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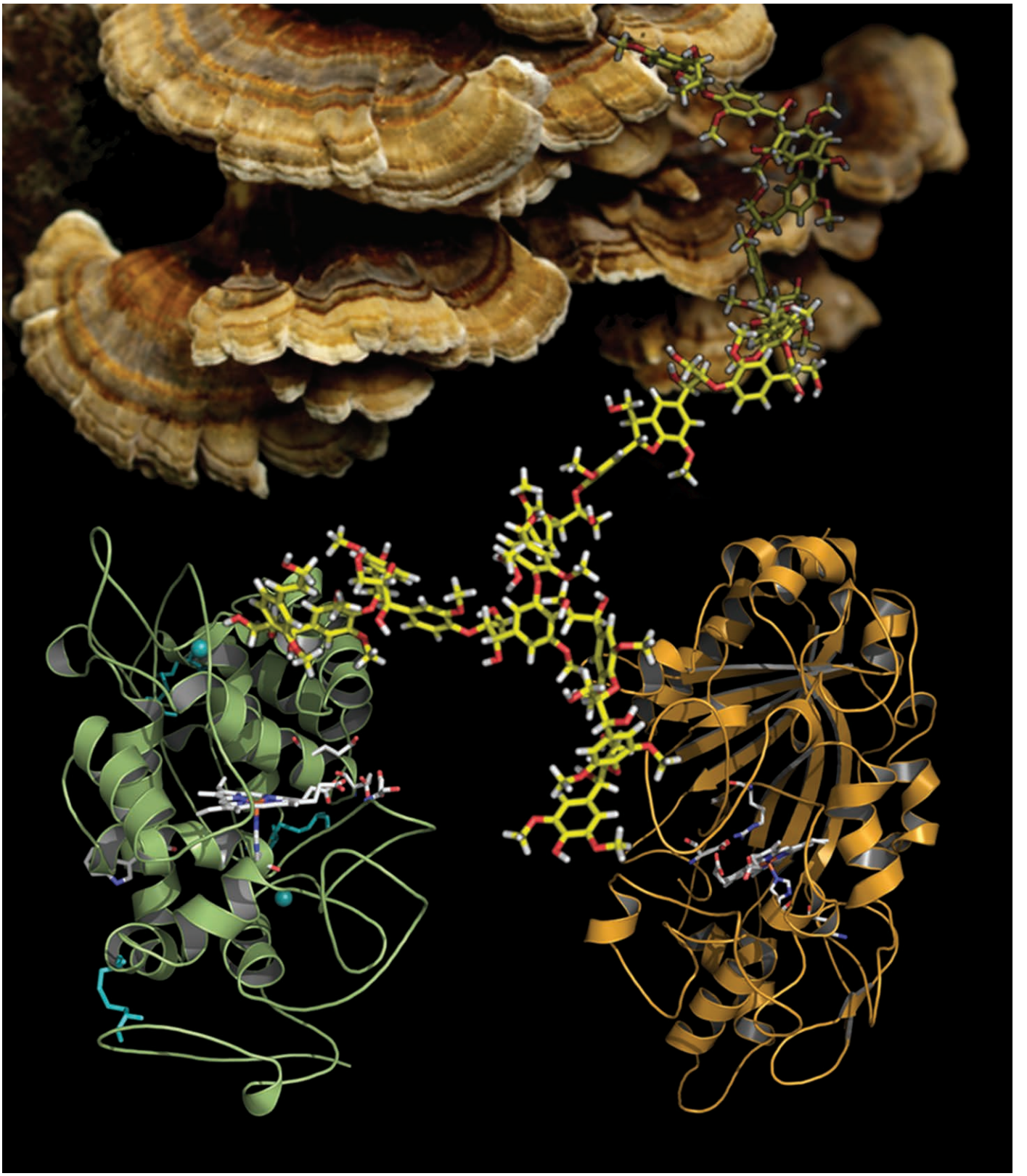

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Phylogenetic and phylogenomic overview of the Polyporales

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Abstract: We present a phylogenetic and phylogenomic overview of the Polyporales. The newly sequenced genomes of *Bjerkandera adusta*, *Ganoderma* sp., and *Phlebia brevispora* are introduced and an overview of 10 currently available Polyporales genomes is provided. The new genomes are 39 500 000–49 900 00 bp and encode for 12 910–16 170 genes. We searched available genomes for single-copy genes and performed phylogenetic informativeness analyses to evaluate their potential for phylogenetic systematics of the Polyporales. Phylogenomic datasets (25, 71, 356 genes) were assembled for the 10 Polyporales species with genome data and compared with the most comprehensive dataset of Polyporales to date (six-gene dataset for 373 taxa, including taxa with missing data). Maximum likelihood and Bayesian phylogenetic analyses of genomic datasets yielded identical topologies, and the corresponding clades also were recovered in the 373-taxa dataset although with different support values in some datasets. Three previously recognized lineages of Polyporales, antrodia, core polyporoid and phlebioid clades, are supported in most datasets, while the status of the residual polyporoid clade remains uncertain and certain taxa (e.g. *Gelatoporia*, *Grifola*, *Tyromyces*) apparently do not belong to any of the major lineages of Polyporales. The most promising candidate single-copy genes are presented, and nodes in the Polyporales phylogeny critical for the suprageneric taxonomy of the order are identified and discussed.

Key words: genomics, new molecular markers, Polyporales, taxonomy

Introduction

The Polyporales is a diverse group of Agaricomycetes including roughly 1800 described species (Kirk et al. 2008). They are key players in the carbon cycle, and the white-rot members of the order are among the most efficient lignin decayers in the biosphere (Floudas et al. 2012). Advances in our understanding of the biodiversity and ecology of this group can be achieved only if coupled with a comprehensive phylogeny for the Polyporales, which brings us back to a long-standing question in systematics: more genes or more taxa? Here we explore both solutions to the problem, which involves the use of whole genome sequence data in comparison to extensively sampled multigene datasets.

A wide variety of basidiocarp types and hymenophore configuration in the Polyporales include bracket-shaped (e.g. *Ganoderma, Trametes*), effused

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resupinate (*Wolfiporia*, *Phlebia*), stipitate with poroid (*Polyporus*) lamellate (*Lentinus*) or smooth (*Podoscypha*) hymenophores. A few species produce shelf-like or flabellate clusters of overlapping basidiocarps (*Laetiporus*, *Sparassis*). Variations of and transitions between basidiocarp types exist, and there is no morphological synapomorphy that unites the Polyporales (Binder et al. 2005). The most common ''polyporoid'' basidiocarps types just mentioned also have evolved convergently in at least 11 additional orders of Agaricomycetes (e.g. *Gloeophyllum* [Gloeophyllales], *Phellinus* [Hymenochaetales], *Heterobasidion* [Russulales] etc.) (Hibbett 2007).

The great majority of Polyporales are saprotrophic wood-decay fungi, while a few are plant pathogens causing timber damage (e.g. *Fomitopsis*, *Ganoderma*, *Phaeolus*). Wood-decayers in the Polyporales can be divided into two major groups: white-rot fungi that are able to decay both lignin and cellulosic compounds and brown-rot fungi that have taken a less costly approach to access cellulose and hemicellulose by either impairing the structural integrity of lignin using oxidative enzymes or secondary metabolites to produce hydrogen peroxide and free radicals (Eastwood et al. 2011). White-rot species allow the carbon in lignin to become readily available in the form of CO_2, which in turn feeds into biogeochemical reactions or functions as substrate for other organisms. Brown-rot species on the other hand leave behind residual lignin that is mostly inert to further microbial and fungal decay for extended periods and leads to carbon sequestration. The enzymatic capabilities of the Polyporales are not only important for the biogeochemical cycles but also make them prime candidates for industrial applications including biomass conversion, mycoremediation, paper pulping and the production of biofuels.

Resolving the taxonomic structure of Polyporales has proven difficult based on the nearly exclusive use of ribosomal DNA sequences (e.g. Binder et al. 2005). More recent studies (e.g. Justo and Hibbett 2011, Miettinen et al. 2011, Sjökvist et al. 2012) have used also protein-coding genes (*rpb1, rpb2, tef1*) for phylogenetic reconstruction showing that many genera and families belonging to the Polyporales are poly- and/or paraphyletic as currently defined. To achieve a taxonomic arrangement of the Polyporales that truly reflects the evolutionary history of the group a collective effort and coordination among researchers will be necessary, especially concerning two different aspects: (i) the building of a well resolved phylogeny ideally should be based on an identical set of genes for as many taxa as possible, with attention to nodes and clades that have been difficult to resolve in the past, and taxa of nomenclatural relevance (type genera and species); (ii) the ''translation'' of the phylogeny into a formal subordinal taxonomy also will require a general consensus about how broadly or narrowly families and genera should be defined. This effort should start with the reevaluation and possibly validation of clade names that have been introduced informally to facilitate communication, such as core polyporoid clade, antrodia clade, phlebioid clade, residual polyporoid clade and cinereomyces clade (Hibbett and Donoghue 1995, Larsson et al. 2004, Binder et al. 2005, Garcia-Sandoval et al. 2010, Miettinen et al. 2011). Justo and Hibbett (2011) further subdivided the core polyporoid clade into three well supported lineages (polyporus, trametoid, dentocorticium clades) without any judgment on the suprageneric taxonomy of this group. Studies using the combined 5.8S and ribosomal nuclear ribosomal LSU DNA (Larsson 2007) or multigene datasets (Miettinen et al. 2011) explored the application of already existing family names. There are 40 validly published and legitimate family names for taxa belonging (or putatively belonging) in the Polyporales (TABLE I). The oldest name is *Polyporaceae* (1839) and the most recent is *Phaeotrametaceae* (2005), but the great majority of family names, a total of 29, were published by Jülich (1981) although, with the exception of *Fomitopsidaceae* and *Phaneorochaetaceae*, they rarely have been used since their creation.

Under the auspices of the Joint Genome Institute (JGI) Fungal Genomics Program (Grigoriev et al. 2011), fungal phylogenomics has experienced rapid advances in recent years. The first sequenced Basidiomycota genome was a member of the Polyporales, the white-rot *Phanerochaete chrysosporium* (Martinez et al. 2004). The data from the genome of *P. chrysosporium* influenced the research of biologists, biochemists and computer scientists alike and allowed the first insight into the white-rot mechanism. This opened the door for comparative genomics with the non-ligninolytic, brown rot-producing *Rhodonia placenta* (= *Postia placenta*) (Martinez et al. 2009) and the white-rot *Gelatoporia subvermispora* (= *Ceriporiopsis subversmispora*) (Fernandez-Fueyo et al. 2012), which in contrast to *P. chrysosporium* delignifies wood selectively. Four other Polyporales genomes (*Dichomitus squalens*, *Fomitopsis pinicola*, *Trametes versicolor*, *Wolfiporia cocos*) were generated in the Saprotrophic Agaricomycotina Project (SAP), which focused on the evolution of the wood-decay apparatus (Floudas et al. 2012), and other independent projects also are contributing new genomes (e.g. Chen et al. 2012) with *Ganoderma lucidum*.

