187689**
Coniophora prasinoides	FP-105969	**GU187519**	**GU187576**	**GU187621**	**GU187450**	**GU187785**	**GU187691**
Coniophora puteana	MUCL1000	**GU187521**	**GU187578**	**GU187631**	**GU187451**	**GU187778**	**GU187693**
Coniophora sp.	Braz-6	**GU187517**	**GU187575**	**GU187628**	**GU187458**	**GU187784**	**GU187697**
Gyrodontium sacchari	MUCL40589	**GU187522**	**GU187579**	**GU187632**	**GU187460**	**GU187764**	**GU187703**
Hydnomerulius pinastri	MD-312	**GU187523**	**GU187580**	**GU187633**	**GU187462**	**GU187787**	**GU187708**
Leucogyrophana arizonica	RLG-9902	**GU187527**	**GU187582**	**GU187636**	**GU187466**	**GU187792**	—
Leucogyrophana lichenicola	DAOM194172	**GU187531**	**GU187583**	**GU187638**	**GU187467**	**GU187789**	**GU187715**
Leucogyrophana mollusca	L-10277	**GU187525**	**GU187584**	**GU187634**	**GU187468**	**GU187817**	—
Leucogyrophana montana	2998a	—	**GU187665**	—	—	—	—
Leucogyrophana olivascens	HHB-11134	**GU187532**	**GU187587**	**GU187639**	**GU187469**	**GU187790**	**GU187717**
Leucogyrophana romellii	T-547	**GU187529**	**GU187586**	**GU187635**	**GU187465**	**GU187794**	**GU187720**
Pseudomerulius aureus	FP-103859	**GU187534**	**GU187590**	**GU187643**	**GU187474**	**GU187799**	**GU187731**
Pseudomerulius curtisii	REH8912	**GU187533**	**GU187589**	**GU187641**	**GU187472**	**GU187796**	**GU187725**
Serpula lacrymans	REG 383	**GU187542**	**GU187596**	**GU187649**	**GU187485**	**GU187809**	**GU187752**
Serpula himantioides	MUCL30528	**GU187545**	**GU187600**	**GU187651**	**GU187480**	**GU187808**	**GU187748**
Serpula himantioides	RLG-12941	**GU187547**	**GU187602**	**GU187654**	**GU187484**	**GU187811**	**GU187750**
Serpula incrassata	DAOM170590	**GU187541**	**GU187595**	**GU187652**	**GU187481**	—	**GU187751**
Serpula similis	MUCL43280	**GU187546**	**GU187601**	**GU187653**	**GU187486**	**GU187812**	**GU187724**
Serpula tignicola	CBS311.54	**GU187543**	**GU187597**	**GU187650**	**GU187487**	—	**GU187753**
Jaapiales							
Jaapia argillacea	CBS252.74	**GU187524**	**GU187581**	AF518581	**GU187463**	**GU187788**	**GU187711**

the GTR model with the substitution rate matrix, transition/transversion rate ratio, character state frequencies, gamma shape parameter α and proportion of invariant sites in each partition calculated independently. Posterior probabilities (PP) were drawn from two runs employing four Metropolis-coupled Markov chain Monte Carlo analyses over 15 000 000 generations each, sampling trees every 1000th generation. The final burn-in of trees that were not accounted for computing the 50% majority rule consensus tree was determined with Tracer 1.4 (Rambaut and Drummond 2007, http://beast.bio.ed.ac.uk/Tracer). Maximum likelihood (ML) searches conducted with RAxML involved 1000 replicates under the GTRGAMMA model, with all model parameters estimated by the program. The tree with the best likelihood value served as the starting tree for the Bayesian analyses. In addition 1000 rapid bootstrap (ML BS) replicates were run with the GTRCAT model.

Results from preliminary analyses provided evidence that the Amylocorticiales consists of a morphologically diverse group of species that may be underrepresented in the six-gene analyses. To obtain all available Amylocorticiales nuc-lsu rRNA sequences BLASTN analyses (Altschul et al. 1997) were conducted. The query sequences at hand were aligned to the best hits scored in GenBank with MacClade and *Jaapia* species were included as outgroups. The nuc-lsu dataset was submitted to the RAxML Blackbox server (Stamatakis et al. 2008) to calculate ML BS support. Parsimony BS analyses were performed locally with 1000 replicates in PAUP*, all characters equally weighted, one random taxon addition sequence and tree bisection reconnection (TBR) branch swapping. Heuristic parsimony searches used the same protocol, but keeping only two trees per replicate. The resulting optimal trees were subjected to TBR branch swapping limiting MAXTREES to 1000.

RESULTS

Efficiency of new Agaricomycetidae rpb1 and rpb2 primers.—The new primers in this study, which were designed as more specific alternatives to existing primers, yielded variable success rates. The *rpb1* primers aA_f and aC_r performed not as well as the pair A_f–C_r. Combinations of both (aA_f–C_r and A_f–aC_r) proved to be more efficient and increased the PCR success rate by roughly 25% compared to A_f–C_r. The hybridization site of aA_f is identical to the one of A_f, while aC_r binds 92 bp downstream of C_r. The third *rpb1* oligonucleotide, i2.2F, binds to a conserved site in intron 2 (338–355 bp downstream of A_f) and is highly efficient as a sequencing primer. In addition i2.2F turned out to be an excellent PCR primer when paired with C_r. The resulting PCR product is approximately 500 bp shorter than the 1380 bp A_f–C_r product.

The *rpb2* target region 5F–11R1 requires two PCR and averages 2215 bp. The new primer a-8.0R was designed as an alternative to b7.1R for use with f5F to amplify a product that provides 111 bp additional overlap with the second product b6.9F–b11R1, a total overlap of 286 bp. The a-8.0R oligonucleotide performed as effectively as b7.1R.

Phylogenetic analyses of the six-gene dataset.—The final concatenated dataset had a length of 6095 aligned nucleotide positions after pruning and had an overall completeness of approximately 91%. The six-gene dataset and the unshortened single-gene matrices have been deposited in TreeBase (S2565), including 5.8S (99% complete, 161 positions), nuc-lsu (100%, 1583), nuc-ssu (95%, 1841), *rpb1* (84%, 2058), *rpb2* (86%, 2472) and *tef1* (82%, 1022). In the searches with RAxML the six-gene alignment had 4034 distinct patterns with a proportion of gaps and undetermined characters of 0.202. Partitioned ML analyses resulted in a best scoring tree with a likelihood of −ln = 239802.52. In addition ML bootstrap analyses were run separately to estimate the support values in RAxML. We used Tracer 1.4 to inspect the log files generated in Bayesian analyses, confirming that the estimated sample sizes for statistics represent appropriate values of posterior distributions. The two Bayesian runs converged to stable likelihood values (−ln 235243.01–−ln 235178.03) after 9 000 000 generations and 6000 stationary trees from each analysis were used to compute a 50% majority rule consensus tree in PAUP* to calculate posterior probabilities. The analyses are summarized (FIG. 1), highlighting ML BS values up to 70% and strong Bayesian posterior probability values (*PP* = 1.0) for the focal groups.

Phylogenetic analyses of the nuc-lsu Amylocorticiales dataset.—The nuc-lsu dataset included 1422 positions; 1049 characters were constant and 250 characters were parsimony informative. Heuristic searches resulted in 18 equally parsimonious trees with a length of 829 steps (CI = 0.56, RI = 0.75), one of which is provided (FIG. 2). Support values for the Amylocorticiales are high (BS, ML BS = 100), but the backbone structure of the clade is not well supported. Nevertheless 13 nodes receive bootstrap support of at least 94% (in either parsimony or ML bootstrap), including six that group multiple species: (i) *Anomoloma* spp.; (ii) *Plicaturopsis crispa* and *Irpicodon pendulus*; (iii) *Amylocorticiellum subillaqueatum* and *Podoserpula pusio*; (iv) *Amylocorticium* spp.; (v) *Athelopsis lacerata*, *Amyloxenasma allantosporum* and *Phlebiella* sp.; and (vi) *Anomoporia bombycina* and *A. vesiculosa* (but not *Anomoporia kamtschatica*). Nine species in the Amylocorticiales nuc-lsu rRNA dataset were represented by multiple accessions. Most of these were grouped with strong support, but two species, *Anomoloma myceliosa* and *Amylocorticium cebennense*, were not resolved as monophyletic, and

two groups of species, *Anomoloma albolutescens* and *A. flavissimum*, and *Anomoporia bombycina* and *A. vesiculosa*, could not be distinguished based on nuc-lsu rRNA sequences (FIG. 2).

TAXONOMY

Jaapiaceae Manfr. Binder, K.H. Larss. & Hibbett fam. nov.

MycoBank MB515501

Basidiomata resupinata. Hymenophora laevia, tenuia, porosa. Systema hypharum monomiticum; hyphae fibulatae. Cystidia semper praesentia. Basidia clavata, tetraspora. Basidiosporae laeves, fusiformes, maturitate crassitunicatae, cyanophilae. Fungi lignicolae.

Typus: *Jaapia* Bres., Ann Mycol 9:428. 1911

Basidiomycetes with effused *basidiomata*. *Hymenophore* smooth, very thin, and porous. *Hyphal system* monomitic and all hyphae nodose-septate. *Cystidia* always present, long and strongly projecting. *Basidia* clavate, producing four spores. *Basidiospores* smooth, fusiform, at maturity thick-walled and strongly cyanophilous. Living on decaying wood.

Exemplar genus: *Jaapia*.

Jaapiales Manfr. Binder, K.H. Larss. & Hibbett, ord. nov.

MycoBank MB515500

Descriptio ordinis eadem est ac descriptio familiae.