In the present paper we bring together phylogenomics and phylogenetics to provide an overview of

TABLE I. Legitimate family names for taxa belonging (or putatively belonging) in the Polyporales

Family	Type species of the type genus	Phylogenetic position
1. *Dacryobolaceae* Jülich 1981	*Dacryobolus sudans* (Alb. & Schwein.) Fr.	Antrodia clade
2. *Daedaleaceae* Jülich 1981	*Daedalea quercina* (L.) Pers.	Antrodia clade
3. *Fomitopsidaceae* Jülich 1981	*Fomitopsis pinicola* (Sw.) P. Karst.	Antrodia clade
4. *Laricifomitaceae* Jülich 1981	*Laricifomes officinalis* (Batsch) Kotl. & Pouzar	Antrodia clade
5. *Phaeolaceae* Jülich 1981	*Phaeolus schweinizii* (Fr.) Pat.	Antrodia clade
6. *Piptoporaceae* Jülich 1981	*Piptoporus betulinus* (Bull.) P. Karst.	Antrodia clade
7. *Sparassidaceae* Herter 1910	*Sparassis crispa* (Wulfen) Fr.	Antrodia clade
8. *Coriolaceae* Singer 1961	*Coriolus versicolor* (L.) Quél.	Core polyporoid clade
9. *Cryptoporaceae* Jülich 1981	*Cryptoporus volvatus* (Peck) Shear	Core polyporoid clade
10. *Echinochaetaceae* Jülich 1981	*Echinochaete brachypora* (Montagne) Ryvarden	Core polyporoid clade
11. *Fomitaceae* Jülich 1981	*Fomes fomentarius* (L.) Fr.	Core polyporoid clade
12. *Ganodermataceae* (Donk) Donk 1948	*Ganoderma lucidum* (Curtis) P. Karst.	Core polyporoid clade
13. *Grammotheleaceae* Jülich 1981	*Grammothele lineata* Berk. & M.A. Curtis	Core polyporoid clade
14. *Microporaceae* Jülich 1981	*Microporus xanthopus* (Fr.) Kuntze	Core polyporoid clade
15. *Pachykytosporaceae* Jülich 1981	*Pachykytospora tuberculosa* (Fr.) Kotl. & Pouzar	Core polyporoid clade
16. *Perenniporiaceae* Jülich 1981	*Perenniporia medulla-panis* (Jacq.) Donk	Core polyporoid clade
17. *Polyporaceae* Corda 1839	*Polyporus tuberaster* (Jacq. ex Pers.) Fr. [a]	Core polyporoid clade
18. *Sparsitubaceae* Jülich 1981	*Sparsitubus nelumbiformis* L.W. Hsu & J.D. Zhao	Core polyporoid clade
19. *Trametaceae* Boidin, Mugnier & Canales 1998	*Trametes suaveolens* (L.) Fr.	Core polyporoid clade
20. *Bjerkanderaceae* Jülich 1981	*Bjerkandera adusta* (Willd.) P. Karst.	Phlebioid clade
21. *Climacodontaceae* Jülich 1981	*Climacodon septentrionalis* (Fries) P. Karsten	Phlebioid clade
22. *Hapalopilaceae* Jülich 1981	*Hapalopilus rutilans* (Pers.) Murrill	Phlebioid clade
23. *Irpicaceae* Spirin & Zmitr. 2003	*Irpex lacteus* (Fr.) Fr.	Phlebioid clade
24. *Meruliaceae* Rea 1922	*Merulius tremellosus* Schrad	Phlebioid clade
25. *Phanerochaetaceae* Jülich 1981	*Phanerochaete velutina* (DC.) P. Karst.	Phlebioid clade
26. *Phlebiaceae* Jülich 1981	*Phlebia radiata* Fr.	Phlebioid clade
27. *Hyphodermataceae* Jülich 1981	*Hyphoderma setigerum* (Fr.) Donk	Residual polyporoid clade
28. *Meripilaceae* Jülich 1981	*Meripilus giganteus* (Pers.) P. Karst	Residual polyporoid clade
29. *Podoscyphaceae* D.A. Reid 1965	*Podoscypha nitidula* (Berk.) Pat.	Residual polyporoid clade
30. *Steccherinaceae* Parmasto 1968	*Steccherinum ochraceum* (Pers. ex J.F. Gmel.) Gray	Residual polyporoid clade
31. *Mycorrhaphiaceae* Jülich 1981	*Mycorrhaphium adustum* (Schwein.) Maas Geest.	Residual polyporoid clade (according to Miettinen et al. 2011)
32. *Grifolaceae* Jülich 1981	*Grifola frondosa* (Dicks.) Gray	Uncertain based on molecular data (probably in or close to the core polyporoid clade)
33. *Ischnodermataceae* Jülich 1981	*Ischnoderma resinosum* (Schrad.) P. Karst	Uncertain based on molecular data (probably in or close to the residual polyporoid clade)
34. *Rigidoporaceae* Jülich 1981	*Rigidoporus lineataus* (Pers.) Ryvarden	No data available for the type species (probably in or close to the residual polyporoid clade)
35. *Lophariaceae* Boidin, Mugnier & Canales 1998	*Lopharia mirabilis* (Berk. & Broome) Pat.	Uncertain
36. *Diachanthodaceae* Jülich 1981	*Diacanthodes novo-guineensis* (Hennings) O. Fidalgo	Unknown
37. *Incrustoporiaceae* Jülich 1981	*Incrustoporia stellae* (Pilát) Domanski	Unknown
38. *Nigrofomitaceae* Jülich 1981	*Nigrofomes melanoporus* (Mont.) Murrill	Unknown
39. *Phaeotrametaceae* Popoff ex Piatek 2005	*Phaeotrametes decipiens* (Berk.) J.E. Wright	Unknown
40. *Haddowiaceae* Jülich 1981	*Haddowia longipes* (Lév.) Steyaert	Unknown (probably = *Ganodermataceae*)

[a] *Polyporus tuberaster* is accepted here as the type species of *Polyporus*, but see also Krüger and Gargas (2004) and Sotome et al. (2008) for a detailed discussion on the problems surrounding the typification of *Polyporus*.

evolutionary relationships in the Polyporales that will serve as road map for future studies in both fields. Our particular foci are: (i) We introduce de novo sequenced genomes of the three white-rot Polyporales species, *Bjerkandera adusta*, *Ganoderma* sp. (*lucidum* complex) and *Phlebia brevispora*; (ii) we use the 10 Polyporales genomes currently available for identification and informativeness profiling of single-copy genes as candidates for future phylogenetic studies in the Polyporales; (iii) we assemble core (nearly complete) and extended (taxa introducing missing data) super matrices with the six AFTOL1 (http://aftol.org/) target regions (5.8S, nrLSU, nrSSU, *rpb1*, *rpb2*, *tef1*), combining published data and newly generated sequences to the largest dataset available of Polyporales to date. We also compare the results from these datasets with three different phylogenomic datasets of the Polyporales (25, 71, 356 genes respectively). Analyses of lignin-degrading peroxidases in Polyporales and other functional aspects are described in accompanying papers (e.g. Ruiz-Dueñas et al. this issue) as well as taxonomic studies on the antrodia clade (Ortiz-Santana et al. this issue).

MATERIALS AND METHODS

New genomes: sources of strains, culture conditions and extraction of nucleic acids.—Cultures of *Bjerkandera adusta* (strain HHB-12826-SP), *Ganoderma* sp. (strain 10597 SS1) and *Phlebia brevispora* (strain HHB-7030) were cultivated in the Hibbett laboratory at Clark University. All cultures are available from the Northern Research Station Laboratory (formerly Forest Products Laboratory, USDA Forest Service, Madison, Wisconsin). These cultures were grown routinely under ambient laboratory conditions at 23 C in daylight and simultaneously in an incubator (Precision, GCA, Thermo Scientific, Asheville, North Carolina) at 28 C in the dark. Liquid nutrient media were used to determine the optimal growth conditions for the fungal isolates, including modified vitamin (VIT) medium (1), potato-dextrose (PD) medium (24 g potato-dextrose [EMD]/L), malt extract (ME) medium (20 g malt extract, 0.5 g yeast extract/L), minimal (MM) medium (0.25 g ammonium tartrate, 0.5 g glucose, 0.5 g yeast extract/L) and Avicel medium (40 g Avicel PH-101 from Fluka analytical, 5 g ammonium tartrate,1 g KH_2PO_4, 0.5 g $MgSO_4$ [$\times$ $7H_2O$], 0.001 g $CaCl_2$ [$\times$ $2H_2O$], 0.1 g yeast extract, 0.88 mg $ZnSO_4$ [$\times$ $7H_2O$], 0.81 mg $MnSO_4$ [$\times$ $4H_2O$], 0.80 mg $FeCl_3$ [$\times$ $6H_2O$]/L). Mycelium used for DNA extractions was grown 1–2 wk and was harvested with a system including a Buchner funnel and Whatman No. 4 filter disks. Up to 10 g (wet) ground mycelium powder were loaded on QIAGEN (Valencia, California) Genomic 500/G tips and processed according to the lysis protocol for tissue in the QIAGEN Blood & Cell Culture DNA Kit, including the RNase and Proteinase K steps. Materials for RNA extraction were filtered after 3–5 d growth. The QIAGEN RNeasy Midi Kit was used to process up to 1 g (wet) of ground mycelium at a time. The extraction followed the protocol for isolation of total RNA from animal tissues (QIAGEN) including on-column DNase digestion and final standard LiCl purification.

Genome sequencing, assembly and automated annotation.—Genomes were sequenced with a hybrid whole-genome shotgun approach using a combination of ABI3730 (fosmids) (Applied Biosystems, Foster City, California), 454-Titanium (454 Life Sciences, Branford, Connecticut) and Illumina GAII (Illumina Inc., San Diego, California) sequencing platforms. Roche 454 sequence was from one or more standard libraries and typically one 4 kb and one 8 kb paired-end library. Illumina reads were collected from nominal 300 bp fragment libraries, sequenced to 76 bp and assembled with Velvet (0.7.55 - REF). The resulting Velvet contigs were shredded into overlapping 800 bp chunks with a 200 bp overlap and used by Newbler (2.5-internal-10Apr08-1) together with 454 standard and paired-end reads and Sanger-sequenced fosmids ends. Gaps were closed in silico with gap resolution. cDNA libraries were constructed with the methods outlined in the Roche cDNA Rapid Library Preparation Method Manual (Roche). The 454 libraries were sequenced with the genome sequencer FLX Instrument (Roche). Ribosomal RNA, low quality and low complexity reads were filtered out, then the remaining reads were assembled with either a JGI specific assembly process or Newbler (2.3-PreRelease-6/30/2009, Roche) with default parameters. Each of the JGI-sequenced genomes introduced here were annotated with the JGI annotation pipeline, which takes multiple inputs (scaffolds, ESTs, known genes) and runs several analytical tools for gene prediction and annotation, and deposits the results in Mycocosm (http://jgi.doe.gov/fungi) for further analysis and manual curation. All genome assemblies and annotations can be accessed interactively through the JGI fungal genome portal Mycocosm (Grigoriev et al. 2012) at http://jgi.doe.gov/fungi. The three new Polyporales genomes discussed here also are deposited to DDBJ/EMBL/GenBank under these accessions numbers: *Phlebia brevispora* HHB-7030 SS6: ANLB00000000, *Ganoderma* sp. 10597 SS1: ANLC00000000, and *Bjerkandera adusta* HHB-12826-SP SB-22: ANLD00000000.

Identification of single-copy genes.—A cluster was assembled in the JGI Mycocosm for the 10 Polyporales genomes available as of Jun 2012 (No. 1262): *Bjerkandera adusta*, *Dichomitus squalens*, *Fomitopsis pinicola*, *Ganoderma* sp. (*G. lucidum* complex), *Gelatoporia subvermispora* (= *Ceriporiopsis subvermispora*), *Phanerochaete chrysosporium*, *Phlebia brevispora*, *Rhodonia placenta* (= *Postia placenta*), *Trametes versicolor* and *Wolfiporia cocos*. BLAST queries in InParanoid (http://inparanoid.sbc.su.se/cgi-bin/index.cgi) and the *Saccharomyces* Genome Database (http://www.yeastgenome.org/) were used to identify the putative orthologs in yeast. KEGG (http://www.genome.jp/kegg/) was used to estimate the higher level functions of the single-copy genes. An ad hoc cluster (No. 1263) was assembled to check for the presence of the identified single-copy genes in the selected outgroups for the phylogenomic analyses: *Fomitiporia*

mediterranea (Hymenochaetales), *Gloeophyllum trabeum* (Gloeophyllales), *Heterobasidion annosum* (Russulales), *Punctularia strigosozonata* (Corticiales), *Stereum hirsutum* (Russulales).

Phylogenomic analyses.—Two datasets were assembled: (i) A 356-gene dataset that combines single-copy genes present in the 10 Polyporales + 5 outgroup dataset obtained from genome data; (ii) Combined 71-gene based on the AFTOL2 genes dataset (Floudas et al. 2012). In both cases amino acid sequences were aligned with MAFFT 6 (Katoh et al. 2005) under the L-INS-I setting and curated with Gblocks under stringent settings (Castresana 2000, Talavera and Castresana 2007). The 356-gene dataset was analyzed under the maximum likelihood criterion (ML) with RAxML 7.2.6 (Stamatakis 2006) and the 71-gene dataset with both ML and Bayesian (BY) PhyloBayes 3 (Lartillot et al. 2009) analyses. We also constructed a third dataset with the 25 genes that performed best in the informativeness profiling analysis (see below), which was analyzed with both ML and Bayesian methods. Searches conducted with RAxML involved 100 rapid bootstrap replicates and ML optimization using the PROTGAMMAWAGF model. This model specifies the WAG amino acid matrix with the Γ model of rate heterogeneity using four discrete rate categories, while RAxML estimates all free model parameters. PhyloBayes was run with the CAT infinite mixture model accounting for site-specific amino acid preferences, employing six MCMC chains sampling data every 1000th cycle. The Bayesian analyses were set to stop after the maximum differences in split frequency between runs reached zero and consensus trees were built with the READPB command.