Typus: *Jaapia* Bres., Ann Mycol 9:428. 1911

Amylocorticiales K.H. Larss., Manfr. Binder & Hibbett, ord. nov.

MycoBank MB515502

Basidiomata resupinata, effuso-reflexa vel stipitata. Hymenophora laevia, merulioidea, irpicoidea vel poroidea. Systema hypharum monomiticum; hyphae fibulatae. Cystidia interdum praesentia. Basidia terminalia vel raro lateralia, tetraspora. Basidiosporae laeves, ellipsoideae, cylindricae vel allantoideae, tenuitunicatae vel crassitunicatae, saepe amyloideae. Fungi lignicolae vel in plantis variis parasitici. Lignum decompositum brunneum vel album.

Typus: *Amylocorticium* Pouzar, Cesk Mykol 13:11. 1959

Basidiomycetes with effused, effused-reflexed to almost pileate, or stipitate *basidiomata*. *Hymenophore* smooth, merulioid, irpicoid or poroid. *Hyphal system* monomitic and all hyphae nodose-septate. *Cystidia* are rare and when present, tube-like and often nodose-septate. *Basidia* are mostly terminal but in one genus born laterally on horizontal hyphae (pleurobasidia), invariably producing four spores. *Basidiospores* smooth, thin-walled or thick-walled, ellipsoid, cylindrical or allantoid, in most species amyloid. Species live saprophytically on decaying wood or as plant parasites. Associated with brown rot or white rot.

Exemplar genera: *Amylocorticium*, *Anomoporia* Pouzar 1966, *Irpicodon* Pouzar 1966, *Podoserpula* D.A. Reid 1963, *Serpulomyces* (Zmitr.) Zmitr. 2002.

DISCUSSION

This study represents the most intensive analysis of the ''basal'' lineages of Agaricomycetidae to date, and it resolves some conflicts arising from studies with more limited sampling of genes or taxa in the Amylocorticiales, Atheliales and Jaapiales (Binder et al. 2005, Larsson 2007, Larsson et al. 2004, Matheny et al. 2006). The Agaricomycetidae is resolved as monophyletic in all six-gene analyses and receives high support values (ML BS = 99%, PP = 1.0; FIG. 1). Strongly supported clades within the Agaricomycetidae include the Amylocorticiales (74%, 1.0), Atheliales (100%, 1.0), Boletales (100%, 1.0) and Agaricales (89%, 1.0). The sister group relationship of the Agaricomycetidae with the lone genus *Jaapia* has been seen in previous analyses with rRNA genes (Hibbett and Binder 2002), but here it receives moderately strong support (71%, 1.0) for the first time. Other strongly supported sister group relationships include the Amylocorticiales-Agaricales clade (90%, 1.0) and the Atheliales-Boletales clade (88%, 1.0).

Amylocorticiales.—The Amylocorticiales contains mostly resupinate forms that have been referred to genera *Anomoporia*, *Amyloathelia*, *Amylocorticiellum*, *Amylocorticium*, *Amyloxenasma*, *Anomoloma*, *Athelia*, *Athelopsis*, *Ceraceomyces*, *Hypochniciellum*, *Leptosporomyces* and *Serpulomyces* (FIG. 2). Several of these taxa appear to be non-monophyletic. Genera that are resolved as having multiple lineages within the Amylocorticiales based on the nuc-lsu rRNA analyses include *Anomoporia*, *Ceraceomyces* and *Hypochniciellum*. Genera that include isolates that have been placed (by this or other studies; Binder et al. 2005, Larsson et al. 2006, Matheny et al. 2006) outside Amylocorticiales include *Athelia* (also resolved in Atheliales), *Hypochniciellum* (Agaricales) and *Ceraceomyces* (Polyporales). Some of these placements might be due to misidentifications, which are particularly likely with corticioid forms (Binder et al. 2005). Alternative generic classifications that could resolve some of the conflicts between phylogeny and taxonomy have been proposed for several of the species analyzed here, including *Amylocorticiellum subillaqueatum* (for *Hypochniciellum subillaqueatum*)

FIG. 1. Phylogenetic relationships of Agaricomycetidae inferred from nuc-ssu, nuc-lsu, and 5.8S rRNA, *rpb1*, *rpb2* and *tef1* genes. Topology, branch lengths and bootstrap values above branches are from RAxML analyses excluding third base positions in protein-coding genes. Thickened branches indicate Bayesian posterior probability = 1.0. Resupinate species are highlighted in boldface. Abbreviations in Amylocorticiales and Atheliales clades: BR (brown rot), ECM (ectomycorrhizal), PLP (plant pathogen), TES (termite symbionts).

and *Serpulomyces borealis* (for *Ceraceomyces borealis*) (Zmitrovich and Spirin 2002). A genus-level revision of the Amylocorticiales is needed, but that will require multilocus data and additional isolates for many of the problematical taxa. Analyses of internal transcribed spacers of nuc rRNA genes could be useful in addressing possible synonymies in the *Anomoporia bombycina/vesiculosa* group and the *Anomoloma albolutescens/flavissium* group (FIGS. 2; 3A–C, E, F).