Informativeness profiling.—To evaluate the potential use of the identified single-copy genes for phylogenetic reconstruction we profiled their phylogenetic informativeness. To compute the rates of evolution of amino acid and nucleotide sites, we specified an ultrametric evolutionary tree. The concatenated amino acid sequences (257 297 aa) were used to estimate the phylogeny with the parallel version of PhyML 20110919 (Guindon and Bascon 2003, Guindon 2010). The LG model was selected assuming an estimated proportion of invariant sites and four gamma-distributed rate categories to account for rate heterogeneity across sites. Equilibrium amino acid frequencies were estimated from the model. We let PhyML optimize substitution model parameters. We used a time-calibrated phylogeny (ultrametric tree). While absolute dates of internal nodes were not relevant to any inferences herein, their relative depths were aligned with the ultrametric profiles for predictive purposes. We obtained the chronogram by passing the phylogenetic tree to r8s software 1.71 (Sanderson 2003). We pruned *Fomitiporia mediterranea* (further root), and the place where this outgroup attached to the rest of the tree became the root node for r8s. We fixed the age of the root to 1. Node heights were estimated by nonparametric rate smoothing (NPRS) with a truncated Powell algorithm in r8s.

Using the alignment data and the ultrametric tree, molecular evolutionary rates were estimated for each gene at each alignment position independently. We used Rate4site (Mayrose et al. 2005) and HyPhy (Pond et al. 2005) to obtain the substitution rates at amino acid and nucleotide sites respectively. In the Rate4site program rates were inferred by ML assuming a JTT model for the topology and branch lengths of the input phylogenetic tree without any optimization. In the HyPhy analysis we assumed a Kimura-2-parameter (K2P) model with transitions twice as likely as transversions.

For each gene, the phylogenetic informativeness profile ρ as a function of time, T, was calculated, substituting the estimated rates λi of evolution of each site (Townsend 2007). This formula provides a metric of the probability that character i would provide an unambiguous synapomorphy lying within an asymptotically short internode between two pairs of sister taxa whose common ancestor is at time T. To convey the informativeness of a particular dataset, the equation was plotted at a continuum of depths, from time 0 to the root, of the phylogenetic trees. The differential phylogenetic informativeness (DPI) of each gene was evaluated quantitatively by integrating on the phylogenetic informativeness profile from the origin (h1) to the terminus (h) of the epochs of interest. Three epochs of interest were used: 0.17–0.49 (from the appearance of the antrodia clade to the most recent split on the tree between *Ganoderma* sp. and *Dichomitus squalens*), 0.49–0.77 (from the origin of the Polyporales to the origin of the antrodia clade) and 0.77–1 (previous to the appearance of the Polyporales). Using DPI, we ranked the genes for each one of the epochs. Both the calculations of the molecular evolutionary rate and the phylogenetic informativeness profiles were performed with the PhyDesign web application (López-Giráldez and Townsend 2011).

To quantify phylogenetic noise as well as signal and calculate a probability of resolution for each gene for the indicated node (t = 0.06, T = 0.68), we applied the analytical solution for probability of resolution from Townsend et al. (2012). Once probabilities of resolution were calculated for the three nodes indicated above we calculated: (i) best overall gene: as the most likely to provide accurate resolution over all three epochs (product of the probabilities); (ii) worst overall gene: as the one most likely to get all three epochs incorrect. (product of [1-the probabilities]); (iii) best recent yet worst ancient was calculated as the ratio of rankings in ancient Pr (probability of resolution) over recent *Pr*; (iv) best ancient yet worst recent was calculated as the ratio of rankings in recent *Pr* over ancient *Pr*.

Rates of DNA and amino acid site evolution were estimated as above. For nucleotide characters, we calculated the results using their nominal state space (four states). For amino acid characters, we used an empirical estimate of their state space (five states; Simmons et al. 2004).

Multigene phylogenetic overview of the Polyporales.—Two major datasets, an extended supermatrix and a core supermatrix, were analyzed. The extended supermatrix combines almost all nrLSU, 5.8S, nrSSU, *rpb1*, *rpb2* and *tef1* data for the Polyporales publicly available in GenBank by the end of Dec 2011 (excluding different copies of the same locus for different isolates of the same species). Only

TABLE II. Assembly statistics for the three new Polyporales genomes

	Bjerkandera adusta	*Ganoderma* sp.	*Phlebia brevispora*
Genome assembly length, Mbp	42.73	39.52	49.96
Number of scaffolds	508	156	1645
Scaffold N50/L50(Mbp)	13/1.03	6/2.73	329
Total contig length, Mbp (percent gap)	40.23(5.8%)	38.53(2.5%)	46.43(7%)
Number of contigs	1263	503	3178
Contig N50/L50(Kbp)	88/124.7	27/375.6	104/66.2
Percent repeats	1.34%	2.53%	4.53%

one set of sequences for any given taxon was included (SUPPLEMENTARY TABLE I). Whenever possible the loci were selected from the same isolate; if not possible the conspecificity of different isolates was assessed indirectly by comparison of loci in common for both isolates and/or the results of BLAST queries. Additional newly generated nrLSU, *rpb2* and *tef1* for selected taxa of Polyporales also were included (SUPPLEMENTARY TABLE II). These sequences were generated with standard DNA extraction, PCR and sequencing methods (e.g. Sjökvist et al. 2012). For the 10 Polyporales and two outgroup species with genomes available the complete sequences for *rpb1, rpb2* and *tef1* extracted from the genome data were used. The supermatrix consists of 373 Polyporales taxa, which are represented by at least nrLSU data, except seven species with *rpb2* but no nrLSU data. Two hundred sixty 5.8S, 91 nrSSU, 44 *rpb1*, 130 *rpb2* and 86 *tef1* were concatenated to the nrLSU data. *Stereum hirsutum* and *Heterobasidion annosum* (Russulales) were used as outgroup taxa. To test for possible conflicts ML analyses were performed in a dataset with ribosomal genes only (nrLSU, 5.8S, nrSSU) and compared to a separate datasets for each protein-coding gene (both nucleotide and amino acid datasets).

The core supermatrix excludes from the extended supermatrix all taxa that lack data for both *rpb1* and *rpb2*. It has 126 Polyporales taxa. For both datasets, maximum likelihood and Bayesian analyses were run. Maximum likelihood analyses (ML) were run in the RAxML servers, 7.2.8 (Stamatakis et al. 2008), under a GTR model with 100 rapid bootstrap replicates. Bayesian analyses (BY) were run with MrBayes 3.2 (Ronquist et al. 2012) for 10 000 000 generations, with four chains, and trees sampled every 100 generations. The initial burn-in was set to 2 500 000 generations, and after examining the graphic representation of the likelihood scores of the sampled trees that was confirmed to be an adequate value for both datasets. A 50% majority rule consensus tree was computed with the remaining trees.

RESULTS

Polyporales genomes.—A general characterization of the genomes of *Bjerkandera adusta*, *Ganoderma* sp. and *Phlebia brevispora* is provided (TABLES II, assembly statistics; III, gene model statistics; IV, EST and protein similarity support; V, KOG and KEGG characterization).

Genome sizes and gene counts of sequenced Polyporales are 35 000 000–50 000 000 bp and 10 000–16 000 genes respectively. The first species of Polyporales to have its genome sequenced, *Phanerochaete chrysosporium* (Martinez et al. 2004), is also the smallest with 35 100 000 bp and 10 048 genes. These numbers correlate in general (SUPPLEMENTARY FIG. 1), but repeats, primarily transposable elements, can introduce significant variation into the genome size. The 50 500 000-bp genome of *Wolfiporia cocos* is the largest among the Polyporales and contains more than 10% repetitive elements and only 12 746 genes. In contrast, the next largest genome of *Phlebia brevispora* (49 960 000 bp) encodes for the largest number of genes (16 170) among the Polyporales.

The 10 sequenced Polyporales offer a range of evolutionary distances reflected in gene content and similarities between pairwise orthologs. The closest pair of Polyporales, *Ganoderma* sp. and *Dichomitus squalens*, share 8137 orthologs with average amino

TABLE III. Predicted gene model statistics for the three new Polyporales

	Bjerkandera adusta	*Ganoderma* sp.	*Phlebia brevispora*
Gene length (median), bp	1424	1541	1347
Transcript length (median), bp	1168	1182	1058
Protein length (median), aa	334	355	329
Exon length (median), bp	153	148	140
Intron length (median)	55	62	57
Exons per gene (median)	4	5	4

TABLE IV. EST and protein similarity support for the predicted genes in the three new Polyporales

	Bjerkandera adusta		*Ganoderma* sp.		*Phlebia brevispora*	
	Number	Percent	Number	Percent	Number	Percent
Total number of genes supported by:	15 473	100.00%	12 910	100.00%	16 170	100.00%
Homologs from Swissprot	8252	53.33%	7857	60.86%	9066	56.07%
Homologs from NCBI NR	11 166	72.16%	10 411	80.64%	12 604	77.95%
Predicted Pfam domain	6788	43.87%	6508	50.41%	7283	45.04%
EST alignment (>75%)	11 978	77.41%	10 553	81.74%	10 128	62.63%

acid identity of 76%, 7289 of them in syntenic regions (SUPPLEMENTARY TABLES III–V). Average identity between orthologs is 60–76% (SUPPLEMENTARY TABLE IV). The number of orthologs within Polyporales pairs can be as low as 5387 (*G. subvermispora-P. chrysosporium*; SUPPLEMENTARY TABLE III), which is comparable to the most distant pairs between Polyporales and Hymenochaetales (SUPPLEMENTARY TABLE V; 5382 in *F. mediterranea-G. subvermispora*, 5167 in *F. mediterranea-P. chrysosporium*). However, the number of syntenic orthologs is twice as high even in the most distant pair of Polyporales (3229 in *G. subvermispora-P. chrysosporium*) than between the groups (1764 in *F. mediterranea-P. chrysosporium*). The latter however depends on quality of assembly and may be lower for highly fragmented assemblies like *F. mediterranea*.

There is also synteny between multiple genomes. The largest group of 65 orthologous families (i.e. 15-member gene family, all of which have best hit of other) reside on the same scaffolds in 15 genomes, encompassing in average 1.2 Mb (max 2.5 Mb, min 0.55 Mb). In addition, 81 pairs of adjacent genes conserved in all 15 genomes.

While the details of the functional gene content were described in depth in companion papers (Ruiz-Dueñas et al. this issue), PFAM domain composition is comparable among all 10 Polyporales (SUPPLEMENTARY TABLES VI, VII). The top PFAM domains include p450, WD40 putatively involved protein-protein interaction, protein kinases involved in signaling, followed by transporters and other functions (SUPPLEMENTARY TABLE VI). HET domain has significant variation in Basidiomycota, often absent but significantly expanded in Polyporales *D. squalens* (112), *Ganoderma* sp. (90) and *Trametes versicolor* (159). PFAM domains, where at least one largest count is from Polyporales (SUPPLEMENTARY TABLE VII), include HET, hydrolases, Fe2 oxidases, iron reductases and others.