Although it is characterized by resupinate forms, there actually has been considerable morphological diversification within the Amylocorticiales. Nonresupinate members of the clade include the ''pagoda fungus'', *Podoserpula pusio* (FIG. 3D), which has a multitiered pileate-stipitate form, and the sister taxa *Plicaturopsis crispa* and *Irpicodon pendulus* (FIG. 3G), which are pileate-sessile (FIGS. 1, 2). *Podosperula* and *Plicaturopsis* both have merulioid hymenophores; *Irpicodon* has an irregularly hydnoid hymenophore, and *Anomoporia* and *Anomoloma* have poroid hymenophores. The inclusion of these morphologically divergent forms in an otherwise corticioid clade is

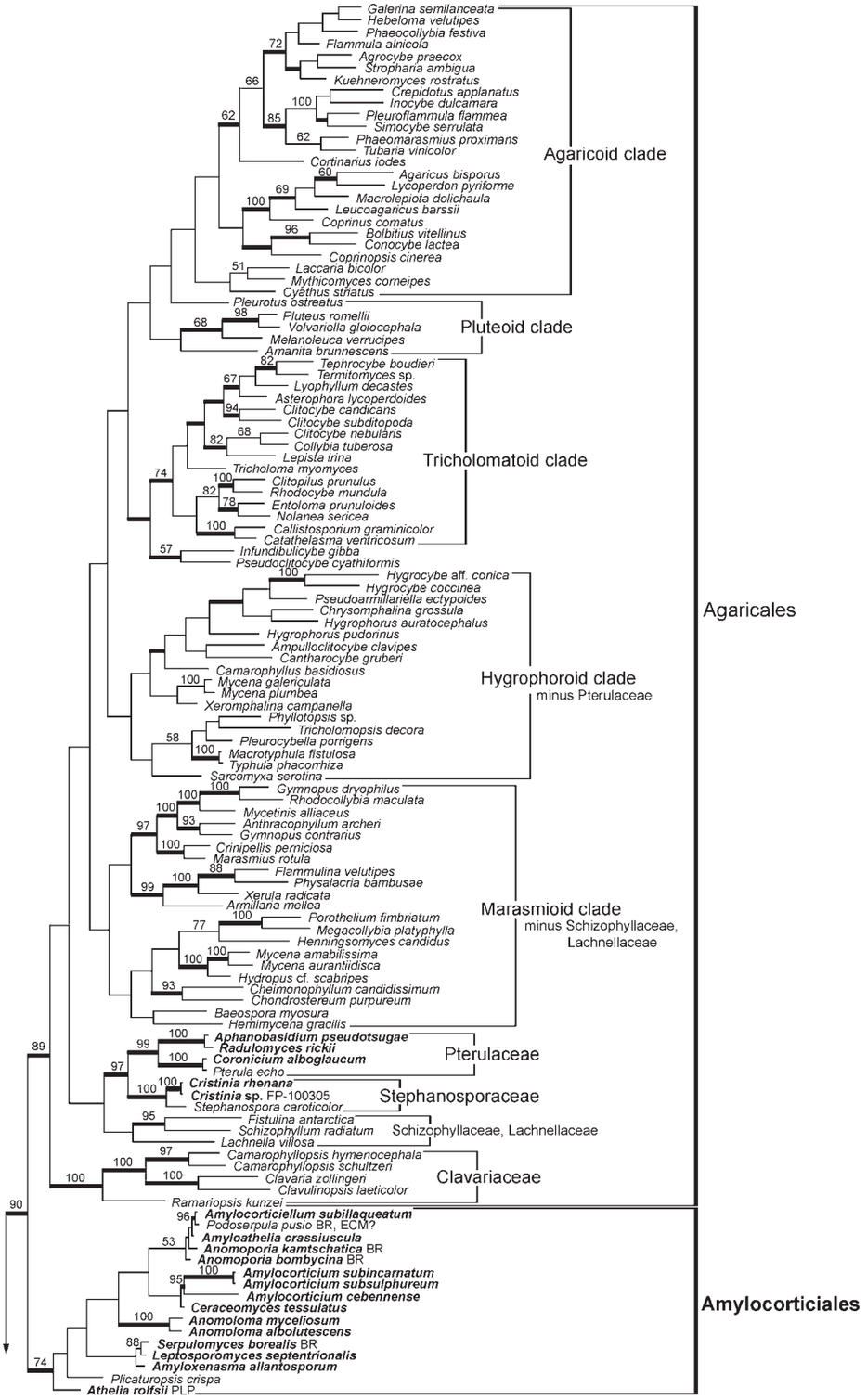

FIG. 1. Continued.

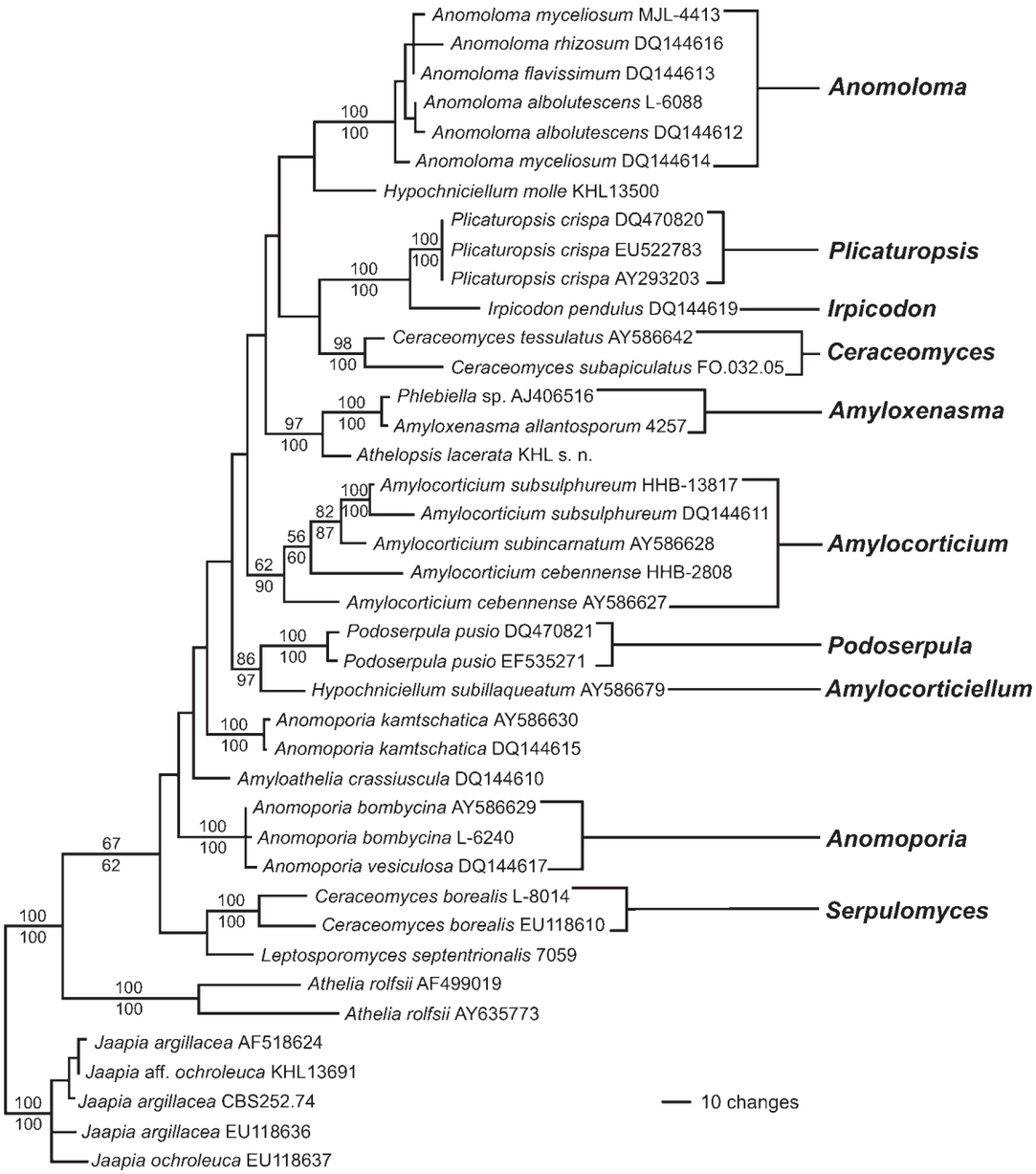

FIG. 2. Phylogenetic relationships of Amylocorticiales inferred from nuc-lsu rRNA gene sequences. One of 18 equally parsimonious trees. Support values above branches are from maximum parsimony bootstrap searches, and values below branches are from rapid bootstrap analyses with RAxML. Strain numbers are provided for species for which sequencing data were generated in this study.

surprising, but except for *Irpicodon* they all are represented by multiple accessions in the nuc-lsu rRNA dataset (FIG. 2), suggesting that misidentifications are not a source of error. An amyloid reaction of the basidiospore wall is present in most Amylocorticiales species but lacking in *Ceraceomyces*, *Podoserpula*, *Serpulomyces* and *Leptosporomyces septentrionalis*. To our knowledge spore amyloidity is not reported for *Athelia rolfsii*. Amyloidity of spores is not unique to Amylocorticiales and is a widespread phenomenon in both Russulales and Agaricales.