To go beyond functionally characterized protein domains, using MCL we clustered 129 895 proteins

TABLE V. Characterization of the new genomes according to the EuKaryotic orthologous groups (KOG) and the Kyoto Encyclopedia of Genes and Genomes (KEGG)

	B. adusta	*Ganoderma* sp.	*P. brevispora*
Total gene models	15 473	12 910	16 170
KOG: cellular processes and signaling	2731	2445	2855
KOG: information storage and processing	1790	1623	1871
KOG: metabolism	2322	2323	2765
KOG: poorly characterized	1676	1559	1792
KEGG: amino acid metabolism	588	581	615
KEGG: biosynthesis of polyketides and nonribosomal peptides	153	146	142
KEGG: biosynthesis of secondary metabolites	327	348	399
KEGG: carbohydrate metabolism	598	643	640
KEGG: energy metabolism	142	133	146
KEGG: glycan biosynthesis and metabolism	245	245	268
KEGG: lipid metabolism	534	546	585
KEGG: metabolism of cofactors and vitamins	465	457	519
KEGG: metabolism of other amino acids	131	127	132
KEGG: nucleotide metabolism	302	291	390
KEGG: overview	372	384	418
KEGG: xenobiotics biodegradation and metabolism	399	425	448

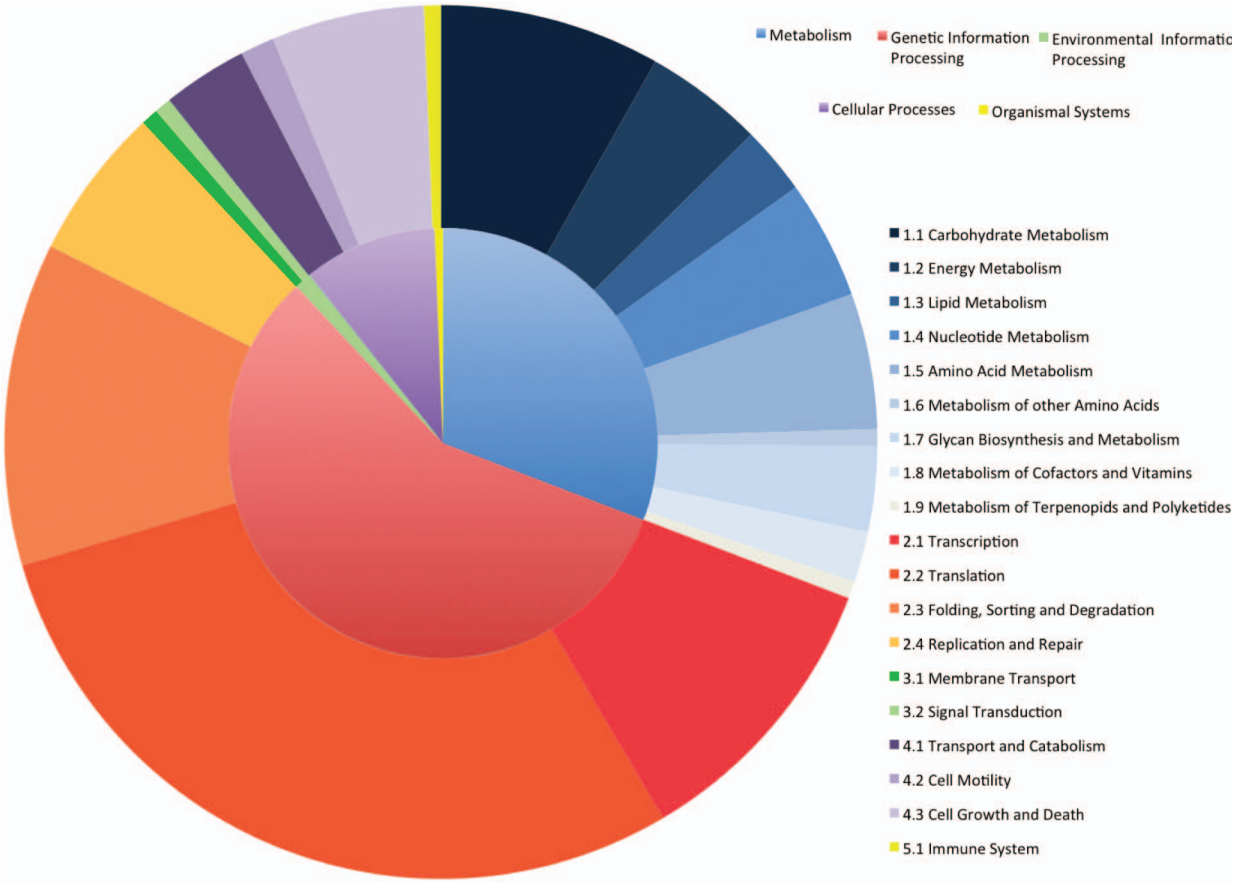

FIG. 1. Graphical overview of functions of single-copy present in the 10 Polyporales genomes according to the KOG classification.

from 10 Polyporales into 11 499 multigene groups and 9424 singletons. The core Polyporales set included 1776 clusters composed of 44 010 proteins from all 10 genomes, 534 of which contain exactly one member from each cluster.

Single-copy genes in Polyporales.—We identified 534 single-copy genes in the cluster including the 10 Polyporales genomes. The putative orthologs in yeasts and the higher functions of the genes are summarized (SUPPLEMENTARY FILE 1, FIG. 1). Higher-level functions could be predicted for only 159 out the 534 genes. For the phylogenomic and informativeness profiling analyses we selected a subset of 356 single-copy genes that were present in Polyporales and the outgroup taxa.

Phylogenomic analyses.—The 356-gene and 71-gene datasets have 145 050 and 44 385 amino acid positions respectively (gaps included). The resulting trees from the ML and BY analyses of both datasets have identical topologies (FIG. 2). In the 356-gene trees all internal nodes of the Polyporales receive 100% RaxML bootstrap support. In the 71-gene trees all nodes receive full support (100% RaxML bootstrap and a PhyloBayes posterior probability of 1.0 except the sister taxa relationship between *Gelatoporia subvermispora* and the antrodia clade (89% BS, 1.0 PP) and the sister taxa relationship between *Rhodonia placenta* and *Wolfiporia cocos* (92% BS and 1.0 PP). Support values for all nodes are provided (FIG. 2).

Informativeness profiling.—The 25 best performing genes in the phylogenetic informativeness profiling are provided (FIG. 3), together with the three AFTOL1 protein-coding genes (*rpb1*, *rpb2*, *tef1*). Information about these 25 genes is summarized (TABLE VI). Note that gene IDs refer to the Mycocosm cluster 1263 (Polyporales + outgroups) that unfortunately is no longer available. The nucleotide and protein sequences of all 356 genes profiled can be retrieved at wordpress.clarku.edu/polypeet/datasets/.

The resulting trees from the phylogenetic analyses of the 25-gene dataset (not shown) have an identical topology to the 356-gene and the 71-gene analyses (FIG. 2). All internal nodes of the Polyporales received full support (100% BS, 1 PP).

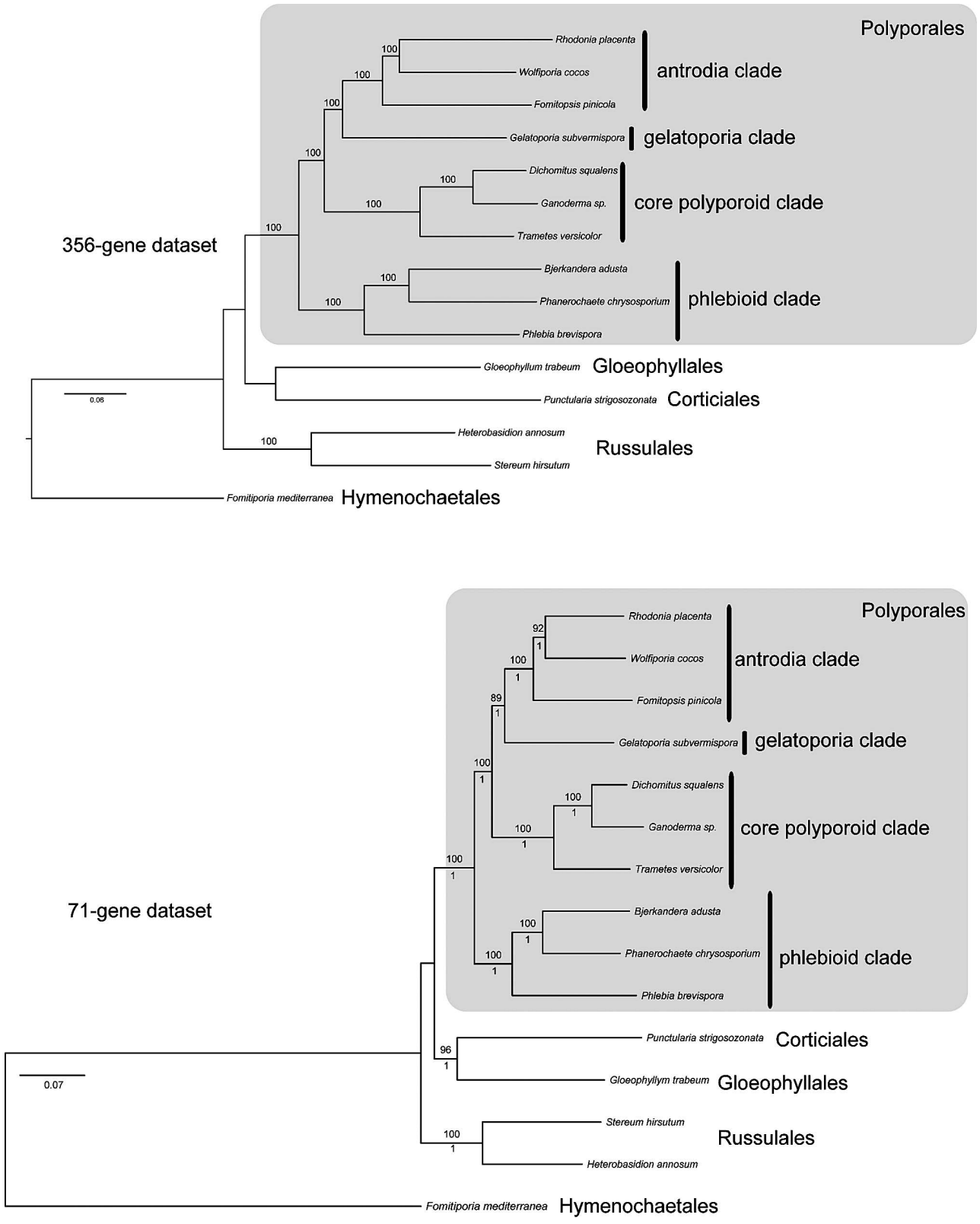

FIG. 2. Best trees from the ML analyses of the 356- and 71-gene datasets.

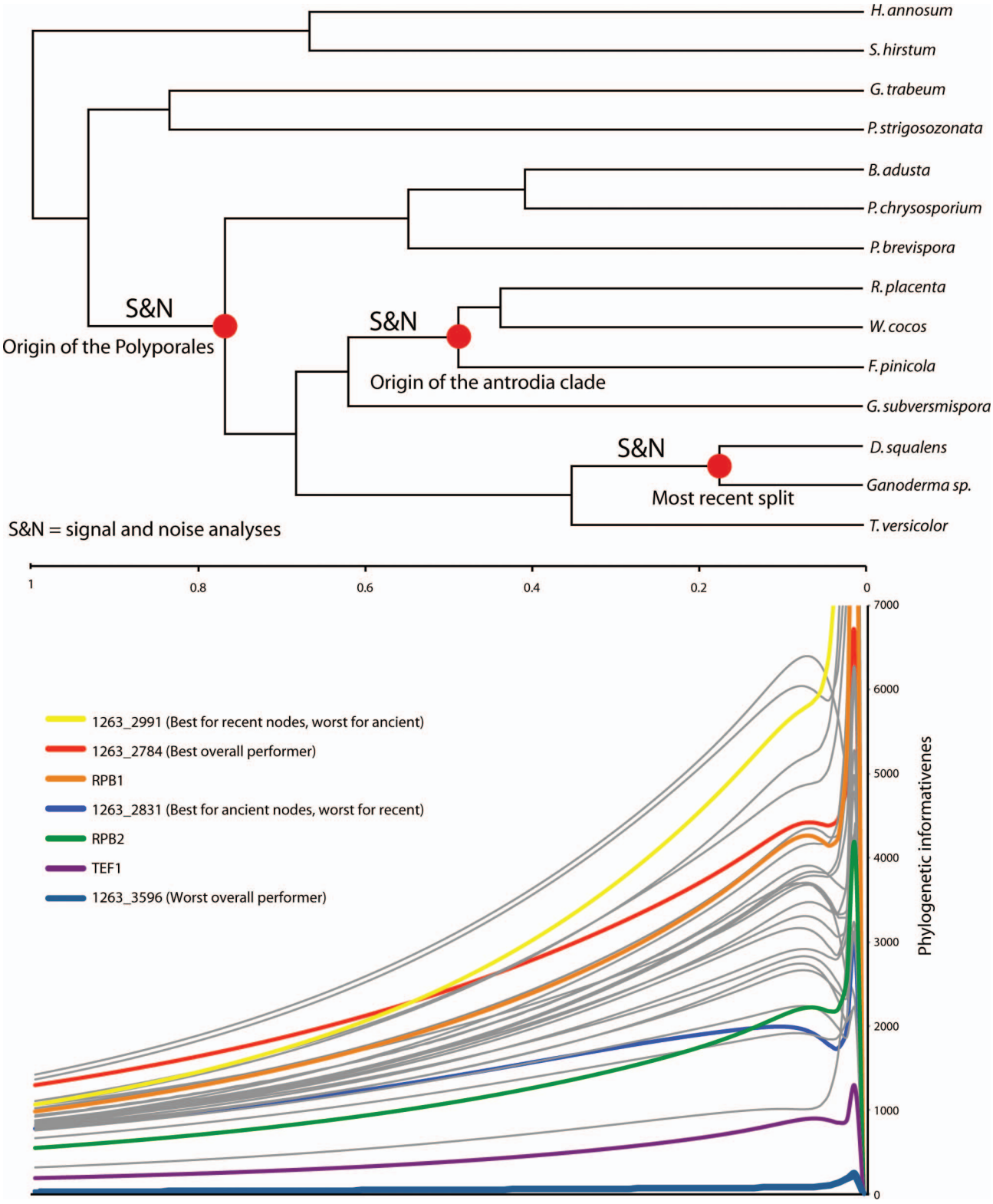

FIG. 3. Graphical overview of the phylogenetic informativeness of the 25 top candidate genes.

TABLE VI. Top 25 genes from the phylogenetic informativeness profiling analysis

Cluster 1263 ID	Nucleotide alignment	Putative yeast ortholog	Description
1263_2751	6946	SEN1	Presumed helicase required for RNA polymerase II transcription termination and processing of RNAs; homolog of Senataxin which causes Ataxia-Oculomotor Apraxia 2 and a dominant form of amyotrophic lateral sclerosis
1263_2765	7073	SWR1	Swi2/Snf2-related ATPase that is the structural component of the SWR1 complex, which exchanges histone variant H2AZ (Htz1p) for chromatin-bound histone
1263_2784	7400	unknown	Signaling protein DOCK180
1263_2789	4720	RRP5	RNA binding protein with preference for single stranded tracts of U's involved in synthesis of both 18S and 5.8S rRNAs; component of both the ribosomal small subunit (SSU) processosome and the 90S preribosome
1263_2790	5057	unknown6	Kinesin-like protein
1263_2794	5690	unknown5 (UBR1 like)	UBR1, related to ubiquitin-protein ligase e3 component
1263_2798	5586	unknown8 (VPS15 like)	VPS15-like, Serine/threonine protein kinase containing WD40 repeats
1263_2799	6110	MON2	Peripheral membrane protein with a role in endocytosis and vacuole integrity, interacts with Arl1p and localizes to the endosome; member of the Sec7p family of proteins
1263_2800	5374	POL1	Catalytic subunit of the DNA polymerase I alpha-primase complex, required for the initiation of DNA replication during mitotic DNA synthesis and premeiotic DNA synthesis
1263_2801	4752	YOR296W	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; expressed during copper starvation
1263_2802	4392	NCR1	Vacuolar membrane protein that transits through the biosynthetic vacuolar protein sorting pathway, involved in sphingolipid metabolism; glycoprotein and functional orthologue of human Niemann Pick C1 (NPC1) protein
1263_2806	6198	DOP1	Golgi-localized, leucine-zipper domain containing protein; involved in endosome to Golgi transport, organization of the ER, establishing cell polarity and morphogenesis; detected in highly purified mitochondria in high-throughput studies
1263_2810	3686	AMS1	Vacuolar alpha mannosidase, involved in free oligosaccharide (fOS) degradation; delivered to the vacuole in a novel pathway separate from the secretory pathway
1263_2817	7230	unknown9	Hypothetical protein
1263_2822	4917	RAV1	Subunit of the RAVE complex (Rav1p, Rav2p, Skp1p), which promotes assembly of the V-ATPase holoenzyme; required for transport between the early and late endosome/PVC and for localization of TGN membrane proteins; potential Cdc28p substrate
1263_2831	4140	SCP160	Essential RNA-binding G protein effector of mating response pathway, mainly associated with nuclear envelope and ER, interacts in mRNA-dependent manner with translating ribosomes via multiple KH domains, similar to vertebrate vigilins
1263_2840	5586	TRS120	One of 10 subunits of the transport protein particle (TRAPP) complex of the cis-Golgi which mediates vesicle docking and fusion; involved in endoplasmic reticulum (ER) to Golgi membrane traffic
1263_2843	4207	SEC8	Essential 121kDa subunit of the exocyst complex (Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, and Exo84p), which has the essential function of mediating polarized targeting of secretory vesicles to active sites of exocytosis

TABLE VI. Continued

Cluster 1263 ID	Nucleotide alignment	Putative yeast ortholog	Description
1263_2856	4722	SKI3	Ski complex component and TPR protein, mediates 3′–5′ RNA degradation by the cytoplasmic exosome; null mutants have superkiller phenotype of increased viral dsRNAs and are synthetic lethal with mutations in 5′–3′ mRNA decay
1263_2902	4404	PDS5	Protein required for establishment and maintenance of sister chromatid condensation and cohesion, co-localizes with cohesin on chromosomes, may function as a protein-protein interaction scaffold; also required during meiosis
1263_2991	7954	SSM4	Ubiquitin-protein ligase involved in ER-associated protein degradation; located in the ER/nuclear envelope; ssm4 mutation suppresses mRNA instability caused by an rna14 mutation
1263_2996	4453	SRO77	Protein with roles in exocytosis and cation homeostasis; functions in docking and fusion of post-Golgi vesicles with plasma membrane; homolog of Sro7p and Drosophila lethal giant larvae tumor suppressor; interacts with SNARE protein Sec9p
1263_3045	6185	unknown51	Hypothetical protein
1263_3046	2822	unknown157	Hypothetical protein
1263_3047	4004	unknown19	Hypothetical protein

Multigene phylogenetic overview of the Polyporales.—The resulting trees from the maximum likelihood analyses, extended supermatrix (FIG. 4) and core supermatrix (FIG. 5), present essentially the same topology. The four major lineages of Polyporales recognized by Binder et al. (2005) are present in our analyses but only the core polyporoid and phlebioid clades appear well supported in both datasets. The composition and support of the antrodia and residual polyporoid clades and the status of some of the smaller lineages (tyromyces clade, gelatoporia clade, *Grifola*) remains unresolved (see DISCUSSION). The nodes that define the antrodia, core polyporoid, gelatoporia and phlebioid clade in the phylogenomic analyses (FIG. 2) are represented by equivalent nodes in the extended and core supermatrix analyses although with varying support. The tyromyces clade (not represented by genomic data) appears nested within the antrodia clade in the multigene analyses (see DISCUSSION). Except for the internal relations between the members of the antrodia clade, the individual taxa relationships obtained in the phylogenomic datasets (FIG. 2) also are reflected in the multigene datasets (FIGS. 4, 5). Throughout the paper clade names are written with no caps and no italics (e.g. antrodia clade) to avoid confusion with formal taxonomic names. Alignments haven been deposited in TreeBase under study number 13783. Supplementary analyses of ribosomal genes vs. protein-coding genes did not reveal any supported conflict between genes. These supplementary analyses and trees also can be retrieved from TreeBase. Phylogenetic and taxonomic implications of the multigene analyses are discussed below.

DISCUSSION

More genes or more taxa?—The increased availability of genome-scale data for Fungi has transformed mycology by opening a new universe of potential target genes to address ecological and systematical questions. Resolving the evolution of genes or gene families of enzymes involved in wood decay is of particular interest and will lead to a better characterization of the major wood-decay modes (Floudas et al. 2012). The number of published Polyporales genomes is limited currently to three brown-rot producing and seven white-rot producing species, and still the amount of data permits comparative work between and within ecological traits. Our previous view of functional boundaries within brown- and white-rot ecology clearly has been affected by the morphology of wood decay. However recent studies (e.g. Fernandez-Fueyo et al. 2012) suggest that upon genome analysis white-rot wood decay in the Polyporales generally may represent ecological variations of adaptations to substrates and colonization succession series, which would make the enzymes involved scalable for industrial applications.

We have identified 534 putative single-copy orthologs in 10 genomes of Polyporales (SUPPLEMENTARY FILE 1) and a subset of 356 genes also is shared by Corticiales, Gloeophyllales, Russulales and Hymenochaetales (FIG. 2). Many of these genes are poorly

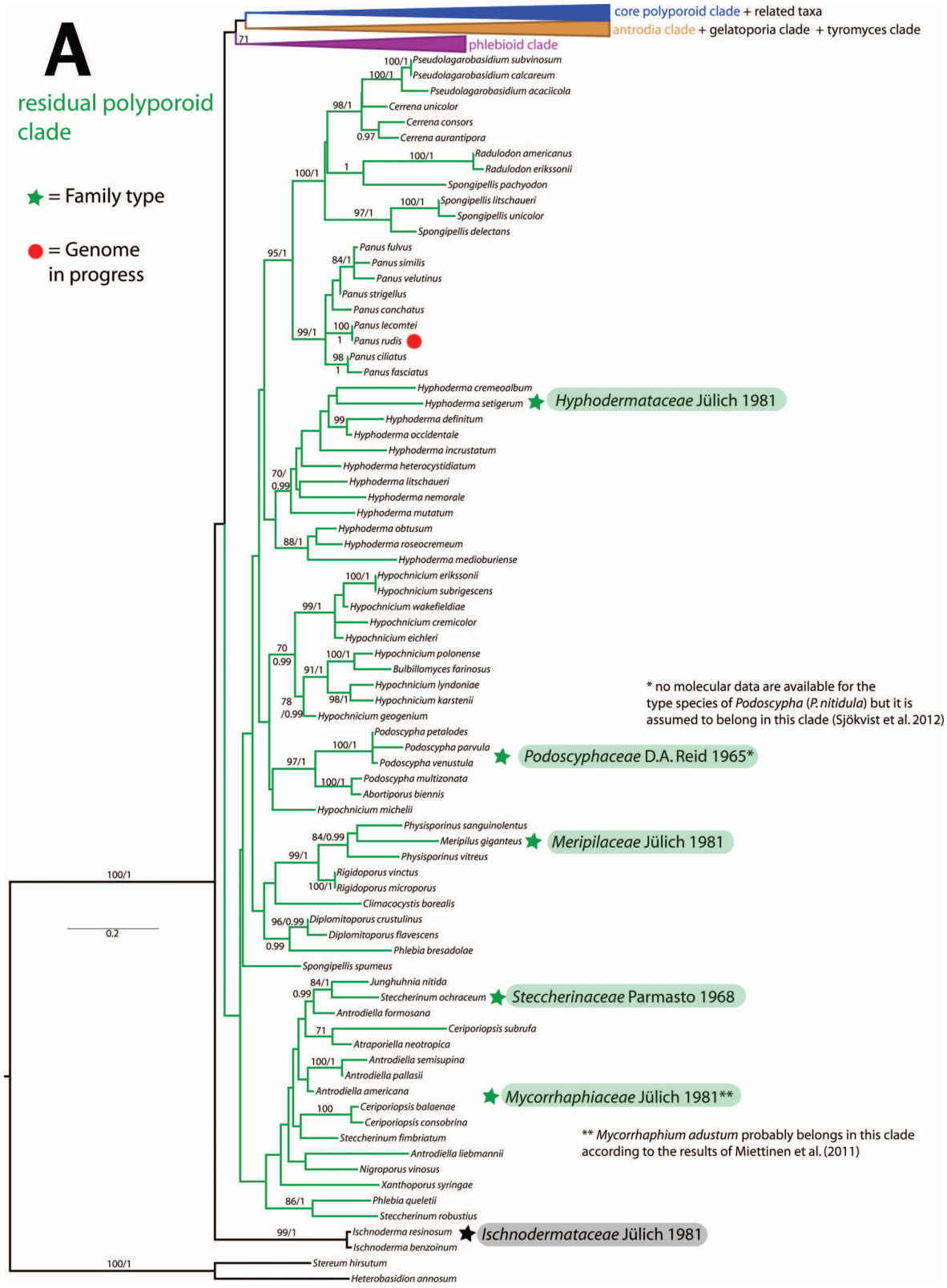

FIG. 4. A–D. Best tree from the ML analyses of the extended supermatrix dataset. Support values are on or below the branches. A. Residual polyporoid clade. B. Phlebioid clade. C. Core polyporoid clade. D. Antrodia clade.