All species in Amylocorticiales have a monomitic hyphal system with clamped hyphae and a thickening hymenium. In typical cases the corticioid taxa develop hymenium over a subiculum of rather loosely interwoven hyphae to create a membranaceous fruiting body structure. There is a tendency for the most basal hyphae in the subiculum to be distinctly wider than in

the subhymenium. Good examples of this fruiting body construction are seen in *Ceraceomyces tessulatus*, *S. borealis* and *Hypochniciellum molle*. This type of structure is not unique for species in Amylocorticiales and is not present in all species. The fruiting body texture of *Amyloxenasma* species differs in that they have a dense hyphal system with narrow, contorted and mostly indistinct hyphae in a subgelatinous matrix. Basidiospores are invariably smooth, allantoid, cylindrical or ellipsoid with thin to thickened spore walls. Cystidia are not common and when present simple, tube-like and sometimes septate. Other kinds of differentiated vegetative organs are lacking, but sclerotia are present in *A. rolfsii*. Conidia are documented only for *Amylocorticiellum subillaqueatum*, which has small cylindrical conidia formed on short subulate outgrowths from vegetative hyphae.

The most parsimonious ancestral state reconstruction (ASR) of fruiting body forms in Amylocorticiales, based on either the nuc-lsu rRNA tree or the six-gene tree (FIGS. 1, 2), suggests that the plesiomorphic fruiting body form of the Amylocorticiales is resupinate with a smooth hymenophore. ASR can be sensitive to analytical method and taxon sampling, so this inference should be viewed with caution. Nevertheless the simplest interpretation of morphological evolution suggests there have been independent gains of pileate-stipitate and pileate-sessile forms, as well as hydnoid, merulioid and poroid hymenophores within the Amylocorticiales.

Most species of the Amylocorticiales are thought to be saprotrophic and occur on wood at various stages of decay. There is diversity in the mode of decay however. Species of *Anomoloma* produce a white rot, and on that basis the genus was segregated from *Anomoporia*, which includes species that are associated with brown rot (Niemelä et al. 2007). Information about decay type among other members of the Amylocorticiales is fragmentary; *Amylocorticium* spp. are reported to produce a brown rot, *P. crispa* produces a white rot and *S. borealis* is reported to produce either a white rot or brown rot (Ginns 1976, Ginns and Lefebvre 1993). Biotrophic nutritional modes also occur in the Amylocorticiales. *Athelia rolfsii* (*Sclerotium rolfsii*) is a soilborne plant pathogen that attacks turf grasses and other herbaceous hosts (Ginns and Lefebvre 1993). Niemelä et al. (2007) suggested that *Anomoloma flavissimum* might be ectomycorrhizal because the fruiting bodies seem to arise from the ground and rhizomorphs are observed in soil and debris, but they also noted that the wood underlying fruiting bodies had white rot. Similarly *P. pusio* grows on old rotten stumps or in litter on the ground next to old stumps and could be a saprotroph or ectomycorrhizal (Bougher and Syme 1998).

Agaricales.—The limits of the Amylocorticiales and Agaricales were poorly resolved in Binder et al. (2005) and Matheny et al. (2006). The analyses presented here suggest that several lineages that previously have been placed as close relatives of the Amylocorticiales actually represent multiple lineages within the Agaricales. One such lineage is the group containing the coralloid *Clavaria zollingeri* and *Clavulinopsis laeticor* and the agaricoid *Camarophyllopsis hymenocephala*, which Matheny et al. (2006) called the Clavariaceae. Matheny et al. included these taxa in a large dataset containing *rpb1*, *rpb2*, nuc-ssu, nuc-lsu and 5.8S rRNA genes that was focused on Agaricales (Boletales and Atheliales also were included). Depending on the analytical method, the Clavariaceae was placed as the sister group of the Amylocorticiales (in Bayesian analyses) or was nested within the Agaricales (parsimony). The Amylocorticiales-Clavariaceae group was referred to as the plicaturopsidoid clade, one of six major clades of Agaricales. The six-gene analyses presented here add *tef1* to the set of loci analyzed by Matheny et al. (2006) and include all three species of Clavariaceae sampled by Matheny et al. plus *Camarophyllopsis schultzeri* and the coralloid *Ramariopsis kunzei*. In the optimal maximum likelihood tree (FIG. 1) the Clavariaceae form a strongly supported (ML BS = 100%, PP = 1.0) clade that is resolved as the sister group of the remainder of the Agaricales. This placement is weakly supported however as are all of the deepest nodes comprising the backbone of the Agaricales. Of the six major clades of Agaricales resolved by Matheny et al. (2006) two are resolved as monophyletic (the agaricoid and tricholomatoid clades) with only the tricholomatoid clade receiving significant support (ML BS = 74%, PP = 1.0). The pluteoid clade is monophyletic, pending exclusion of the Pleurotaceae, and the hygrophoroid clade is monophyletic with the exclusion of the Pterulaceae.

Binder et al. (2005) performed analyses of a supermatrix containing mostly nuc-lsu rRNA genes, along with nuc-ssu, mt-lsu and mt-ssu rRNA genes. Two members of the Amylocorticiales, *Plicaturopsis crispa* and *Phlebiella* sp., were grouped in a weakly supported clade containing three members of the Atheliales (labeled as the athelioid clade) and a heterogeneous assemblage of coralloid (*Lentaria albovinacea*), corticioid (*Radulomyces molaris*), hydnoid (*Deflexula subsimplex*) and gasteroid (*Stephanos-Stephanospora caroticolor*) forms. *Lentaria albovinacea* was not included in the six-gene analyses in the present study, but it was included in an analysis of *rpb1* and nuc-lsu rRNA genes focused on Agaricales (Garnica et al. 2007), in which it was placed with weak support (bootstrap < 50%, PP = 0.85) as the sister group of *Hygrophorus chrysodon*. Garnica et al. (2007)

FIG. 3. Representatives of Amylocorticiales and Jaapiales. A. *Anomoporia vesiculosa* (picture by Yu-Cheng Dai) Changbaishan Nat. Res., Lilin Prov., China. B. *Anomoloma myceliosum* (by Yu-Cheng Dai) Changbaishan Nat. Res., Lilin Prov., China. C. *Anomoporia bombycina* (by Yu-Cheng Dai) Jiuzhai Nat. Res., Sichuan Prov., China. D. *Podoserpula pusio* (by Kevin Thiele and Tom May), Jensens Creek at Malinns, Bonang Road, Victoria, Australia. E. *Anomoloma flavissimum* (by Yu-

did not include any members of the Amylocorticiales, but parsimony and maximum likelihood analyses of a nuc-lsu rRNA dataset including Amylocorticiales and relevant Agaricales are consistent with the placement of *L. albovinacea* close to or nested within the Hygrophoraceae (Binder and Hibbett unpubl). However genus *Lentaria* is polyphyletic with *Lentaria michneri*, *L. pinicola* and *L. dendroidea* having been shown to be in the Gomphales, Phallomycetidae (Binder et al. 2005, Hosaka et al. 2006; Larsson 2007).

Radulomyces and *Deflexula* are members of the Pterulaceae, a family placed by Matheny et al. (2006) in the hygrophoroid clade of the Agaricales. Munkacsi et al. (2004) showed that the Pterulaceae includes the coralloid genus *Pterula* and fungal cultivars of the Neotropical ant *Apterostigma pilosum*, and Larsson (2007) suggested that the Pterulaceae also includes the corticioid genera *Aphanobasidium*, *Coronicium* and *Merulicium*. The six-gene analysis presented here found strong support (ML BS = 99%, PP = 1.0) for a Pterulaceae clade containing *Pterula echo*, *Radulomyces rickii*, *Coronicium alboglaucum* and *Aphanobasidium pseudotsugae*. The agaricoid wood decayer *Phyllotopsis nidulans* was placed in the Pterulaceae by Matheny et al. (2006), but the present analyses suggest (with weak support) that it is related to *Pleurocybella porrigens* and *Phyllotopsis* sp., which are also agaricoid wood decayers.

Stephanospora caroticolor is a hypogeous gasteromycete that has anatomical similarities to the corticioid species *Lindtneria trachyspora*. Larsson (2007) showed that *Lindtneria* and the corticioid taxa *Cristinia helvetica* and *Athelidium aurantiacum* form a clade, which he called the Stephanosporaceae. The present analysis strongly supports (ML BS = 100%, PP = 1.0) the monophyly of the Stephanosporaceae, represented by *S. caroticolor*, *Cristinia rhenana* and *Cristinia* sp. (FIG. 1). The Stephanosporaceae is strongly supported (ML BS = 97%, PP = 1.0) as the sister group of the Pterulaceae in the six-gene analysis (FIG. 1). The same relationship was recovered by Larsson (2007) based on nuc-lsu rRNA sequences but with weak support.

Atheliales-Boletales clade.—The results of the six-gene analyses presented here are consistent with Binder and Hibbett (2006), Larsson et al. (20040 and Larsson (2007) with regard to the composition of the Atheliales and Boletales and their sister group relationship. Even with more than 6 kilobases of data however the deepest divergences within the Atheliales are not resolved with confidence. This lack of resolution makes it difficult to infer patterns of evolution in nutritional modes in Atheliales, which includes a remarkable diversity of ectomycorrhizal species, saprotrophs, termite symbionts, lichen parasites and cyanobacterial symbionts (Agerer 1987–1998; Danielson and Pruden 1989; Eriksson and Ryvarden 1973; Erland and Taylor 1999; Gilbert 1988; Horton et al. 2005; Jülich 1978; Lilleskov et al. 2004; Lindsey and Gilbertson 1978; Matsuura et al. 2000; Munkacsi et al. 2004; Oberwinkler 1970; Yurchenko and Golubkov 2003). Moreover this study did not sample all taxa of Atheliales (missing taxa include the ectomycorrhizal *Tylospora asterophora* and *Byssocorticium pulchrum*) or its apparent sister group, the lichen-forming Lepidostromataceae (Ertz et al. 2008).