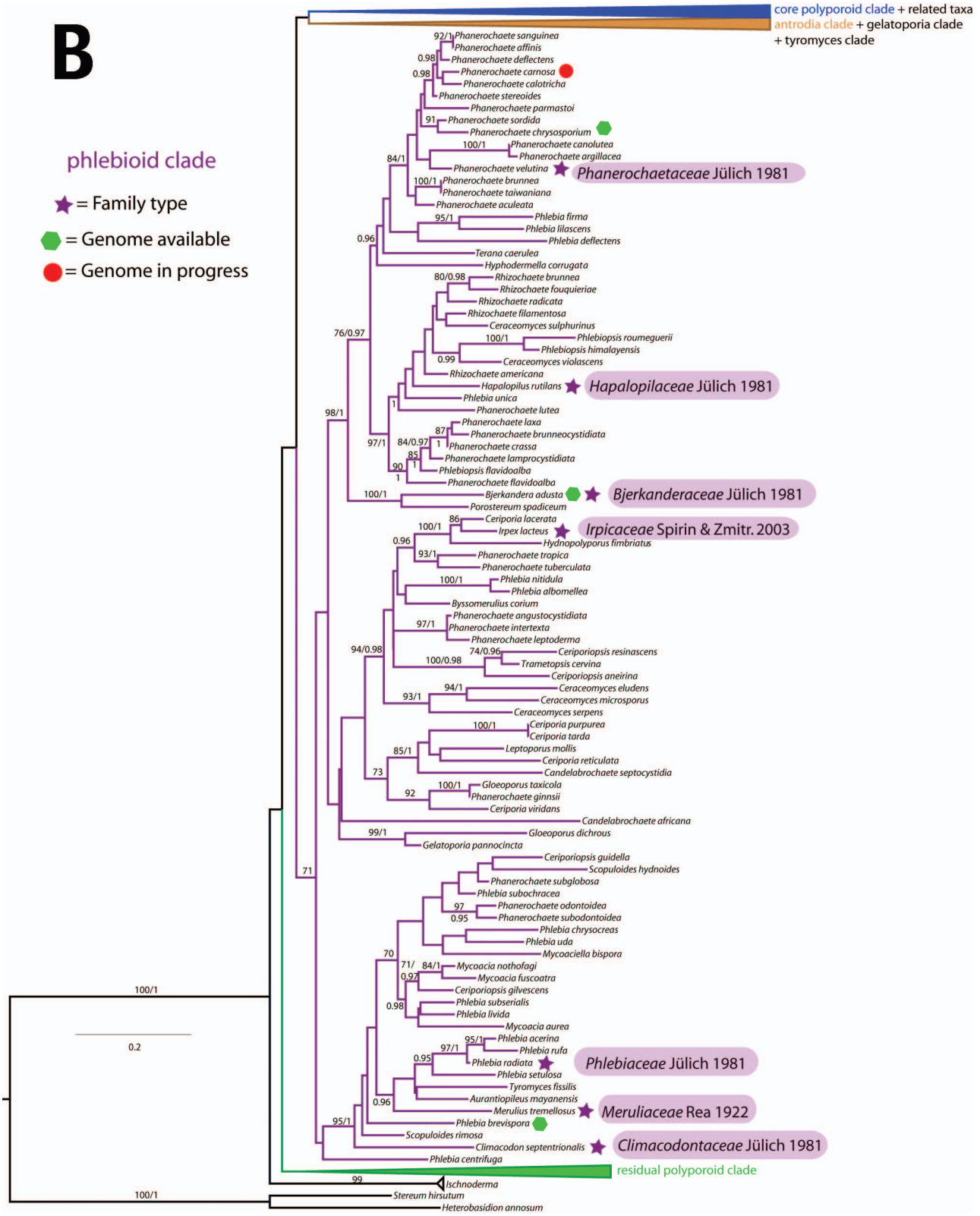

FIG. 4. Continued.

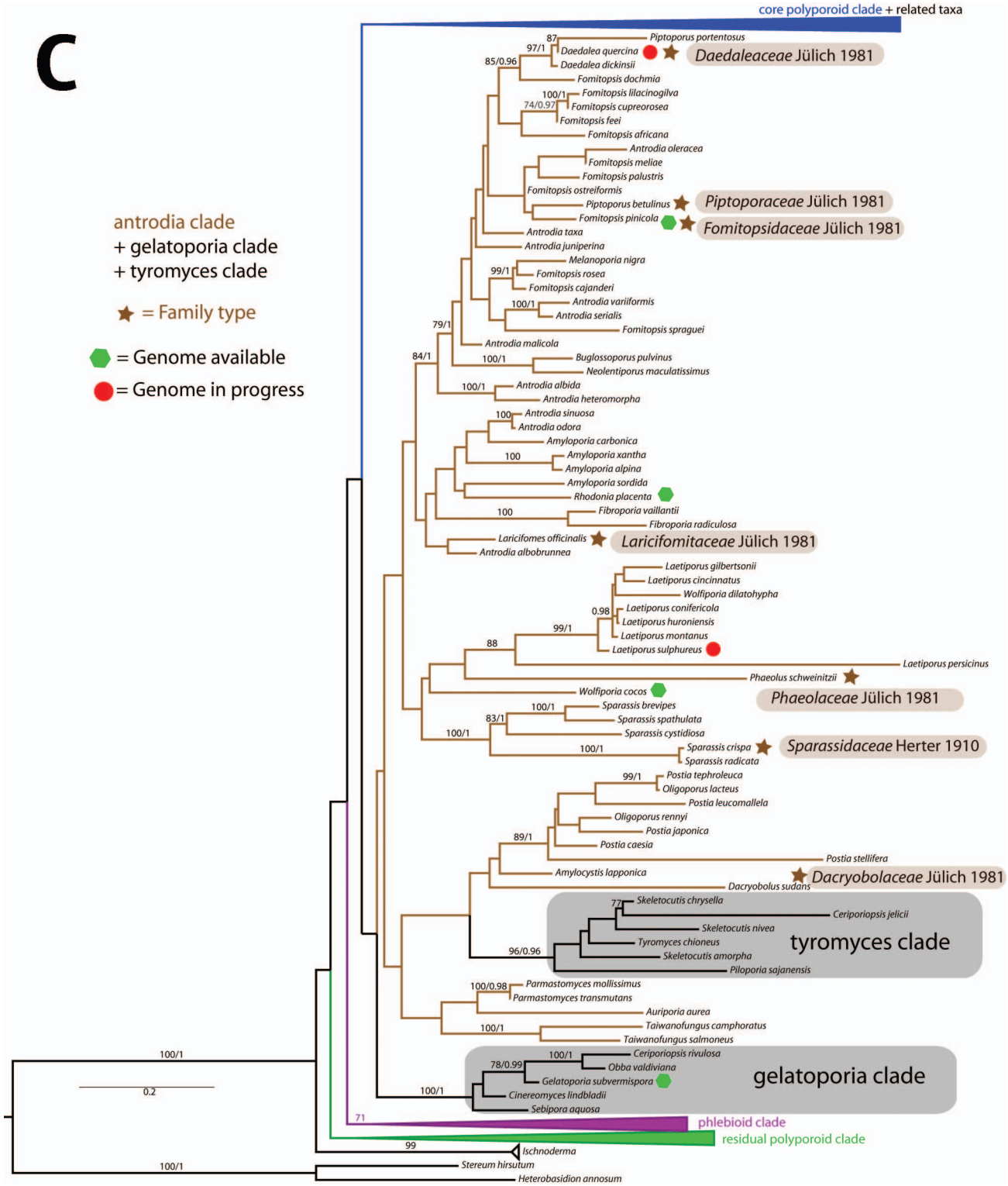

FIG. 4. Continued.

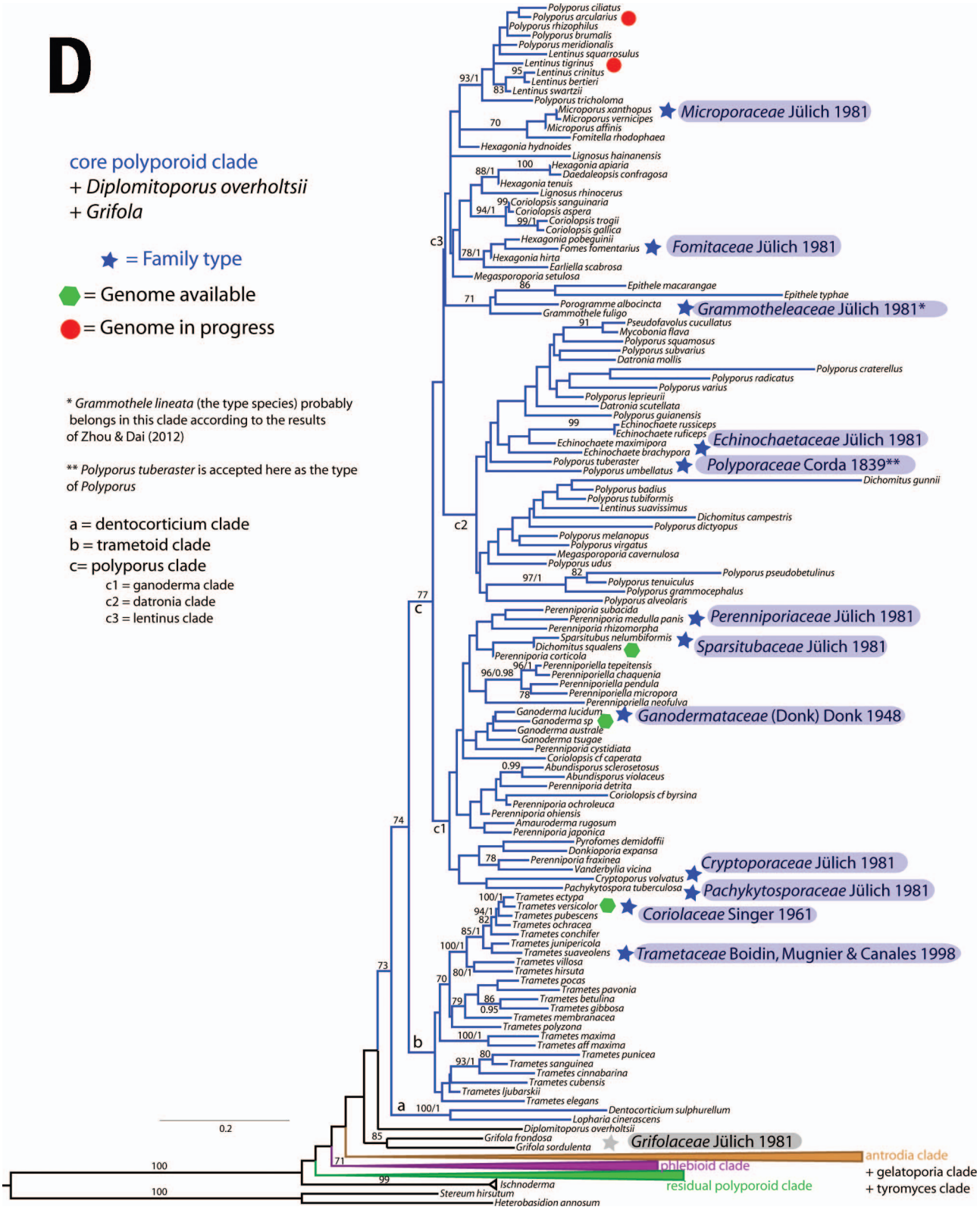

FIG. 4. Continued.

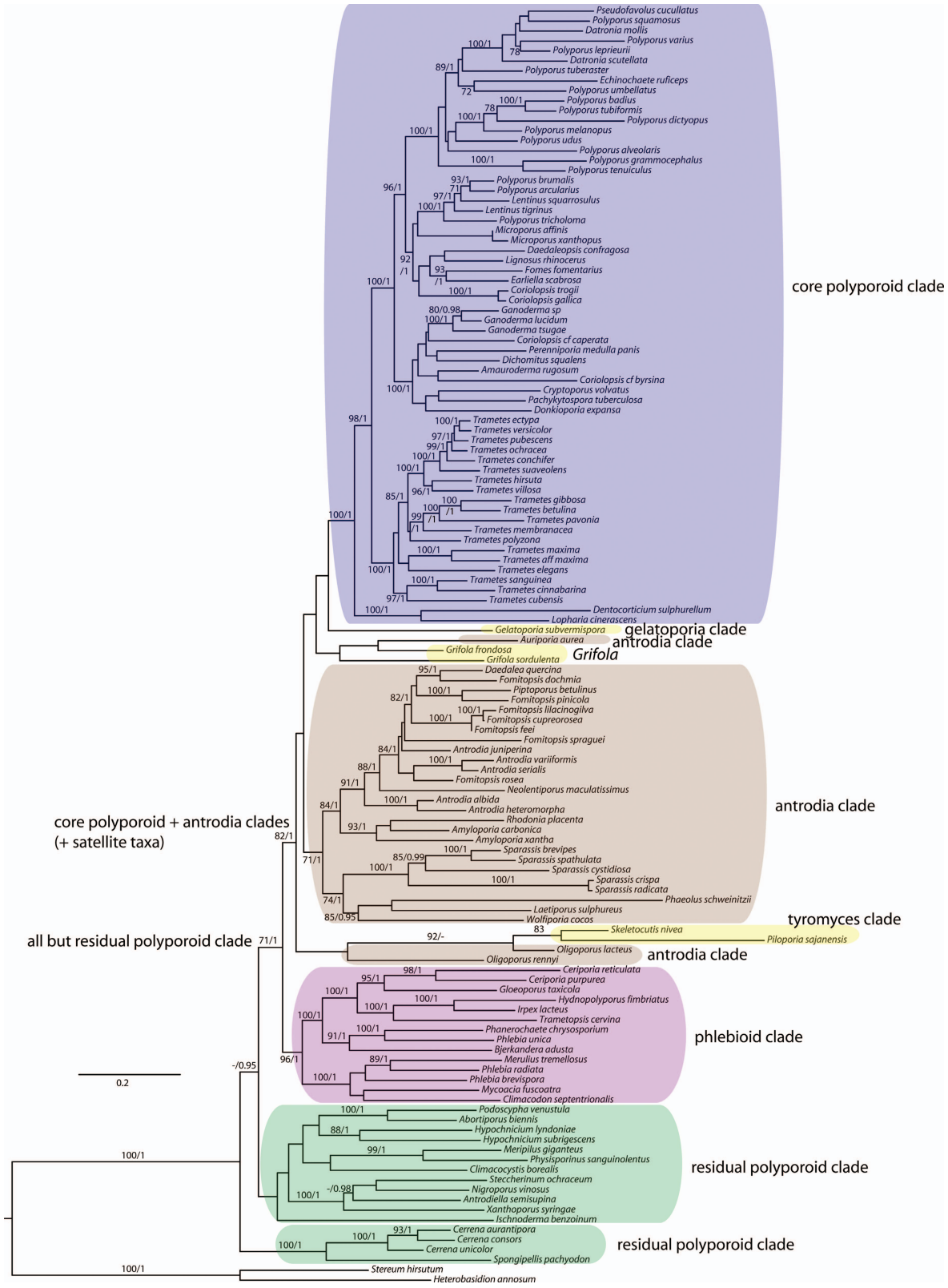

FIG. 5. Best tree from the ML analyses of the core supermatrix dataset. Support values are on or below branches.

characterized and labeled with ''unknown function'' or ''hypothetical protein'', and we only were able to assign 29% to higher level KEGG pathways (FIG. 1). The mechanisms and constraints for the maintenance of single-copy genes is not completely understood and their evolutionary rates can differ considerably, which may lead to topological incongruence (Wapinski et al. 2007, Feau et al. 2011). Nevertheless, single-copy genes hold great potential to improve phylogenetic resolution in clades that remain difficult based on analyses of commonly used universal loci (Aguileta et al. 2008). The most popular nonribosomal loci include *TEF1*, calmodulin, chitin synthase I, γ-actin (Carbone and Kohn 1999, Rehner and Buckley 2005), *atp6* (Kretzer and Bruns 1999), *RPB2* (Liu et al. 1999), *RPB1* (Matheny et al. 2002), β-tubulin and GPDH (Glass and Donaldson 1995). As illustrated in Feau et al. (2011), the use of these genes, individually or in combination, is disparate among studies and the resulting datasets are rarely overlap between the major fungal groups. From lessons learned we cannot expect to find a magic bullet that solves all our problems. In an attempt to answer the original question, ''more genes or more taxa?'', Rokas and Carroll (2005) concluded by making inferences from a dataset of 14 yeast genomes that the number of genes used is more important than the number of species. A denser sampling of larger, identical sets of genes has great potential to improve phylogenetic resolution and support (FIG. 2). In the present study we have shown that analyses of a (nearly complete) core dataset of Polyporales (FIG. 5) produce a better supported tree topology than in previous studies (e.g. Binder et al. 2005), which used four genes (nuclear and mitochondrial large and small subunits) and sampled ca. 125 Polyporales taxa (with only 47 represented by all four genes), and while the major groups discussed here were already apparent in that study there was no statistical support for any of them or for the relationships between them, only some nodes within those lineages received statistical support (cf. Binder et al. 2005 FIG. 4).

The phylogenetic trees presented here now can be used to select target species for the next round of whole genome sequencing. Our results from the extended core dataset (FIG. 4) also suggest that the introduction of missing data has its limitations and cannot be continued arbitrarily because it affects statistical support. Phylogenomics and phylogenetics are synergistic disciplines and the newly generated data will help build a robust backbone tree for the Polyporales.

We were able to reconstruct a fully resolved tree inferred from 25 out of 356 proteins identified through phylogenetic informativeness profiling (FIG. 3) (López-Giráldez and Townsend 2011), which will warrant further investigation in Polyporales and their allies. One option is individual primer design for the best performing genes, but this is a labor-intense, time-consuming process and there is no guarantee of finding a sufficient number of conservative sites to effectively cover larger parts of the genes. In addition, a broader applicability of newly designed primers has to stand the test of time and promising candidate genes, for example *FG1093* and *MS204* (Walker et al. 2012) or *mcm7* and *tsr1* (Schmitt et al. 2009), are still not widely used. Non PCR-based technologies are quickly advancing and offer an alternative to primer design (Faircloth et al. 2012, Lemmon et al. 2012). These methods use targeted hybrid DNA enrichment that makes it possible to analyze more than 500 genes simultaneously, which is roughly the size of the single-copy gene data for Polyporales, for up to 100 species. The objective is to identify conserved areas flanked by more variable regions in single-copy genes in small datasets of well studied genomes that serve as target probes. The probes then are tiled in small increments across the loci and combined across a species to generate a single probe set (Faircloth et al. 2012, Lemmon et al. 2012). This procedure has the advantage in that once a set of probes is produced, tiling permits its application to more distantly related species and libraries that have been constructed from the probe set can be subjected to high-throughput sequencing (Lemmon et al. 2012). Hybrid enrichment methods have the potential to tip the scales in favor of investigating more genes and more taxa in the imminent future.

Informativeness profiling.—All nodes of interest in the phylogeny are more ancient than the peaks of all of the informativeness profiles, so noise cannot be ignored and as a quantitative measure of utility the signal and noise-base probability of resolution should be used. The probabilities of resolution at each node of interest for all single-copy genes (SUPPLEMENTARY FILE 2) were used to sort genes by best performance across all epochs (FIG. 3). The overall best performer was 1263_2784; the overall worst was 1263_3596; the best performing for resolving recent nodes yet worst for ancient was 1263_2991 (ranked 18th overall); and the best performing for resolving ancient nodes yet worst for recent was 1263_2831 (ranked 9th overall).

It is worth noting the comparison between 1263_2991 and 1263_2831 because a cursory look at the informativeness profiles (FIG. 3) might give the impression that 1263_2991 would be better (it is always higher across the time scale plotted), but signal and noise tells us that is not so: noise from the high early peak makes it a poor subject for ancient resolution, whereas 1263_2831 is good at ancient

resolution, and despite being the biggest changer in the other direction it is a better overall choice because it still has sufficient information to resolve early nodes with some confidence.

The three AFTOL1 protein-coding genes, *rpb1*, *rpb2* and *tef1*, ranked 8th, 71st and 270th respectively (FIG. 3) in their overall phylogenetic resolution. Despite the great resolution power of *rpb1* across the epochs, it is by far the worst represented gene in GenBank with only 44 sequences of Polyporales available for this study.

Taxonomy of the Polyporales.—Our discussion centers around the major lineages recognized in the analyses of the extended supermatrix, core supermatrix and/or the phylogenomic datasets, the possible correspondence of those lineages with available family names in the Polyporales (TABLE I) and the recognition of critical nodes in the Polyporales phylogeny that need further attention. During the analyses it became evident that sets of sequences deposited in GenBank under different homotypic synonyms (e.g. *Phlebia deflectens*/*Phanerochete deflectens*; *Phanerochete flavidoalba*/*Phlebiopsis flavidoalba*) actually represent different species and that some taxa listed under different names (e.g. *Ischoderma benzoinum*/*Ischnoderma resinosum*; *Panus rudis*/*Panus lecomtei*) in fact might be the same species, however these species taxonomic problems are beyond the scope of the present study.

Residual polyporoid clade. Representative genera: *Abortiporus*, *Antrodiella*, *Atraporiella*, *Bulbillomyces*, *Cerrena*, *Climacocystis*, *Diplomitoporus*, *Hyphoderma*, *Hypochnicium*, *Ischnoderma*, *Junghuhnia*, *Meripilus*, *Nigroporus*, *Panus*, *Physisporinus*, *Podoscypha*, *Pseudolagarobasidium*, *Radulodon*, *Spongipellis*, *Steccherinum*, *Xanthoporus*. (The monophyly of many genera of the Polyporales mentioned in the discussion remains uncertain).

The name ''residual polypores'' was used first by Binder et al. (2005) to refer to a heterogeneous group of Polyporales that did not belong in any of the other lineages recognized by the authors (antrodia, core polyporoid and phlebioid clades). While the name is a practical way to refer to this group the monophyly of this lineage as a whole has yet to be proven. In our extended supermatrix tree *Ischnoderma* is placed as sister to all other Polyporales, and in the core supermatrix tree *Cerrena* and *Spongipellis* occupy that position. In both cases neither the placement of these taxa nor the grouping of the remainder residual polypores receives significant statistical support. In the core supermatrix tree (FIG. 5) the grouping of all Polyporales other than the residual polypores receives moderate to high statistical support (71% BS, 1 PP; FIG. 4A).

This group morphologically is extremely diverse including pileate-stipitate forms with gilled (*Panus*) or poroid hymenium (*Xanthoporus*), pileate-sessile (*Cerrena*), corticioid forms with smooth hymenium (*Hyphoderma*), stipitate-stereoid taxa (*Podoscypha*), resupinate-hydnoid (*Steccherinum*), resupinate-poroid (*Ceriporiopsis*) etc. The variation in microscopic characters is equally great with mono-, di- and trimitic taxa, clamp connections present or absent, cystidia present or absent etc. All taxa, however, are known to produce a white-rot wood decay (Ryvarden 1990, Bernicchia 2005, Bernicchia and Gorjón 2010).

Other than the grouping of *Cerrena*, *Panus*, *Pseudolagarobasidum*, *Radulodon* and *Spongipellis* in one clade the relationships between genera in this group remain largely unresolved. If research resolves the residual polyporoid clade as a monophyletic group and if one single family name is to be used for it then the name *Podoscyphaceae* is the oldest available. If several families are recognized for this lineage(s) there are at least five other available family names (FIG. 4A, TABLE I).

Resolving the phylogenetic relationships in this group is critical for identifying the earliest-diverging lineages in the Polyporales that eventually will allow for a detailed study of character evolution in the order. Unfortunately at present no genomic data for members of the residual polyporoid clade are available, but the genome sequencing of *Panus rudis* is in progress.

Phlebioid clade. Representative genera: *Aurantiopileus*, *Bjerkandera*, *Byssomerulius*, *Candelabrochaete*, *Ceriporia*, *Climacodon*, *Gloeoporus*, *Hapalopilus*, *Hydnopolyporus*, *Hyphodermella*, *Irpex*, *Leptoporus*, *Merulius*, *Mycoacia*, *Mycoaciella*, *Phanerochaete*, *Phlebia*, *Phlebiopsis*, *Porostereum*, *Rhizochaete*, *Scopuloides*, *Terana*, *Trametopsis*.