The suborders and unplaced families within the Boletales recognized in Binder and Hibbett (2006) using *atp6*, mt-lsu, nuc-lsu, nuc-ssu and 5.8S rRNA genes, are all strongly supported in the present study (FIG. 1). Several higher-level groupings that were not resolved previously (or that were not robust) receive strong support here, including a clade uniting the Boletineae (including ''Paxillineae'') and Sclerodermatineae (ML BS = 99%, PP = 1.0) and another clade uniting the Tapinellineae and Serpulaceae (ML BS = 90%, PP = 1.0). The latter appears to be the sister group of the remainder of the Boletales (ML BS = 75%, PP = 1.0). The Boletales is morphologically diverse, containing pileate-stipitate forms with tubular or lamellate hymenophores, resupinate and effused-reflexed forms and gasteromycetes. The typical boletoid fruiting body form (pileate-stipitate with a tubular hymenophore) is restricted to the strongly supported (ML BS = 99%, PP =1.0) Boletineae/Sclerodermatineae/Suillineae clade and may be a synapomorphy of that group. Resupinate to effused-reflexed wood decayers occur throughout the Tapinellineae, Coniophorineae, Serpulaceae and Hygrophoropsidaceae (including *Leucogyrophana*, which is polyphyletic, *Coniophora*, and *Serpula*), which is consistent with the view that resupinate forms and saprotrophic living strategies could be plesiomorphic in both Boletales (Binder and Hibbett 2006) and Atheliales.

←

Cheng Dai) Fenglin Nat. Res., Heilongjiang Prov. China. F. *Anomoloma albolutescens* (by Yu-Cheng Dai) Changbaishan Nat. Res., Lilin Prov., China. G. *Irpicodon pendulus* (by Olli Manninen), Tyresta National Park, Sweden. H. *Jaapia ochroleuca* (by Kilian Mühlebach), Dalpe Ti Pian di mezzo, Switzerland.

Conclusions.—The Agaricales and Boletales, two large clades of mostly pileate-stipitate forms that contain more than half of the known species of Agaricomycetes, are nested within a paraphyletic assemblage of three relatively small clades containing mostly resupinate forms. One of these is the newly recognized Amylocorticiales. Although it is a small group the Amylocorticiales manifests many of the evolutionary trends evident across the Agaricomycetes as a whole. The Amylocorticiales appears to be primitively corticioid and to have undergone repeated elaboration in fruiting body form and hymenophore configuration, although no known agaricoid forms are in the group. The Amylocorticiales is also of interest from a physiological perspective, being one of six major clades of Agaricomycotina in which the brown rot mode of wood decay has evolved (the others are the Polyporales, Gloeophyllales, Boletales, Agaricales [*Fistulina*] and Dacrymycetales; Hibbett and Donoghue 2001). The occurrence of plant pathogens and possibly ectomycorrhizal taxa in the group further exemplifies the ecological adaptability of Agaricomycetes and invites investigations into the mechanistic basis of shifts in nutritional modes.

The present study reinforces the view that corticioid fungi, while accounting for a relatively small fraction of described species, contain a disproportionately large number of the major clades of Agaricomycetes (Larsson et al. 2004, Larsson 2007) and could represent the plesiomorphic form of the entire class (Hibbett and Binder 2002, Hibbett 2004). There is a striking imbalance in the number of described species in the Amylocorticiales (roughly 70 species), Atheliales (ca. 95 spp.), and Jaapiales (two spp., *Jaapia argillacea* and *J. ochroleuca* [FIG. 3H]) compared to the Agaricales (ca. 13 000 spp.) and Boletales (ca. 1300 spp.). To some extent this is probably due to under sampling of corticioid fungi, but it is hard to believe that taxonomic bias alone could account for the magnitude of the disparity. Instead it is likely that there have been repeated shifts in diversification rates in the Agaricomycetidae, with speciation rates increasing (or extinction rates decreasing) in the Agaricales and Boletales relative to the Amylocorticiales, Atheliales and Jaapiales. It is tempting to speculate that the evolution of agaricoid and boletoid fruiting body forms, which are lacking in the Amylocorticiales, Atheliales and Jaapiales, could have spurred diversification in the Agaricales and Boletales. The evolution of ectomycorrhizal living strategies also could have been a ''key innovation'' in the Agaricomycetidae, but this does not explain why the largely ectomycorrhizal Atheliales have remained such a small group. The present study reconstructed phylogenetic relationships, but it did not include formal analyses that could detect shifts in diversification rates (Maddison et al. 2007) or address their causes. Such analyses are under way and will be reported elsewhere.

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