The term ''phlebioid clade'' first was introduced by Larsson et al. (2004) to refer to a group of corticioid and resupinate genera including *Phlebia*, *Byssomerulius* and *Hyphoderma* among others. Binder et al. (2005) maintained the use of the name but excluded from the phlebioid clade *Hyphoderma* and related taxa in that they were placed in the residual polyporoid clade, a result that is supported in the analyses presented here (FIG. 4B).

The phlebioid clade as defined here is only moderately supported in the extended supermatrix dataset (71% BS; FIG. 4B) but receives better support in the core supermatrix dataset (96% BS, 1 PP; FIG. 5) and also by the genomic dataset where *Bjerkandera adusta*, *Phanerochaete chrysosporium* and *Phlebia brevispora* are represented (FIG. 2). In all datasets the phlebioid clade is the sister group of the clade containing the antrodia and core polyporoid clades and their related lineages.

The taxonomy of many of the genera belonging in the phlebioid clade is far from settled, and a case in point example is the genus *Phlebia*. A total 26 taxa currently accepted in the genus *Phlebia*, including *Mycoacia*, *Mycoaciella* and *Merulius* (Nakasone and Burdsall 1984; Nakasone 1997, 2002; Bernicchia and Gorjón 2010; Moreno et al. 2011), were included in the extended supermatrix analyses, and they appear widely distributed in and out of the phlebioid clade; (i) *P. bresadolae* and *P. queletii* are placed in the residual polyporoid clade; (ii) the type species (*P. radiata*) groups with several *Phlebia*, *Merulius*, *Mycoacia* and *Mycoaciella* species, but taxa belonging to other genera (*Climacodon*, *Tyromyces*, *Scopuloides*, *Aurantiopileus*, *Ceriporiopsis* p.p.) appear intermixed with *Phlebia* species; (iii) *P. nitidula* and *P. albomellea* are more closely related to the lineage including *Ceraceomyces*, *Irpex* and *Trametopsis* than to other *Phlebia* species; (iv) *P. unica* appears in the same group as *Rhizochaete* and *Phlebiopsis*; (v) *P. deflectens*, *P. firma* and *P. lilascens* are closely related to *Phanerochaete*.

Corticioid and resupinate forms predominate in the phlebioid clade, but pileate forms also occur (e.g. *Hapalopilus*). There is an extensive variation in types of hymenium and microscopic characters. All taxa included in the phlebioid clade produce white-rot decay with one notable exception, *Leptoporus mollis*, which is placed in the extended dataset closely related to *Ceriporia*. *L. mollis* is described as causing a ''brown cubical rot on dead conifers'' (Gilbertson and Ryvarden 1986). The same authors wrote, ''The microscopic characters of *L. mollis* suggest relationships with species of *Ceriporia*, all white-rot fungi. Because *L. mollis* does not show close affinities to other brown-rot fungi it is best kept as a monotypic genus.'' The seemingly anomalous phylogenetic position of *Leptoporus* was noted by Lindner and Banik (2008) and, if confirmed in subsequent analyses, it would represent an independent origin of the brown-rot ecology in the Polyporales outside the antrodia clade.

The name *Meruliaceae* is the oldest available at the family level for the phlebioid clade. If several families are recognized in this lineage there are six other available family names (FIG. 4B, TABLE I). Three genomes currently are available for taxa in the phlebioid clade: *Bjerkandera adusta*, *Phlebia brevispora*, and *Phanerochaete chrysosporium* and the genome of *Phanerochaete carnosa* is currently in progress.

Antrodia clade, gelatoporia clade, tyromyces clade and Grifola. Representative genera: *Amylocystis*, *Amyloporia*, *Antrodia*, *Auriporia*, *Buglossoporus*, *Cinereomyces*, *Dacryobolus*, *Daedalea*, *Fibroporia*, *Fomitopsis*, *Gelatoporia*, *Grifola*, *Laetiporus*, *Laricifomes*, *Neolentiporus*, *Obba*, *Oligoporus*, *Parmastomyces*, *Phaeolus*, *Piloporia*, *Piptoporus*, *Postia*, *Rhodonia*, *Sebipora*, *Skeletocutis*, *Sparassis*, *Taiwanofungus*, *Tyromyces*, *Wolfiporia*.

The antrodia clade was introduced first by Hibbett and Donoghue (2001) and further delineated by Binder et al. (2005). All taxa putatively belonging in the clade share brown-rot wood decay. The composition and support of the antrodia clade has been, and remains, one of the major issues in the phylogeny of the Polyporales. In the analyses by Binder et al. (2005) isolates of the white-rot *Climacocystis* sp., *Grifola frondosa* and (in some topologies) *Ischnoderma benzoinum* were nested inside the antrodia clade. In the analyses by Garcia-Sandoval et al. (2011) *Grifola frondosa* again was nested inside the antrodia clade.

In our extended supermatrix analysis none of these taxa appears nested in the antrodia clade but a small clade of white-rot polypores (the tyromyces clade, FIG. 4C) is nested within two brown-rot lineages. This placement receives no statistical support but brings to our attention this recurring problem and the need to clarify the phylogenetic position of the lineages that are not brown rot (antrodia clade); neither are supported as belonging in the core polyporoid clade (discussed below). Some of these lineages have been studied in detail by Miettinen and Rajchenberg (2012) who, based on nrLSU and ITS data, placed the gelatoporia clade (''*Cinereomyces* clade'' in their study) as the sister group of the core polyporoid clade and the tyromyces clade as sister to a clade containing the residual, phlebioid and antrodia clades plus *Grifola*. In our analyses the phylogenetic position of these lineages is not well resolved: (i) the gelatoporia clade is placed as the sister group of the antrodia clade + tyromyces clade (extended supermatrix, FIG. 4C) or as sister to the core polyporoid clade (core supermatrix, FIG. 5). In the genomic analyses *Gelatoporia subvermispora* is sister to the antrodia clade (FIG. 2); (ii) The tyromyces clade appears nested in the antrodia clade (extended supermatrix, FIG. 4C) or intermixed with *Oligoporus* species that cluster separately from the rest of the antrodia clade (core supermatrix, FIG. 5); (iii) *Grifola* appears as sister to the core polyporoid + *Diplomitoporus overholtsii* (extended supermatrix, FIG. 4D) or intermixed with *Auriporia aurea* and sister to the core polyporoid + gelatoporia clades (core supermatrix, FIG. 5).

The clade containing these satellite lineages plus the antrodia and core polyporoid clades is relatively well supported in the core-supermatrix dataset (82% BS, 1 PP; FIG. 5) and receives full support in the genomic analyses (FIG. 2). Resolving the internal relations among the lineages in this clade is critical for elucidating the origins of brown-rot polypores and how many transitions between white and brown rot

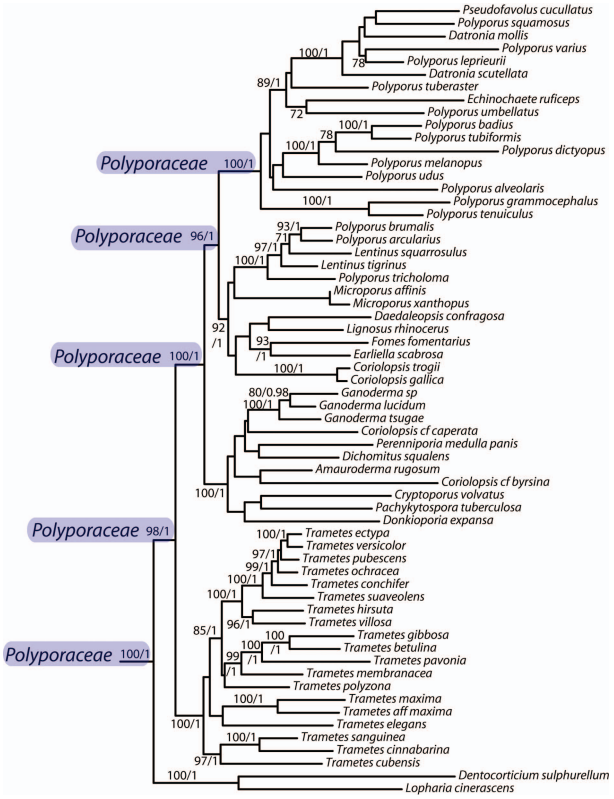

FIG. 6. Alternative circumscriptions of the *Polyporaceae* based on the results of the core supermatrix dataset.

have occurred in the Polyporales. Our results do not support the previously suggested reversal to white rot from brown rot for *Grifola* (García-Sandoval et al. 2011), but the unresolved position of these small white-rot lineages with respect to the brown-rot antrodia clade needs further study.

Pileate-stipitate (e.g. *Phaeolus*), pileate-sessile (e.g. *Oligoporus*) and resupinate forms (e.g. *Amylocystis*) with poroid hymenophores are predominant in the antrodia clade, but true corticioids with smooth hymenophores (e.g. *Dacryobolus*), taxa with daedaleoid hymenophores (*Daedalea*) and species with cauliflower-like basidiocarps (*Sparassis*) also exist. A detailed phylogenetic overview of the genus taxonomy of the antrodia clade is given by Ortiz-Santana et al. (this issue) Three genomes are available for taxa in the antrodia clade: *Fomitopsis pinicola*, *Rhodonia placenta* and *Wolfiporia cocos* and two more are in progress for *Daedalea quercina* and *Laetiporus sulphureus*.

If the antrodia clade is resolved as monophyletic and one family name is used for it, the name *Sparassidaceae* is the oldest available. If several families are recognized for this lineage(s), there are six other available family names (FIG. 4C). The name *Grifolaceae* is available for *Grifola* that apparently does not belong to either the antrodia or core polyporoid clades. No family names are available for the gelatoporia and tyromyces clades.

Core polyporoid clade. Representative genera: *Abundisporus*, *Amauroderma*, *Coriolopsis* sensu lato, *Cryptoporus*, *Datronia*, *Dichomitus*, *Donkioporia*, *Earliella*, *Echinochaete*, *Epithele*, *Fomes*, *Fomitella*, *Ganoderma*, *Grammothele*, *Hexagonia*, *Lentinus*, *Lignosus*, *Megasporoporia*, *Microporus*, *Pachykytospora*, *Perenniporia*, *Perenniporiella*, *Polyporus* sensu lato, *Porogramme*, *Pyrofomes*, *Sparsitubus*, *Trametes*, *Vanderbylia*.

The term ''core polyporoid clade'' was introduced first by Binder et al. (2005), although it was recognized under different names before that, for example ''polyporoid clade'' in Larsson et al. (2004), *Polyporaceae* in Kim and Jung (2000). The core polyporoid clade receives only moderate support in the extended supermatrix dataset (73% BS, FIG. 4C) and full support in the core supermatrix and genomic datasets (FIGS. 2, 5). The three major lineages of the core polyporoid clade recognized by Justo and Hibbett (2011) are present in the extended and core datasets (FIGS. 4D, 5), but they receive only significant support in the later: (i) dentocorticium clade. Includes *Dentocorticium sulphurellum* and *Lopharia cinerascens*. This is very likely the earliest diverging lineage in the core polyporoid clade; (ii) trametoid clade. Corresponds to *Trametes* in the sense of Justo and Hibbett (2011). Other authors (e.g. Welti et al. 2012) have proposed a different taxonomic organization for this clade; (iii) polyporus clade. The three lineages named by Justo and Hibbett (2011) as datronia clade, ganoderma clade and lentinus clade are present in the extended and core datasets, with good support values in the later.

Pileate-stipitate and pileate-sessile basidiocarps with poroid hymenophores are predominant in the core polyporoid clade, but taxa with lamellate hymenophores (e.g. *Lentinus*, *Trametes*) and resupinate forms (e.g. *Dentocorticium*, *Grammothele*) also exist. Most of the taxa have di- or trimitic hyphal systems and tetrapolar mating systems. All taxa in the core polyporoid clade produce a white-rot wood decay.

The use of the family name *Polyporaceae* perfectly exemplifies the need for a consensus among taxonomists for a formal suprageneric taxonomic arrangement in the Polyporales. Assuming that the phylogeny of the core polyporoid clade presented here is supported in future studies, five well supported nodes in the core polyporoid clade can define the limits of the *Polyporaceae* (FIG. 6). Three genomes are available for taxa in the core polyporoid clade, *Dichomitus squalens*, *Ganodema* sp. and *Trametes versicolor*, and two more are in progress for *Lentinus tigrinus* and *Polyporus arcularius*.

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